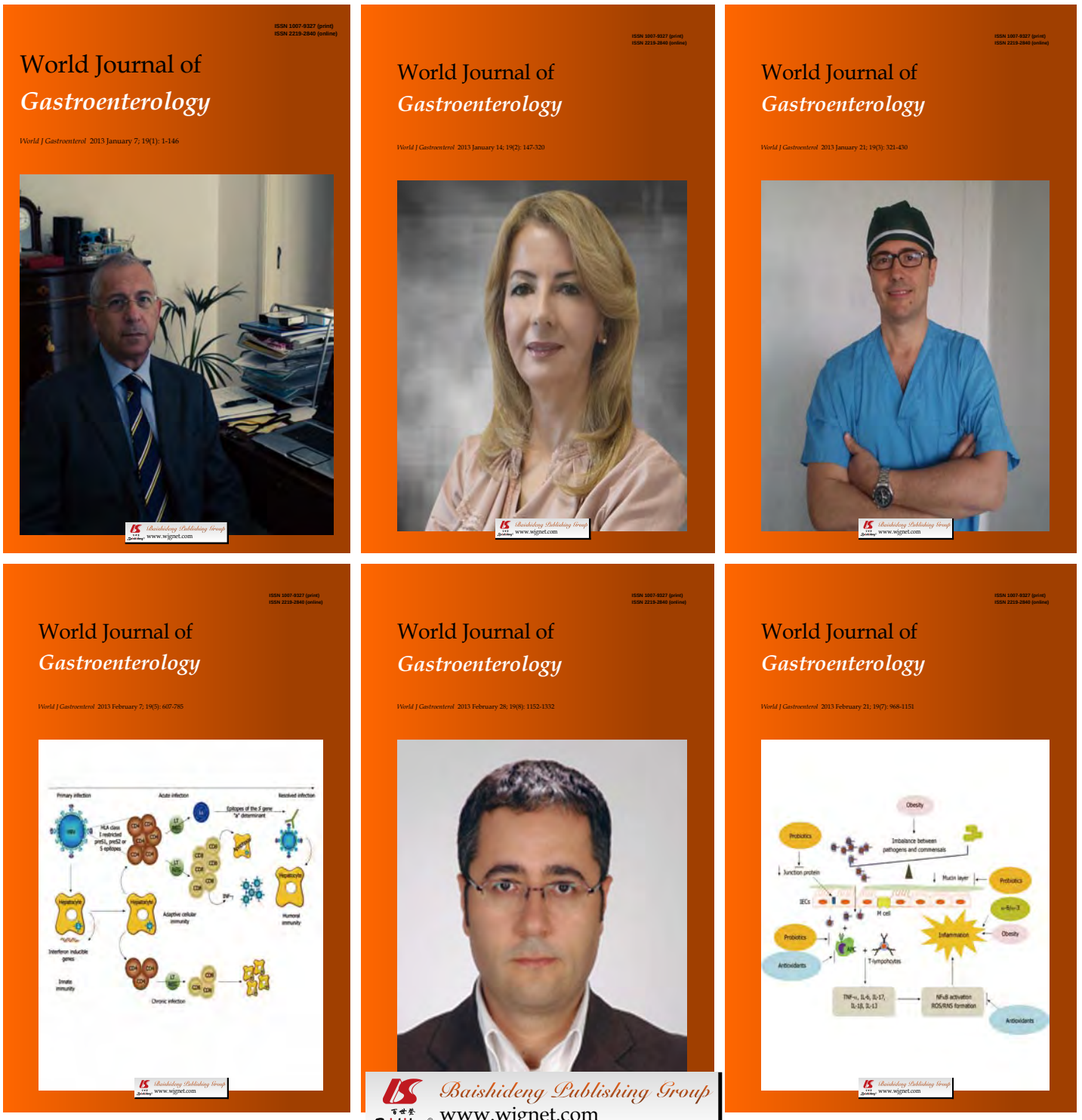
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

2013 Bound Volume 19 Issue 1-8: 1-1332



Baishideng Publishing Group
www.wjgnet.com

WJG World Journal of Gastroenterology

A peer-reviewed, online, open-access journal of gastroenterology and hepatology

Editorial Board

2010-2013

The *World Journal of Gastroenterology* Editorial Board consists of 1352 members, representing a team of worldwide experts in gastroenterology and hepatology. They are from 64 countries, including Albania (1), Argentina (8), Australia (33), Austria (15), Belgium (14), Brazil (13), Brunei Darussalam (1), Bulgaria (2), Canada (21), Chile (3), China (82), Colombia (1), Croatia (2), Cuba (1), Czech (6), Denmark (9), Ecuador (1), Egypt (4), Estonia (2), Finland (8), France (29), Germany (87), Greece (22), Hungary (11), India (32), Indonesia (2), Iran (10), Ireland (6), Israel (13), Italy (124), Japan (140), Jordan (2), Kuwait (1), Lebanon (4), Lithuania (2), Malaysia (1), Mexico (11), Morocco (1), Moldova (1), Netherlands (32), New Zealand (2), Norway (13), Pakistan (2), Poland (11), Portugal (6), Romania (4), Russia (1), Saudi Arabia (3), Serbia (3), Singapore (11), Slovenia (1), South Africa (3), South Korea (46), Spain (43), Sri Lanka (1), Sweden (17), Switzerland (12), Thailand (1), Trinidad and Tobago (1), Turkey (30), United Arab Emirates (2), United Kingdom (95), United States (285), and Uruguay (1).

HONORARY EDITORS-IN-CHIEF

James L Boyer, *New Haven*
Ke-Ji Chen, *Beijing*
Martin H Floch, *New Haven*
Bo-Rong Pan, *Xi'an*
Eamonn M Quigley, *Cork*
Rafiq A Sheikh, *Sacramento*
Nicholas J Talley, *Rochester*

EDITOR-IN-CHIEF

Ferruccio Bonino, *Pisa*
Myung-Hwan Kim, *Seoul*
Kjell Öberg, *Uppsala*
Matt Rutter, *Stockton-on-Tees*
Andrzej S Tarnawski, *Long Beach*

STRATEGY ASSOCIATE EDITORS-IN-CHIEF

You-Yong Lu, *Beijing*
Peter Draganov, *Florida*
Hugh J Freeman, *Vancouver*
Maria Concepción Gutiérrez-Ruiz, *Mexico*
Kazuhiro Hanazaki, *Kochi*
Akio Inui, *Kagoshima*
Kalpesh Jani, *Baroda*
Javier San Martin, *Punta del Este*
Natalia A Osna, *Omaha*
Wei Tang, *Tokyo*
Alan BR Thomson, *Edmonton*
Harry Hua-Xiang Xia, *Livingston*
John M Luk, *Hong Kong*
Hiroshi Shimada, *Yokohama*

GUEST EDITORIAL BOARD MEMBERS

Jiunn-Jong Wu, *Tainan*
Cheng-Shyong Wu, *Chia-Yi*
Ta-Sen Yeh, *Taoyuan*
Tsung-Hui Hu, *Kaohsiung*
Chuah Seng-Kee, *Kaohsiung*
I-Rue Lai, *Taipei*
Jin-Town Wang, *Taipei*
Ming-Shiang Wu, *Taipei*
Teng-Yu Lee, *Taichung*
Yang-Yuan Chen, *Changhua*
Po-Shiuan Hsieh, *Taipei*
Chao-Hung Hung, *Kaohsiung*
Hon-Yi Shi, *Kaohsiung*
Hui-kang Liu, *Taipei*
Jen-Hwey Chiu, *Taipei*
Chih-Chi Wang, *Kaohsiung*
Wan-Long Chuang, *Kaohsiung*
Wen-Hsin Huang, *Taichung*
Hsu-Heng Yen, *Changhua*
Ching Chung Lin, *Taipei*
Chien-Jen Chen, *Taipei*
Jaw-Ching Wu, *Taipei*
Ming-Chih Hou, *Taipei*
Kevin Cheng-Wen Hsiao, *Taipei*
Chiun Hsu, *Taipei*
Yu-Jen Chen, *Taipei*
Chen Hsiu-Hsi Chen, *Taipei*
Liang-Shun Wang, *Taipei*
hun-Fa Yang, *Taichung*
Min-Hsiung Pan, *Kaohsiung*
Chun- Hung Lin, *Taipei*
Ming-Whei Yu, *Taipei*
Chuen Hsueh, *Taoyuan*
Hsiu-Po Wang, *Taipei*
Lein-Ray Mo, *Tainan*
Ming-Lung Yu, *Kaohsiung*

MEMBERS OF THE EDITORIAL BOARD



Albania

Bashkim Resuli, *Tirana*



Argentina

Julio H Carri, *Córdoba*
Bernabe Matias Quesada, *Buenos Aires*
Bernardo Frider, *Buenos Aires*
Maria Ines Vaccaro, *Buenos Aires*
Eduardo de Santibañes, *Buenos Aires*
Adriana M Torres, *Rosario*
Carlos J Pirola, *Buenos Aires*
Silvia Sookoian, *Buenos Aires*



Australia

Finlay A Macrae, *Victoria*
David Ian Watson, *Bedford Park*
Jacob George, *Sydney*
Leon Anton Adams, *Nedlands*
Minoti V Apte, *Liverpool*
Andrew V Biankin, *Sydney*
Filip Braet, *Sydney*
Guy D Eslick, *Sydney*
Michael A Fink, *Melbourne*
Mark D Gorrell, *Sydney*
Michael Horowitz, *Adelaide*
John E Kellow, *Sydney*
Daniel Markovich, *Brisbane*

Phillip S Oates, *Perth*
Ross C Smith, *Sydney*
Kevin J Spring, *Brisbane*
Philip G Dinning, *Koagarah*
Christopher Christophi, *Melbourne*
Cuong D Tran, *North Adelaide*
Shan Rajendra, *Tasmania*
Rajvinder Singh, *Adelaide*
William Kemp, *Melbourne*
Phil Sutton, *Melbourne*
Richard Anderson, *Victoria*
Vance Matthews, *Melbourne*
Alexander G Heriot, *Melbourne*
Debbie Trinder, *Fremantle*
Ian C Lawrance, *Perth*
Adrian G Cummins, *Adelaide*
John K Olynyk, *Fremantle*
Alex Boussioutas, *Melbourne*
Emilia Prakoso, *Sydney*
Robert JL Fraser, *Daw Park*



Austria

Wolfgang Mikulits, *Vienna*
Alfred Gangl, *Vienna*
Dietmar Öfner, *Salzburg*
Georg Roth, *Vienna*
Herwig R Cerwenka, *Graz*
Ashraf Dahaba, *Graz*
Markus Raderer, *Vienna*
Alexander M Hirschl, *Wien*
Thomas Wild, *Kapellerfeld*
Peter Ferenci, *Vienna*
Valentin Fuhrmann, *Vienna*
Kurt Lenz, *Linz*
Markus Peck-Radosavljevic, *Vienna*
Michael Trauner, *Vienna*
Stefan Riss, *Vienna*



Belgium

Rudi Beyaert, *Gent*
Inge I Depoortere, *Leuven*
Olivier Detry, *Liège*
Benedicte Y De Winter, *Antwerp*
Etienne M Sokal, *Brussels*
Marc Peeters, *De Pintelaan*
Eddie Wisse, *Keerbergen*
Jean-Yves L Reginster, *Liège*
Mark De Ridder, *Brussel*
Freddy Penninckx, *Leuven*
Kristin Verbeke, *Leuven*
Lukas Van Oudenhove, *Leuven*
Leo van Grunsven, *Brussels*
Philip Meuleman, *Ghent*



Brazil

Heitor Rosa, *Goiania*
Roberto J Carvalho-Filho, *Sao Paulo*
Damiao Carlos Moraes Santos, *Rio de Janeiro*
Marcelo Lima Ribeiro, *Braganca Paulista*
Eduardo Garcia Vilela, *Belo Horizonte*
Jaime Natan Eisig, *São Paulo*
Andre Castro Lyra, *Salvador*
José Liberato Ferreira Caboclo, *Brazil*
Yukie Sato-Kuwabara, *São Paulo*
Raquel Rocha, *Salvador*
Paolo R Salvalaggio, *Sao Paulo*
Ana Cristina Simões e Silva, *Belo Horizonte*
Joao Batista Teixeira Rocha, *Santa Maria*



Brunei Darussalam

Vui Heng Chong, *Bandar Seri Begawan*



Bulgaria

Zahariy Krastev, *Sofia*
Mihaela Petrova, *Sofia*



Canada

Eldon Shaffer, *Calgary*
Nathalie Perreault, *Sherbrooke*
Philip H Gordon, *Montreal*
Ram Prakash Galwa, *Ottawa*
Baljinder Singh Salh, *Vancouver*
Claudia Zwingmann, *Montreal*
Alain Bitton, *Montreal*
Pingchang Yang, *Hamilton*
Michael F Byrne, *Vancouver*
Andrew L Mason, *Alberta*
John K Marshall, *Hamilton Ontario*
Kostas Pantopoulos, *Montreal*
Waliul Khan, *Ontario*
Eric M Yoshida, *Vancouver*
Geoffrey C Nguyen, *Toronto*
Devendra K Amre, *Montreal*
Tedros Bezabeh, *Winnipeg*
Wangxue Chen, *Ottawa*
Qiang Liu, *Saskatoon*



Chile

De Aretxabala Xabier, *Santiago*
Marcelo A Beltran, *La Serena*
Silvana Zanlungo, *Santiago*



China

Chi-Hin Cho, *Hong Kong*
Chun-Qing Zhang, *Jinan*
Ren Xiang Tan, *Nanjing*
Fei Li, *Beijing*
Hui-Jie Bian, *Xi'an*
Xiao-Peng Zhang, *Beijing*
Xing-Hua Lu, *Beijing*
Fu-Sheng Wang, *Beijing*
An-Gang Yang, *Xi'an*
Xiao-Ping Chen, *Wuhan*
Zong-Jie Cui, *Beijing*
Ming-Liang He, *Hong Kong*
Yuk-Tong Lee, *Hong Kong*
Qin Su, *Beijing*
Jian-Zhong Zhang, *Beijing*
Paul Kwong-Hang Tam, *Hong Kong*
Wen-Rong Xu, *Zhenjiang*
Chun-Yi Hao, *Beijing*
San-Jun Cai, *Shanghai*
Simon Law, *Hong Kong*
Yuk Him Tam, *Hong Kong*
De-Liang Fu, *Shanghai*
Eric WC Tse, *Hong Kong*
Justin CY Wu, *Hong Kong*
Nathalie Wong, *Hong Kong*
Jing Yuan Fang, *Shanghai*
Yi-Min Mao, *Shanghai*
Wei-Cheng You, *Beijing*
Xiang-Dong Wang, *Shanghai*
Xuan Zhang, *Beijing*
Zhao-Shen Li, *Shanghai*
Guang-Wen Cao, *Shanghai*
En-min Li, *Shantou*
Yu-Yuan Li, *Guangzhou*
Fook Hong Ng, *Hong Kong*
Hsiang-Fu Kung, *Hong Kong*
Wai Lun Law, *Hong Kong*
Eric CH Lai, *Hong Kong*
Jun Yu, *Hong Kong*
Ze-Guang Han, *Shanghai*
Bian zhao-xiang, *Hong Kong*
Wei-Dong Tong, *Chongqing*



Colombia

Germán Campuzano-Maya, *Medellín*



Croatia

Tamara Cacev, *Zagreb*
Marko Duvnjak, *Zagreb*



Cuba

Damian C Rodriguez, *Havana*



Czech

Milan Jirsa, *Praha*
Pavel Trunečka, *Prague*
Jan Bures, *Hradec Kralove*
Marcela Kopacova, *Hradec Kralove*
Ondrej Slaby, *Brno*
Radan Bruha, *Prague*



Denmark

Asbjørn M Drewes, *Aalborg*
Leif Percival Andersen, *Copenhagen*
Jan Mollenhauer, *Odense C*
Morten Frisch, *Copenhagen S*
Jorgen Rask-Madsen, *Skodsborg*
Morten Hylander Møller, *Holte*
Søren Rafaelsen, *Vejle*
Vibeke Andersen, *Aabenraa*
Ole Haagen Nielsen, *Herlev*



Ecuador

Fernando E Sempértegui, *Quito*



Egypt

Zeinab Nabil Ahmed Said, *Cairo*
Hussein M Atta, *El-Minia*
Asmaa Gaber Abdou, *Shebein Elkom*

Maha Maher Shehata, *Mansoura*



Estonia

Riina Salupere, *Tartu*
Tamara Vorobjova, *Tartu*



Finland

Saila Kauhanen, *Turku*
Pauli Antero Puolakkainen, *Turku*
Minna Nyström, *Helsinki*
Juhani Sand, *Tampere*
Jukka-Pekka Mecklin, *Jyvaskyla*
Lea Veijola, *Helsinki*
Kaija-Leena Kolho, *Helsinki*
Thomas Kietzmann, *Oulu*



France

Boris Guiu, *Dijon*
Baumert F Thomas, *Strasbourg*
Alain L Servin, *Châtenay-Malabry*
Patrick Marcellin, *Paris*
Jean-Jacques Tuech, *Rouen*
Francoise L Fabiani, *Angers*
Jean-Luc Faucheron, *Grenoble*
Philippe Lehours, *Bordeaux*
Stephane Supiot, *Nantes*
Lionel Bueno, *Toulouse*
Flavio Maina, *Marseille*
Paul Hofman, *Nice*
Abdel-Majid Khatib, *Paris*
Annie Schmid-Alliana, *Nice cedex 3*
Frank Zerbib, *Bordeaux Cedex*
Rene Gerolami Santandera, *Marseille*
Sabine Colnot, *Paris*
Catherine Daniel, *Lille Cedex*
Thabut Dominique, *Paris*
Laurent Huwart, *Paris*
Alain Braillon, *Amiens*
Bruno Bonaz, *Grenoble*
Evelyne Schvoerer, *Strasbourg*
M Coeffier, *Rouen*
Mathias Chamaillard, *Lille*
Hang Nguyen, *Clermont-Ferrand*
Veronique Vitton, *Marseille*
Alexis Desmoulière, *Limoges*
Juan Iovanna, *Marseille*



Germany

Hans L Tillmann, *Leipzig*
Stefan Kubicka, *Hannover*
Elke Cario, *Essen*
Hans Scherubl, *Berlin*
Harald F Teutsch, *Ulm*
Peter Konturek, *Erlangen*
Thilo Hackert, *Heidelberg*
Jurgen M Stein, *Frankfurt*
Andrej Khandoga, *Munich*
Karsten Schulmann, *Bochum*
Jutta Elisabeth Lüttges, *Riegelsberg*
Wolfgang Hagmann, *Heidelberg*
Hubert Blum, *Freiburg*
Thomas Bock, *Berlin*
Christa Buechler, *Regensburg*
Christoph F Dietrich, *Bad Mergentheim*
Ulrich R Fölsch, *Kiel*
Nikolaus Gassler, *Aachen*
Markus Gerhard, *Munich*
Dieter Glebe, *Giessen*
Klaus R Herrlinger, *Stuttgart*
Eberhard Hildt, *Berlin*
Joerg C Hoffmann, *Ludwigshafen*
Joachim Labenz, *Siegen*
Peter Malfertheiner, *Magdeburg*
Sabine Mihm, *Göttingen*
Markus Reiser, *Bochum*
Steffen Rickes, *Magdeburg*
Andreas G Schreyer, *Regensburg*
Henning Schulze-Bergkamen, *Heidelberg*
Ulrike S Stein, *Berlin*
Wolfgang R Stremmel, *Heidelberg*
Fritz von Weizsäcker, *Berlin*
Stefan Wirth, *Wuppertal*
Dean Bogoevski, *Hamburg*
Bruno Christ, *Halle/Saale*
Peter N Meier, *Hannover*
Stephan Johannes Ott, *Kiel*
Arndt Vogel, *Hannover*
Dirk Haller, *Freising*
Jens Standop, *Bonn*
Jonas Mudter, *Erlangen*
Jürgen Büning, *Lübeck*
Matthias Ocker, *Erlangen*
Joerg Trojan, *Frankfurt*
Christian Trautwein, *Aachen*
Jorg Kleeff, *Munich*
Christian Rust, *Munich*
Claus Hellerbrand, *Regensburg*
Elke Roeb, *Giessen*
Erwin Biecker, *Siegburg*
Ingmar Königsrainer, *Tübingen*
Jürgen Borlak, *Hannover*
Axel M Gressner, *Aachen*
Oliver Mann, *Hamburg*
Marty Zdichavsky, *Tübingen*
Christoph Reichel, *Bad Brückenau*
Nils Habbe, *Marburg*
Thomas Wex, *Magdeburg*
Frank Ulrich Weiss, *Greifswald*
Manfred V Singer, *Mannheim*
Martin K Schilling, *Homburg*
Philip D Hard, *Giessen*
Michael Linnebacher, *Rostock*
Ralph Graeser, *Freiburg*
Rene Schmidt, *Freiburg*
Robert Obermaier, *Freiburg*
Sebastian Mueller, *Heidelberg*
Andrea Hille, *Goettingen*
Klaus Mönkemüller, *Bottrop*
Elfriede Bollschweiler, *Köln*
Siegfried Wagner, *Deggendorf*
Dieter Schilling, *Mannheim*
Joerg F Schlaak, *Essen*
Michael Keese, *Frankfurt*
Robert Grützmann, *Dresden*
Ali Canbay, *Essen*
Dirk Domagk, *Muenster*
Jens Hoeppner, *Freiburg*
Frank Tacke, *Aachen*
Patrick Michl, *Marburg*
Alfred A Königsrainer, *Tübingen*
Kilian Weigand, *Heidelberg*
Mohamed Hassan, *Duesseldorf*
Gustav Paumgartner, *Munich*
Philipe N Khalil, *Munich*
Martin Storr, *Munich*



Greece

Andreas Larentzakis, *Athens*
Tsianos Epameinondas, *Ioannina*
Elias A Kouroumalis, *Heraklion*
Helen Christopoulou-Aletra, *Thessaloniki*
George Papatheodoridis, *Athens*
Ioannis Kanellos, *Thessaloniki*
Michael Koutsilieris, *Athens*
T Choli-Papadopoulou, *Thessaloniki*
Emanuel K Manesis, *Athens*
Evangelos Tsiambas, *Ag Paraskevi Attiki*
Konstantinos Mimidis, *Alexandroupolis*
Spilios Manolakopoulos, *Athens*
Spiros Sgouros, *Athens*
Ioannis E Koutroubakis, *Heraklion*
Stefanos Karagiannis, *Athens*
Spiros Ladas, *Athens*
Elena Vezali, *Athens*
Dina G Tiniakos, *Athens*
Ekaterini Chatzaki, *Alexandroupolis*
Dimitrios Roukos, *Ioannina*
George Sgourakis, *Athens*
Maroulio Talieri, *Athens*



Hungary

Peter L Lakatos, *Budapest*
Yvette Mándi, *Szeged*
Ferenc Sipos, *Budapest*
György M Buzás, *Budapest*
László Czakó, *Szeged*
Peter Hegyi, *Szeged*
Zoltan Rakonczay, *Szeged*
Gyula Farkas, *Szeged*
Zsuzsa Szondy, *Debrecen*
Gabor Veres, *Budapest*
Zsuzsa Schaff, *Budapest*



India

Philip Abraham, *Mumbai*
Sri P Misra, *Allahabad*
Ramesh Roop Rai, *Jaipur*
Nageshwar D Reddy, *Hyderabad*
Rakesh Kumar Tandon, *New Delhi*
Jai Dev Wig, *Chandigarh*
Uday C Ghoshal, *Lucknow*
Pramod Kumar Garg, *New Delhi*
Barjesh Chander Sharma, *New Delhi*
Gopal Nath, *Varanasi*
Bhupendra Kumar Jain, *Delhi*
Devinder Kumar Dhawan, *Chandigarh*
Ashok Kumar, *Lucknow*
Benjamin Perakath, *Tamil Nadu*
Debidas Ghosh, *Midnpore*
Pankaj Garg, *Panchkula*
Samiran Nundy, *New Delhi*
Virendra Singh, *Chandigarh*
Bikash Medhi, *Chandigarh*
Radha K Dhiman, *Chandigarh*
Vandana Panda, *Mumbai*
Vineet Ahuja, *New Delhi*
SV Rana, *Chandigarh*

Deepak N Amarapurkar, *Mumbai*
Abhijit Chowdhury, *Kolkata*
Jasbir Singh, *Kurukshetra*
B Mittal, *Lucknow*
Sundeep Singh Saluja, *New Delhi*
Pradyumna Kumar Mishra, *Mumbai*
Runu Chakravarty, *Kolkata*
Nagarajan Perumal, *New Delhi*



Indonesia

David handojo Muljono, *Jakarta*
Andi Utama, *Tangerang*



Iran

Seyed-Moayed Alavian, *Tehran*
Reza Malekzadeh, *Tehran*
Peyman Adibi, *Isfahan*
Alireza Mani, *Tehran*
Seyed Mohsen Dehghani, *Shiraz*
Mohammad Abdollahi, *Tehran*
Majid Assadi, *Bushehr*
Arezoo Aghakhani, *Tehran*
Marjan Mohammadi, *Tehran*
Fariborz Mansour-Ghanaei, *Rasht*



Ireland

Ross McManus, *Dublin*
Billy Bourke, *Dublin*
Catherine Greene, *Dublin*
Ted Dinan, *Cork*
Marion Rowland, *Dublin*



Israel

Abraham R Eliakim, *Haifa*
Simon Bar-Meir, *Tel Hashomer*
Ami D Sperber, *Beer-Sheva*
Boris Kirshtein, *Beer Sheva*
Mark Pines, *Bet Dagan*
Menachem Moshkowitz, *Tel-Aviv*
Ron Shaoul, *Haifa*
Shmuel Odes, *Beer Sheva*
Sigal Fishman, *Tel Aviv*
Alexander Becker, *Afula*
Assy Nimer, *Safed*
Eli Magen, *Ashdod*
Amir Shlomai, *Tel-Aviv*



Italy

Mauro Bortolotti, *Bologna*
Gianlorenzo Dionigi, *Varese*
Fiorucci Stefano, *Perugia*
Roberto Berni Canani, *Naples*
Ballarin Roberto, *Modena*
Bruno Annibale, *Roma*
Vincenzo Stanghellini, *Bologna*
Giovanni B Gaeta, *Napoli*
Claudio Bassi, *Verona*
Mauro Bernardi, *Bologna*
Giuseppe Chiarioni, *Valeggio*
Michele Cicala, *Rome*
Dario Conte, *Milano*
Francesco Costa, *Pisa*
Giovanni D De Palma, *Naples*
Giammarco Fava, *Ancona*
Francesco Feo, *Sassari*
Edoardo G Giannini, *Genoa*
Fabio Grizzi, *Milan*
Salvatore Gruttadauria, *Palermo*
Pietro Invernizzi, *Milan*
Ezio Laconi, *Cagliari*
Giuseppe Montalto, *Palermo*
Giovanni Musso, *Torino*
Gerardo Nardone, *Napoli*
Valerio Nobili, *Rome*
Raffaele Pezzilli, *Bologna*
Alberto Piperno, *Monza*
Anna C Piscaglia, *Roma*
Piero Portincasa, *Bari*
Giovanni Tarantino, *Naples*
Cesare Tosetti, *Porretta Terme*
Alessandra Ferlini, *Ferrara*
Alessandro Ferrero, *Torino*
Donato F Altomare, *Bari*
Giovanni Milito, *Rome*
Giuseppe Sica, *Rome*
Guglielmo Borgia, *Naples*
Giovanni Latella, *L'Aquila*
Salvatore Auricchio, *Naples*
Alberto Biondi, *Rome*
Alberto Tommasini, *Trieste*
Antonio Basoli, *Roma*
Giuliana Decorti, *Trieste*
Marco Silano, *Roma*
Michele Reni, *Milan*
Pierpaolo Sileri, *Rome*
Achille Iolascon, *Naples*
Alessandro Granito, *Bologna*
Angelo A Izzo, *Naples*
Giuseppe Currò, *Messina*
Pier Mannuccio Mannucci, *Milano*
Marco Vivarelli, *Bologna*
Massimo Levrero, *Rome*
Massimo Rugge, *Padova*
Paolo Angeli, *Padova*
Silvio Danese, *Milano*
Antonello Trecca, *Rome*
Antonio Gasbarrini, *Rome*
Cesare Ruffolo, *Treviso*
Massimo Falconi, *Verona*
Fausto Catena, *Bologna*
Francesco Manguso, *Napoli*
Giancarlo Mansueto, *Verona*
Luca Morelli, *Trento*
Marco Scarpa, *Padova*
Mario M D'Elios, *Florence*
Francesco Luzza, *Catanzaro*
Franco Roviello, *Siena*
Guido Torzilli, *Rozzano Milano*
Luca Frulloni, *Verona*
Lucia Malaguarnera, *Catania*
Lucia Ricci Vitiani, *Rome*
Mara Massimi, *L'Aquila*
Mario Pescatori, *Rome*
Mario Rizzetto, *Torino*
Mirko D'Onofrio, *Verona*
Nadia Peparini, *Rome*
Paola De Nardi, *Milan*
Paolo Aurello, *Rome*
Piero Amodio, *Padova*
Riccardo Nascimbeni, *Brescia*
Vincenzo Villanacci, *Brescia*
Vittorio Ricci, *Pavia*
Silvia Fargion, *Milan*
Luigi Bonavina, *Milano*
Oliviero Riggio, *Rome*
Fabio Pace, *Milano*
Gabrio Bassotti, *Perugia*
Giulio Marchesini, *Bologna*
Roberto de Franchis, *Milano*
Giovanni Monteleone, *Rome*
C armelo Scarpignato, *Parma*
Luca VC Valenti, *Milan*
Urgesi Riccardo, *Rome*
Marcello Persico, *Naples*
Antonio Moschetta, *Bari*
Luigi Muratori, *Bologna*
Angelo Zullo, *Roma*
Vito Annese, *Florence*
Simone Lanini, *Rome*
Alessandro Grasso, *Savona*
Giovanni Targher, *Verona*
Domenico Girelli, *Verona*
Alessandro Cucchetti, *Bologna*
Fabio Marra, *Florence*
Michele Milella, *Rome*
Francesco Franceschi, *Rome*
Giuseppina De Petro, *Brescia*
Salvatore Leonardi, *Catania*
Cristiano Simone, *Santa Maria Imbaro*
Bernardino Rampone, *Salerno*
Francesco Crea, *Pisa*
Walter Fries, *Messina*
Antonio Craxì, *Palermo*
Gerardo Rosati, *Potenza*
Mario Guslandi, *Milano*
Gianluigi Giannelli, *Bari*
Paola Loria, *Modena*
Paolo Sorrentino, *Avellino*
Armando Santoro, *Rozzano*
Gabriele Grassi, *Trieste*
Antonio Orlacchio, *Rome*



Japan

Tsuneo Kitamura, *Chiba*
Katsutoshi Yoshizato, *Higashihiroshima*
Masahiro Arai, *Tokyo*
Shinji Tanaka, *Hiroshima*
Keiji Hirata, *Kitakyushu*
Yoshio Shirai, *Niigata*
Susumu Ohmada, *Maebashi*
Kenichi Ikejima, *Tokyo*
Masatoshi Kudo, *Osaka*
Yoshiaki Murakami, *Hiroshima*
Masahiro Tajika, *Nagoya*
Kentaro Yoshika, *Toyoake*
Kyoichi Adachi, *Izumo*
Yasushi Adachi, *Sapporo*
Takafumi Ando, *Nagoya*
Akira Andoh, *Otsu*
Hitoshi Asakura, *Tokyo*
Mitsuhiro Fujishiro, *Tokyo*
Toru Hiyama, *Higashihiroshima*
Yutaka Inagaki, *Kanagawa*
Hiromi Ishibashi, *Nagasaki*
Shunji Ishihara, *Izumo*
Toru Ishikawa, *Niigata*
Yoshiaki Iwasaki, *Okayama*
Terumi Kamisawa, *Tokyo*

Norihiro Kokudo, *Tokyo*
Shin Maeda, *Tokyo*
Yasushi Matsuzaki, *Ibaraki*
Kenji Miki, *Tokyo*
Hiroto Miwa, *Hyogo*
Yoshiharu Motoo, *Kanazawa*
Kunihiko Murase, *Tusima*
Atsushi Nakajima, *Yokohama*
Yuji Naito, *Kyoto*
Hisato Nakajima, *Tokyo*
Hiroki Nakamura, *Yamaguchi*
Shotaro Nakamura, *Fukuoka*
Mikio Nishioka, *Niihama*
Hirohide Ohnishi, *Akita*
Kazuichi Okazaki, *Osaka*
Morikazu Onji, *Ehime*
Satoshi Osawa, *Hamamatsu*
Hidetsugu Saito, *Tokyo*
Yutaka Saito, *Tokyo*
Yasushi Sano, *Kobe*
Tomohiko Shimatani, *Kure*
Yukihiro Shimizu, *Toyama*
Shinji Shimoda, *Fukuoka*
Masayuki Sho, *Nara*
Hidekazu Suzuki, *Tokyo*
Shinji Togo, *Yokohama*
Satoshi Yamagiwa, *Niigata*
Takayuki Yamamoto, *Yokkaichi*
Hiroshi Yoshida, *Tokyo*
Norimasa Yoshida, *Kyoto*
Akihito Nagahara, *Tokyo*
Hiroaki Takeuchi, *Kochi*
Keiji Ogura, *Tokyo*
Kotaro Miyake, *Tokushima*
Mitsunori Yamakawa, *Yamagata*
Naoaki Sakata, *Sendai*
Naoya Kato, *Tokyo*
Satoshi Mamori, *Hyogo*
Shogo Kikuchi, *Aichi*
Shoichiro Sumi, *Kyoto*
Susumu Ikehara, *Osaka*
Taketo Yamaguchi, *Chiba*
Tokihiko Sawada, *Tochigi*
Tomoharu Yoshizumi, *Fukuoka*
Toshiyuki Ishiwata, *Tokyo*
Yasuhiro Fujino, *Akashi*
Yasuhiro Koga, *Isehara city*
Yoshihisa Takahashi, *Tokyo*
Yoshitaka Takuma, *Okayama*
Yutaka Yata, *Maebashi-city*
Itaru Endo, *Yokohama*
Kazuo Chijiiwa, *Miyazaki*
Kouhei Fukushima, *Sendai*
Masahiro Iizuka, *Akita*
Mitsuyoshi Urashima, *Tokyo*
Munechika Enjoji, *Fukuoka*
Takashi Kojima, *Sapporo*
Takumi Kawaguchi, *Kurume*
Yoshiyuki Ueno, *Sendai*
Yuichiro Eguchi, *Saga*
Akihiro Tamori, *Osaka*
Atsushi Masamune, *Sendai*
Atsushi Tanaka, *Tokyo*
Hitoshi Tsuda, *Tokyo*
Takashi Kobayashi, *Tokyo*
Akimasa Nakao, *Nagogya*
Hiroyuki Uehara, *Osaka*
Masahito Uemura, *Kashihara*
Satoshi Tanno, *Sapporo*
Toshinari Takamura, *Kanazawa*
Yohei Kida, *Kainan*
Masanori Hatakeyama, *Tokyo*
Satoru Kakizaki, *Gunma*
Shuhei Nishiguchi, *Hyogo*
Yuichi Yoshida, *Osaka*
Manabu Morimoto, *Japan*
Mototsugu Kato, *Sapporo*
Naoki Ishii, *Tokyo*
Noriko Nakajima, *Tokyo*
Nobuhiro Ohkohchi, *Tsukuba*
Takanori Kanai, *Tokyo*
Kenichi Goda, *Tokyo*
Mitsugi Shimoda, *Mibu*
Zenichi Morise, *Nagoya*
Hitoshi Yoshiji, *Kashihara*
Takahiro Nakazawa, *Nagoya*
Utaroh Motosugi, *Yamanashi*
Nobuyuki Matsuhashi, *Tokyo*
Yasuhiro Kodera, *Nagoya*
Takayoshi Ito, *Tokyo*
Yasuhito Tanaka, *Nagoya*
Haruhiko Sugimura, *Hamamatsu*
Hiroki Yamaue, *Wakayama*
Masao Ichinose, *Wakayama*
Takaaki Arigami, *Kagoshima*
Nobuhiro Zaima, *Nara*
Naoki Tanaka, *Matsumoto*
Satoru Motoyama, *Akita*
Tomoyuki Shibata, *Toyoake*
Tatsuya Ide, *Kurume*
Tsutomu Fujii, *Nagoya*
Osamu Kanauchi, *Toky*
Atsushi Irisawa, *Aizuwakamatsu*
Hikaru Nagahara, *Tokyo*
Keiji Hanada, *Onomichi*
Keiichi Mitsuyama, *Fukuoka*
Shin Maeda, *Yokohama*
Takuya Watanabe, *Niigata*
Toshihiro Mitaka, *Sapporo*
Yoshiki Murakami, *Kyoto*
Tadashi Shimoyama, *Hirosaki*



Jordan

Ismail Matalka, *Irbid*
Khaled Jadallah, *Irbid*



Kuwait

Islam Khan, *Safat*



Lebanon

Bassam N Abboud, *Beirut*
Rami Moucari, *Beirut*
Ala I Sharara, *Beirut*
Rita Slim, *Beirut*



Lithuania

Giedrius Barauskas, *Kaunas*
Limas Kupcinskas, *Kaunas*



Malaysia

Andrew Seng Boon Chua, *Ipoh*



Mexico

Saúl Villa-Trevio, *Mexico*
Omar Vergara-Fernandez, *Mexico*
Diego Garcia-Compean, *Monterrey*
Arturo Panduro, *Jalisco*
Miguel Angel Mercado, *Distrito Federal*
Richard A Awad, *Mexico*
Aldo Torre Delgadillo, *Mexico*
Paulino Martínez Hernández Magro, *Celaya*
Carlos A Aguilar-Salinas, *Mexico*
Jesus K Yamamoto-Furusho, *Mexico*



Morocco

Samir Ahboucha, *Khouribga*



Moldova

Igor Mishin, *Kishinev*



Netherlands

Ulrich Beuers, *Amsterdam*
Albert Frederik Pull ter Gunne, *Tilburg*
Jantine van Baal, *Heidelberglaan*
Wendy Wilhelmina Johanna de Leng, *Utrecht*
Gerrit A Meijer, *Amsterdam*
Lee Bouwman, *Leiden*
J Bart A Crusius, *Amsterdam*
Frank Hoentjen, *Haarlem*
Servaas Morré, *Amsterdam*
Chris JJ Mulder, *Amsterdam*
Paul E Sijens, *Groningen*
Karel van Erpecum, *Utrecht*
BW Marcel Spanier, *Arnhem*
Misha Luyer, *Sittard*
Pieter JF de Jonge, *Rotterdam*
Robert Christiaan Verdonk, *Groningen*
John Plukker, *Groningen*
Maarten Tushuizen, *Amsterdam*
Wouter de Herder, *Rotterdam*
Erwin G Zoetendal, *Wageningen*
Robert J de Knegt, *Rotterdam*
Albert J Bredenoord, *Nieuwegein*
Annemarie de Vries, *Rotterdam*
Astrid van der Velde, *Ede*
Lodewijk AA Brosens, *Utrecht*
James CH Hardwick, *Leiden*
Loes van Keimpema, *Nijmegen*
WJ de Jonge, *Amsterdam*
Zuzana Zelinkova, *Rotterdam*
LN van Steenbergen, *Eindhoven*
Frank G Schaap, *Amsterdam*
Jeroen Maljaars, *Leiden*



New Zealand

Andrew S Day, *Christchurch*
Max S Petrov, *Auckland*



Norway

Espen Melum, *Oslo*

Trine Olsen, *Tromsø*
Eyvind J Paulssen, *Tromsø*
Rasmus Goll, *Tromsø*
Asle W Medhus, *Oslo*
Jon Arne Søreide, *Stavanger*
Kjetil Soreide, *Stavanger*
Reidar Fossmark, *Trondheim*
Trond Peder Flaten, *Trondheim*
Olav Dalgard, *Oslo*
Ole Høie, *Arendal*
Magdy El-Salhy, *Bergen*
Jørgen Valeur, *Oslo*



Pakistan

Shahab Abid, *Karachi*
Syed MW Jafri, *Karachi*



Poland

Beata Jolanta Jablońska, *Katowice*
Halina Cichoż-Lach, *Lublin*
Tomasz Brzozowski, *Cracow*
Hanna Gregorek, *Warsaw*
Marek Hartleb, *Katowice*
Stanislaw J Konturek, *Krakow*
Andrzej Dabrowski, *Bialystok*
Jan Kulig, *Kraków*
Julian Swierczynski, *Gdansk*
Marek Bebenek, *Wroclaw*
Dariusz M Lebensztejn, *Bialystok*



Portugal

Ricardo Marcos, *Porto*
Guida Portela-Gomes, *Estoril*
Ana Isabel Lopes, *Lisboa Codex*
Raquel Almeida, *Porto*
Rui Tato Marinho, *Lisbon*
Ceu Figueiredo, *Porto*



Romania

Dan L Dumitrascu, *Cluj*
Adrian Saftoiu, *Craiova*
Andrada Seicean, *Cluj-Napoca*
Anca Trifan, *Iasi*



Russia

Vasiliy I Reshetnyak, *Moscow*



Saudi Arabia

Ibrahim A Al Mofleh, *Riyadh*
Abdul-Wahed Meshikhes, *Qatif*
Faisal Sanai, *Riyadh*



Serbia

Tamara M Alempijevic, *Belgrade*
Dusan M Jovanovic, *Sremska Kamenica*
Zoran Krivokapic, *Belgrade*



Singapore

Brian Kim Poh Goh, *Singapore*
Khek-Yu Ho, *Singapore*
Fock Kwong Ming, *Singapore*
Francis Seow-Choen, *Singapore*
Kok Sun Ho, *Singapore*
Kong Weng Eu, *Singapore*
Madhav Bhatia, *Singapore*
London Lucien Ooi, *Singapore*
Wei Ning Chen, *Singapore*
Richie Soong, *Singapore*
Kok Ann Gwee, *Singapore*



Slovenia

Matjaz Homan, *Ljubljana*



South Africa

Rosemary Joyce Burnett, *Pretoria*
Michael Kew, *Cape Town*
Roland Ndip, *Alice*



South Korea

Byung Chul Yoo, *Seoul*
Jae J Kim, *Seoul*
Jin-Hong Kim, *Suwon*
Marie Yeo, *Suwon*
Jeong Min Lee, *Seoul*
Eun-Yi Moon, *Seoul*
Joong-Won Park, *Goyang*
Hoon Jai Chun, *Seoul*
Myung-Gyu Choi, *Seoul*
Sang Kil Lee, *Seoul*
Sang Yeoup Lee, *Gyeongsangnam-do*
Won Ho Kim, *Seoul*
Dae-Yeul Yu, *Daejeon*
Donghee Kim, *Seoul*
Sang Geon Kim, *Seoul*
Sun Pyo Hong, *Geonggi-do*
Sung-Gil Chi, *Seoul*
Yeun-Jun Chung, *Seoul*
Ki-Baik Hahm, *Incheon*
Ji Kon Ryu, *Seoul*
Kyu Taek Lee, *Seoul*
Yong Chan Lee, *Seoul*
Seong Gyu Hwang, *Seongnam*
Seung Woon Paik, *Seoul*
Sung Kim, *Seoul*
Hong Joo Kim, *Seoul*
Hyoung-Chul Oh, *Seoul*
Nayoung Kim, *Seongnam-si*
Sang Hoon Ahn, *Seoul*
Seon Hahn Kim, *Seoul*
Si Young Song, *Seoul*
Young-Hwa Chung, *Seoul*
Hyo-Cheol Kim, *Seoul*
Kwang Jae Lee, *Swon*
Sang Min Park, *Seoul*
Young Chul Kim, *Seoul*
Do Hyun Park, *Seoul*
Dae Won Jun, *Seoul*
Dong Wan Seo, *Seoul*
Soon-Sun Hong, *Incheon*
Hoguen Kim, *Seoul*
Ho-Young Song, *Seoul*
Joo-Ho Lee, *Seoul*
Jung Eun Lee, *Seoul*
Jong H Moon, *Bucheon*



Spain

Eva Vaquero, *Barcelona*
Andres Cardenas, *Barcelona*
Laureano Fernández-Cruz, *Barcelona*
Antoni Farré, *Spain*
Maria-Angeles Aller, *Madrid*
Raul J Andrade, *Málaga*
Fernando Azpiroz, *Barcelona*
Josep M Bordas, *Barcelona*
Antoni Castells, *Barcelona*
Vicente Felipo, *Valencia*
Isabel Fabregat, *Barcelona*
Angel Lanas, *Zaragoza*
Juan-Ramón Larrubia, *Guadalajara*
María IT López, *Jaén*
Jesús M Prieto, *Pamplona*
Mireia Miquel, *Sabadell*
Ramon Bataller, *Barcelona*
Fernando J Corrales, *Pamplona*
Julio Mayol, *Madrid*
Matias A Avila, *Pamplona*
Juan Macías, *Seville*
Juan Carlos Laguna Egea, *Barcelona*
Juli Busquets, *Barcelona*
Belén Beltrán, *Valencia*
José Manuel Martin-Villa, *Madrid*
Lisardo Boscá, *Madrid*
Luis Grande, *Barcelona*
Pedro Lorenzo Majano Rodriguez, *Madrid*
Adolfo Benages, *Valencia*
Domínguez-Muñoz JE, *Santiago de Compostela*
Gloria González Aseguinolaza, *Navarra*
Javier Martin, *Granada*
Luis Bujanda, *San Sebastián*
Matilde Bustos, *Pamplona*
Luis Aparisi, *Valencia*
José Julián calvo Andrés, *Salamanca*
Benito Velayos, *Valladolid*
Javier Gonzalez-Gallego, *León*
Ruben Ciria, *Córdoba*
Francisco Rodriguez-Frias, *Barcelona*
Manuel Romero-Gómez, *Sevilla*
Albert Parés, *Barcelona*
Joan Roselló-Catafau, *Barcelona*



Sri Lanka

Arjuna De Silva, *Kelaniya*



Sweden

Stefan G Pierzynowski, *Lund*
Hanns-Ulrich Marschall, *Stockholm*
Lars A Pahlman, *Uppsala*
Helena Nordenstedt, *Stockholm*
Bobby Tingstedt, *Lund*
Evangelos Kalaitzakis, *Gothenburg*
Lars Erik Agréus, *Huddinge*
Annika Lindblom, *Stockholm*

Roland Andersson, *Lund*
Zongli Zheng, *Stockholm*
Mauro D'Amato, *Huddinge*
Greger Lindberg, *Stockholm*
Pär Erik Myrelid, *Linköping*
Sara Lindén, *Göteborg*
Sara Regnér, *Malmö*
Åke Nilsson, *Lund*



Switzerland

Jean L Frossard, *Geneva*
Andreas Geier, *Zürich*
Bruno Stieger, *Zürich*
Pascal Gervaz, *Geneva*
Paul M Schneider, *Zurich*
Felix Stickel, *Berne*
Fabrizio Montecucco, *Geneva*
Inti Zlobec, *Basel*
Michelangelo Foti, *Geneva*
Pascal Bucher, *Geneva*
Andrea De Gottardi, *Berne*
Christian Toso, *Geneva*



Thailand

Weekitt Kittisupamongkol, *Bangkok*



Trinidad and Tobago

Shivananda Nayak, *Mount Hope*



Turkey

Tarkan Karakan, *Ankara*
Yusuf Bayraktar, *Ankara*
Ahmet Tekin, *Mersin*
Aydin Karabacakoglu, *Konya*
Osman C Ozdogan, *Istanbul*
Özlem Yilmaz, *Izmir*
Bülent Salman, *Ankara*
Can GONEN, *Kutahya*
Cuneyt Kayaalp, *Malatya*
Ekmel Tezel, *Ankara*
Eren Ersoy, *Ankara*
Hayrullah Derici, *Balıkesir*
Mehmet Refik Mas, *Etlik-Ankara*
Sinan Akay, *Tekirdag*
A Mithat Bozdayi, *Ankara*
Metin Basaranoglu, *Istanbul*
Mesut Tez, *Ankara*
Orhan Sezgin, *Mersin*
Mukaddes Esrefoglu, *Malatya*
Ilker Tasci, *Ankara*
Kemal Kismet, *Ankara*
Selin Kapan, *Istanbul*
Seyfettin Köklü, *Ankara*
Murat Sayan, *Kocaeli*
Sabahattin Kaymakoglu, *Istanbul*
Yucel Ustundag, *Zonguldak*
Can Gonen, *Istanbul*
Yusuf Yilmaz, *Istanbul*
Müge Tecder-Ünal, *Ankara*
İlhami Yüksel, *Ankara*



United Arab Emirates

Fikri M Abu-Zidan, *Al-Ain*
Sherif M Karam, *Al-Ain*



United Kingdom

Anastasios Koulaouzidis, *Edinburgh*
Sylvia LF Pender, *Southampton*
Hong-Xiang Liu, *Cambridge*
William Dickey, *Londonderry*
Simon D Taylor-Robinson, *London*
James Neuberger, *Birmingham*
Frank I Tovey, *London*
Kevin Robertson, *Glasgow*
Chew Thean Soon, *Manchester*
Geoffrey Burnstock, *London*
Vamsi R Velchuru, *United Kingdom*
Simon Afford, *Birmingham*
Navneet K Ahluwalia, *Stockport*
Lesley A Anderson, *Belfast*
Anthony TR Axon, *Leeds*
Jim D Bell, *London*
Alastair D Burt, *Newcastle*
Tatjana Crnogorac-Jurcevic, *London*
Daniel R Gaya, *Edinburgh*
William Greenhalf, *Liverpool*
Indra N Guha, *Southampton*
Stefan G Hübscher, *Birmingham*
Robin Hughes, *London*
Pali Hungin, *Stockton*
Janusz AZ Jankowski, *Oxford*
Peter Karayiannis, *London*
Patricia F Lalor, *Birmingham*
Giorgina Mieli-Vergani, *London*
D Mark Pritchard, *Liverpool*
Marco Senzolo, *Padova*
Roger Williams, *London*
M H Ahmed, *Southampton*
Christos Paraskeva, *Bristol*
Emad M El-Omar, *Aberdeen*
A M El-Tawil, *Birmingham*
Anne McCune, *Bristol*
Charles B Ferguson, *Belfast*
Chin Wee Ang, *Liverpool*
Clement W Imrie, *Glasgow*
Dileep N Lobo, *Nottingham*
Graham MacKay, *Glasgow*
Guy Fairbairn Nash, *Poole*
Ian Lindsey, *Oxford*
Jason CB Goh, *Birmingham*
Jeremy FL Cobbold, *London*
Julian RF Walters, *London*
Jamie Murphy, *London*
John Beynon, *Swansea*
John B Schofield, *Kent*
Anil George, *London*
Aravind Suppiah, *East Yorkshire*
Basil Ammori, *Salford*
Catherine Walter, *Cheltenham*
Chris Briggs, *Sheffield*
Jeff Butterworth, *Shrewsbury*
Nawfal Hussein, *Nottingham*
Patrick O'Dwyer, *Glasgow*
Rob Glynne-Jones, *Northwood*
Sharad Karandikar, *Birmingham*
Venkatesh Shanmugam, *Derby*
Yeng S Ang, *Wigan*
Alberto Quaglia, *London*
Andrew Fowell, *Southampton*
Gianpiero Gravante, *Leicester*
Piers Gatenby, *London*
Kondragunta Rajendra Prasad, *Leeds*
Sunil Dolwani, *Cardiff*
Andrew McCulloch Veitch, *Wolverhampton*
Brian Green, *Belfast*
Noriko Suzuki, *Middlesex*
Richard Parker, *North Staffordshire*
Shahid A Khan, *London*
Akhilesh B Reddy, *Cambridge*
Jean E Crabtree, *Leeds*
John S Leeds, *Sheffield*
Paul Sharp, *London*
Sumita Verma, *Brighton*
Thamara Perera, *Birmingham*
Donald Campbell McMillan, *Glasgow*
Kathleen B Bamford, *London*
Helen Coleman, *Belfast*
Eyad Elkord, *Manchester*
Mohammad Ilyas, *Nottingham*
Simon R Carding, *Norwich*
Ian Chau, *Sutton*
Claudio Nicoletti, *Norwich*
Hendrik-Tobias Arkenau, *London*
Muhammad Imran Aslam, *Leicester*
Giuseppe Orlando, *Oxford*
John S Leeds, *Aberdeen*
S Madhusudan, *Nottingham*
Amin Ibrahim Amin, *Dunfermline*
David C Hay,*Edinburgh*
Alan Burns, *London*



United States

Tauseef Ali, *Oklahoma City*
George Y Wu, *Farmington*
Josef E Fischer, *Boston*
Thomas Clancy, *Boston*
John Morton, *Stanford*
Luca Stocchi, *Cleveland*
Kevin Michael Reavis, *Orange*
Shiu-Ming Kuo, *Buffalo*
Gary R Lichtenstein, *Philadelphia*
Natalie J Torok, *Sacramento*
Scott A Waldman, *Philadelphia*
Georgios Papachristou, *Pittsburgh*
Carla W Brady, *Durham*
Robert CG Martin, *Louisville*
Eugene P Ceppa, *Durham*
Shashi Bala, *Worcester*
Imran Hassan, *Springfield*
Klaus Thaler, *Columbia*
Andreas M Kaiser, *Los Angeles*
Shawn D Safford, *Norfolk*
Massimo Raimondo, *Jacksonville*
Kazuaki Takabe, *Richmond VA*
Stephen M Kavic, *Baltimore*
T Clark Gamblin, *Pittsburgh*
BS Anand, *Houston*
Ananthanarayanan M, *New York*
Anthony J Bauer, *Pittsburgh*
Edmund J Bini, *New York*
Xian-Ming Chen, *Omaha*
Ramsey Chi-man Cheung, *Palo Alto*
Parimal Chowdhury, *Arkansas*
Mark J Czaja, *New York*

Conor P Delaney, *Cleveland*
Sharon DeMorrow, *Temple*
Bijan Eghtesad, *Cleveland*
Alessandro Fichera, *Chicago*
Glenn T Furuta, *Aurora*
Jean-Francois Geschwind, *Baltimore*
Shannon S Glaser, *Temple*
Ajay Goel, *Dallas*
James H Grendell, *New York*
Anna S Gukovskaya, *Los Angeles*
Jamal A Ibdah, *Columbia*
Atif Iqbal, *Omaha*
Hajime Isomoto, *Rochester*
Hartmut Jaeschke, *Kansas*
Leonard R Johnson, *Memphis*
Rashmi Kaul, *Tulsa*
Ali Keshavarzian, *Chicago*
Miran Kim, *Providence*
Burton I Korelitz, *New York*
Richard A Kozarek, *Seattle*
Alyssa M Krasinskas, *Pittsburgh*
Ming Li, New *Orleans*
Zhiping Li, *Baltimore*
Chen Liu, *Gainesville*
Michael R Lucey, *Madison*
James D Luketich, *Pittsburgh*
Patrick M Lynch, *Houston*
Willis C Maddrey, *Dallas*
Mercedes Susan Mandell, *Aurora*
Wendy M Mars, *Pittsburgh*
Laura E Matarese, *Pittsburgh*
Lynne V McFarland, *Washington*
Stephan Menne, *New York*
Didier Merlin, *Atlanta*
George Michalopoulos, *Pittsburgh*
James M Millis, *Chicago*
Pramod K Mistry, *New Haven*
Emiko Mizoguchi, *Boston*
Peter L Moses, *Burlington*
Masaki Nagaya, *Boston*
Robert D Odze, *Boston*
Stephen JD O'Keefe, *Pittsburgh*
Zhiheng Pei, *New York*
Raymund R Razonable, *Minnesota*
Basil Rigas, *New York*
Richard A Rippe, *Chapel Hill*
Philip Rosenthal, *San Francisco*
Stuart Sherman, *Indianapolis*
Christina Surawicz, *Seattle*
Wing-Kin Syn, *Durham*
Yvette Taché, *Los Angeles*
K-M Tchou-Wong, *New York*
George Triadafilopoulos, *Stanford*
Chung-Jyi Tsai, *Lexington*
Andrew Ukleja, *Florida*
Arnold Wald, *Wisconsin*
Irving Waxman, *Chicago*
Steven D Wexner, *Weston*
Jackie Wood, *Ohio*
Jian Wu, *Sacramento*
Zobair M Younossi, *Virginia*
Liqing Yu, *Winston-Salem*
Ruben Zamora, *Pittsburgh*
Michael E Zenilman, *New York*
Michael A Zimmerman, *Colorado*
Beat Schnüriger, *California*
Clifford S Cho, *Madison*
R Mark Ghobrial, *Texas*
Anthony T Yeung, *Philadelphia*
Chang Kim, *West Lafayette*
Balamurugan N Appakalai, *Minneapolis*
Aejaz Nasir, *Tampa*
Ashkan Farhadi, *Irvine*
Kevin E Behrns, *Gainesville*
Joseph J Cullen, *Iowa City*
David J McGee, *Shreveport*
Anthony J Demetris, *Pittsburgh*
Dimitrios V Avgerinos, *New York*
Dong-Hui Li, *Houston*
Eric S Hungness, *Chicago*
Giuseppe Orlando, *Winston Salem*
Hai-Yong Han, *Phoenix*
Huanbiao Mo, *Denton*
Jong Park, *Tampa*
Justin MM Cates, *Nashville*
Charles P Heise, *Madison*
Craig D Logsdon, *Houston*
Ece A Mutlu, *Chicago*
Jessica A Davila, *Houston*
Rabih M Salloum, *Rochester*
Amir Maqbul Khan, *Marshall*
Bruce E Sands, *Boston*
Chakshu Gupta, *Saint Joseph*
Ricardo Alberto Cruciani, *New York*
Mariana D Dabeva, *Bronx*
Edward L Bradley III, *Sarasota*
Martín E Fernández-Zapico, *Rochester*
Henry J Binder, *New Haven*
John R Grider, *Richmond*
Ronnie Fass, *Tucson*
Dinesh Vyas, *Washington*
Wael El-Rifai, *Nashville*
Craig J McClain, *Louisville*
Christopher Mantyh, *Durham*
Daniel S Straus, *Riverside*
David A Brenner, *San Diego*
Eileen F Grady, *San Francisco*
Ekihiro Seki, *La Jolla*
Fang Yan, *Nashville*
Fritz Francois, *New York*
Giamila Fantuzzi, *Chicago*
Guang-Yin Xu, *Galveston*
Jianyuan Chai, *Long Beach*
JingXuan Kang, *Charlestown*
Le Shen, *Chicago*
Lin Zhang, *Pittsburgh*
Mitchell L Shiffman, *Richmond*
Douglas K Rex, *Indianapolis*
Bo Shen, *Cleveland*
Edward J Ciaccio, *New York*
Jean S Wang, *Saint Louis*
Bao-Ting Zhu, *Kansas*
Tamir Miloh, *Phoenix*
Eric R Kallwitz, *Chicago*
Yujin Hoshida, *Cambridge*
C Chris Yun, *Atlanta*
Alan C Moss, *Boston*
Oliver Grundmann, *Gainesville*
Linda A Feagins, *Dallas*
Chanjuan Shi, *Nashville*
Xiaonan Han, *Cincinnati*
William R Brugge, *Boston*
Richard W McCallum, *El Paso*
Lisa Ganley-Leal, *Boston*
Lin-Feng Chen, *Urbana*
Elaine Y Lin, *New York*
Julian Abrams, *New York*
Arun Swaminath, *New York*
Huiping Zhou, *Richmond*
Korkut Uygun, *Boston*
Anupam Bishayee, *Signal Hill*
C Bart Rountree, *Hershey*
Avinash Kambadakone, *Boston*
Courtney W Houchen, *Oklahoma*
Joshua R Friedman, *Philadelphia*
Justin H Nguyen, *Jacksonville*
Sophoclis Alexopoulos, *Los Angeles*
Suryakanth R Gurudu, *Scottsdale*
Wei Jia, *Kannapolis*
Yoon-Young Jang, *Baltimore*
Ourania M Andrisani, *West Lafayette*
Roderick M Quiros, *Bethlehem*
Timothy R Koch, *Washington*
Adam S Cheifetz, *Boston*
Lifang Hou, *Chicago*
Thiru vengadam Muniraj, *Pittsburgh*
Dhiraj Yadav, *Pittsburgh*
Ying Gao, *Rockville*
John F Gibbs, *Buffalo*
Aaron Vinik, *Norfolk*
Charles Thomas, *Oregon*
Robert Jensen, *Bethesda*
John W Wiley, *Ann Arbor*
Jonathan Strosberg, *Tampa*
Randeep Singh Kashyap, *New York*
Kaye M Reid Lombardo, *Rochester*
Lygia Stewart, *San Francisco*
Martin D Zielinski, *Rochester*
Matthew James Schuchert, *Pittsburgh*
Michelle Lai, *Boston*
Million Mulugeta, *Los Angeles*
Patricia Sylla, *Boston*
Pete Muscarella, *Columbus*
Raul J Rosenthal, *Weston*
Robert V Rege, *Dallas*
Roberto Bergamaschi, *New York*
Ronald S Chamberlain, *Livingston*
Alexander S Rosemurgy, *Tampa*
Run Yu, *Los Angeles*
Samuel B Ho, *San Diego*
Sami R Achem, *Florida*
Sandeep Mukherjee, *Omaha*
Santhi Swaroop Vege, *Rochester*
Scott Steele, *Fort Lewis*
Steven Hochwald, *Gainesville*
Udayakumar Navaneethan, *Cincinnati*
Radha Krishna Yellapu, *New York*
Rupjyoti Talukdar, *Rochester*
Shi-Ying Cai, *New Haven*
Thérèse Tuohy, *Salt Lake City*
Tor C Savidge, *Galveston*
William R Parker, *Durham*
Xiaofa Qin, *Newark*
Zhang-Xu Liu, *Los Angeles*
Adeel A Butt, *Pittsburgh*
Dean Y Kim, *Detroit*
Denesh Chitkara, *East Brunswick*
Mohamad A Eloubeidi, *Alabama*
JiPing Wang, *Boston*
Oscar Joe Hines, *Los Angeles*
Jon C Gould, *Madison*
Kirk Ludwig, *Wisconsin*
Mansour A Parsi, *Cleveland*

Perry Shen, *Winston-Salem*
Piero Marco Fisichella, *Maywood*
Marco Giuseppe Patti, *Chicago*
Michael Leitman, *New York*
Parviz M Pour, *Omaha*
Florencia Georgina Que, *Rochester*
Richard Hu, *Los Angeles*
Robert E Schoen, *Pittsburgh*
Valentina Medici, *Sacramento*
Wojciech Blonski, *Philadelphia*
Yuan-Ping Han, *Los Angeles*
Grigoriy E Gurvits, *New York*
Robert C Moesinger, *Ogden*
Mark Bloomston, *Columbus*
Bronislaw L Slomiany, *Newark*
Laurie DeLeve, *Los Angeles*
Michel M Murr, *Tampa*
John Marshall, *Columbia*
Wilfred M Weinstein, *Los Angeles*
Jonathan D Kaunitz, *Los Angeles*
Josh Korzenik, *Boston*
Kareem M Abu-Elmagd, *Pittsburgh*
Michael L Schilsky, *New Haven*
John David Christein, *Birmingham*
Mark A Zern, *Sacramento*
Ana J Coito, *Los Angeles*
Golo Ahlenstiel, *Bethesda*
Smruti R Mohanty, *Chicago*
Victor E Reyes, *Galveston*
CS Pitchumoni, *New Brunswick*
Yoshio Yamaoka, *Houston*
Sukru H Emre, *New Haven*
Branko Stefanovic, *Tallahassee*
Jack R Wands, *Providence*
Wen Xie, *Pittsburgh*
Robert Todd Striker, *Madison*
Shivendra Shukla, *Columbia*
Laura E Nagy, *Cleveland*
Fei Chen, *Morgantown*
Kusum K Kharbanda, *Omaha*
Pal Pacher, *Rockville*
Pietro Valdastri, *Nashville*

ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

World J Gastroenterol 2013 January 7; 19(1): 1-146



Baishideng Publishing Group
www.wjgnet.com

World Journal of Gastroenterology

Contents

Weekly Volume 19 Number 1 January 7, 2013

EDITORIAL

1 Precut sphincterotomy: A reliable salvage for difficult biliary cannulation
Saritas U, Ustundag Y, Harmandar F

FIELD OF VISION

8 Tumor-infiltrating B cells come into vogue
Linnebacher M

12 Is endoscopic therapy the treatment of choice in all patients with chronic pancreatitis?
Jabłońska B

TOPIC HIGHLIGHT

17 Clinical significance of cytomegalovirus infection in patients with inflammatory bowel disease
Garrido E, Carrera E, Manzano R, Lopez-Sanroman A

REVIEW

26 Current pathogenetic aspects of hepatic encephalopathy and noncirrhotic hyperammonemic encephalopathy
Cichoż-Lach H, Michalak A

35 Retroperitoneal fibrosis associated with immunoglobulin G4-related disease
Fujimori N, Ito T, Igarashi H, Oono T, Nakamura T, Niina Y, Hijioka M, Lee L, Uchida M, Takayanagi R

42 Nestin: A novel angiogenesis marker and possible target for tumor angiogenesis
Matsuda Y, Hagio M, Ishiwata T

BRIEF ARTICLE

49 Performance of liver stiffness measurements by transient elastography in chronic hepatitis
Ferraioli G, Tinelli C, Dal Bello B, Zicchetti M, Lissandrin R, Filice G, Filice C, Above E, Barbarini G, Brunetti E, Calderon W, Di Gregorio M, Gulminetti R, Lanzarini P, Ludovisi S, Maiocchi L, Malfitano A, Michelone G, Minoli L, Mondelli M, Novati S, Patruno SFA, Perretti A, Poma G, Sacchi P, Zanaboni D, Zaramella M

57 Comparison of fatty liver index with noninvasive methods for steatosis detection and quantification
Zelber-Sagi S, Webb M, Assy N, Blendis L, Yeshua H, Leshno M, Ratziu V, Halpern Z, Oren R, Santo E

65 Prolonged QT dispersion in inflammatory bowel disease
Yorulmaz E, Sezgin A, Yorulmaz H, Adali G, Ciftci H

72 Improvement of prognosis for unresectable biliary tract cancer
Sasaki T, Isayama H, Nakai Y, Takahara N, Sasahira N, Kogure H, Mizuno S, Yagioka H, Ito Y, Yamamoto N, Hirano K, Toda N, Tada M, Omata M, Koike K

78 Alcohol, postprandial plasma glucose, and prognosis of hepatocellular carcinoma
Abe H, Aida Y, Ishiguro H, Yoshizawa K, Miyazaki T, Itagaki M, Sutoh S, Aizawa Y

86 Short and long-term outcomes of endoscopic balloon dilatation for Crohn's disease strictures
Endo K, Takahashi S, Shiga H, Kakuta Y, Kinouchi Y, Shimosegawa T

92 Index of convexity: A novel liver function index using Tc-GSA scintigraphy
Miki K, Matsui Y, Teruya M, Kaminishi M, Kokudo N

97 *Helicobacter pylori* seropositivity in diabetic patients is associated with microalbuminuria
Chung GE, Heo NJ, Park MJ, Chung SJ, Kang HY, Kang SJ

103 A pilot study of single-use endoscopy in screening acute gastrointestinal bleeding
Cho JH, Kim HM, Lee S, Kim YJ, Han KJ, Cho HG, Song SY

108 Double guidewire technique *vs* transpancreatic precut sphincterotomy in difficult biliary cannulation
Yoo YW, Cha SW, Lee WC, Kim SH, Kim A, Cho YD

CASE REPORT

115 Vanishing bile duct syndrome in human immunodeficiency virus infected adults: A report of two cases
Oppenheimer AP, Koh C, McLaughlin M, Williamson JC, Norton TD, Laudadio J, Heller T, Kleiner DE, High KP, Morse CG

122 Atresia of the appendix vermiformis: A rare case of developmental abnormality
Yaylak F, Algin MC, Gungor Y, Korkmaz M

125 Russell body duodenitis in a patient with retroperitoneal metastasis of ureteral cancer
Takahashi Y, Shimizu S, Uraushihara K, Fukusato T

129 Small hepatocellular carcinoma with ring calcification: A case report and literature review
Murakami T, Morioka D, Takakura H, Miura Y, Togo S

133 Biliary phytobezoar resulting in intestinal obstruction
KimY, Park BJ, Kim MJ, Sung DJ, Kim DS, Yu YD, Lee JH

137 Endoscopic resection of co-existing severe dysplasia and a small esophageal leiomyoma
Ahn SY, Jeon SW

LETTERS TO THE EDITOR

141 Somatic second-hit mutations leads to polycystic liver diseases
Banales JM, Munoz-Garrido P, Bujanda L

144 Is exon mutation analysis needed for adjuvant treatment of gastrointestinal stromal tumor?
Şendur MAN, Özdemir NY, Akıncı MB, Uncu D, Zengin N, Aksoy S

APPENDIX I-VI Instructions to authors

ABOUT COVER Editorial Board Member of *World Journal of Gastroenterology*, Donato F Altomare, MD, Professor, Department of Emergency and Organ Transplantation, University of Bari, Piazza G Cesare, 11-70124 Bari, Italy

AIMS AND SCOPE

World Journal of Gastroenterology (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. *WJG* was established on October 1, 1995. It is published weekly on the 7th, 14th, 21st, and 28th each month. The *WJG* Editorial Board consists of 1352 experts in gastroenterology and hepatology from 64 countries.

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

FLYLEAF I-IX Editorial Board

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Shuai Ma*
Responsible Electronic Editor: *Li Xiong*
Proofing Editor-in-Chief: *Lian-Sheng Ma*
Responsible Science Editor: *Ling-Ling Wen*
Proofing Editorial Office Director: *Jian-Xia Cheng*

NAME OF JOURNAL
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

EDITOR-IN-CHIEF
Ferruccio Bonino, MD, PhD, Professor of Gastroenterology, Director of Liver and Digestive Disease Division, Department of Internal Medicine, University of Pisa, Director of General Medicine 2 Unit University Hospital of Pisa, Via Roma 67, 56124 Pisa, Italy

Myung-Hwan Kim, MD, PhD, Professor, Head, Department of Gastroenterology, Director, Center for Biliary Diseases, University of Ulsan College of Medicine, Asan Medical Center, 388-1 Pungnap-2dong, Songpa-gu, Seoul 138-736, South Korea

Kjell Öberg, MD, PhD, Professor, Department of Endocrine Oncology, Uppsala University Hospital, SE-751 85 Uppsala, Sweden

Matt D Rutter, MBBS, MD, FRCP, Consultant Gastroenterologist, Senior Lecturer, Director, Tees Bowel Cancer Screening Centre, University Hospital of North Tees, Durham University, Stockton-on-Tees, Cleveland TS19 8PE, United Kingdom

Andrzej S Tarnawski, MD, PhD, DSc (Med), Professor of Medicine, Chief Gastroenterology, VA Long Beach Health Care System, University of California, Irvine, CA, 5901 E. Seventh Str., Long Beach, CA 90822, United States

EDITORIAL OFFICE
Jian-Xia Cheng, Director
Jin-Lei Wang, Vice Director
World Journal of Gastroenterology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Co., Limited
Room 1701, 17/F, Henan Building,
No.90 Jaffe Road, Wanchai, Hong Kong, China
Fax: +852-31158812
Telephone: +852-58042046
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

PUBLICATION DATE
January 7, 2013

COPYRIGHT
© 2013 Baishideng. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT
All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i1.1

World J Gastroenterol 2013 January 7; 19(1): 1-7
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

EDITORIAL

Precut sphincterotomy: A reliable salvage for difficult biliary cannulation

Ulku Saritas, Yucel Ustundag, Ferda Harmandar

Ulku Saritas, Yucel Ustundag, Ferda Harmandar, Department of Internal Medicine, Gastroenteorology Clinics, Zonguldak Karaelmeas University Hospital, 67600 Zonguldak, Turkey
Author contributions: Saritas U contributed to conception, design and preparation of manuscript; Ustundag Y contributed to revision process; Harmandar F helped in drafting the article.
Correspondence to: Yucel Ustundag, MD, Professor of Gastroenterology, Department of Internal Medicine, Gastroenteorology Clinics, Zonguldak Karaelmeas University Hospital, 67600 Zonguldak, Turkey. yucel_u@yahoo.com
Telephone: +90-535-9543556 Fax: +90-372-6660090
Received: May 15, 2012 Revised: September 10, 2012
Accepted: September 19, 2012
Published online: January 7, 2013

Abstract

Even experienced endoscopists have 90% success in achieving deep biliary cannulation with standard methods. Biliary cannulation may become difficult in 10%-15% of patients with biliary obstruction and precut (access) sphincterotomy is frequently chosen as a rescue treatment in these cases. Generally, precut sphincterotomy ensures a rate of 90%-100% successful deep biliary cannulation. The precut technique has been performed as either a fistulotomy with a needle knife sphincterotome or as a transpapillary septotomy with a standard sphincterotome. Both methods have similar efficacy and complication rates when administered to the proper patient. Although precut sphincterotomy ensures over 90% success of biliary cannulation, it has been characterized as an independent risk factor for pancreatitis. The complications of the precut technique are not limited to pancreatitis. Two more important ones, bleeding and perforation, are also reported in some publications as being observed more commonly than during standard sphincterotomy. It is also reported that precut sphincterotomy increases morbidity when performed in patients without dilatation of their biliary tract. Nevertheless, precut sphincterotomy is a good alternative as a rescue method in the setting of a failed standard cannulation method. This paper discusses the technical details, timing, efficacy and potential complications of precut sphincterotomy.

© 2013 Baishideng. All rights reserved.

Key words: Biliary cannulation; Salvage; Precut sphincterotomy; Needle knife; Transpancreatic septotomy

Saritas U, Ustundag Y, Harmandar F. Precut sphincterotomy: A reliable salvage for difficult biliary cannulation. *World J Gastroenterol* 2013; 19(1): 1-7 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i1/1.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i1.1

INTRODUCTION

With the development of increasingly sophisticated imaging methods, endoscopic retrograde cholangiopancreatography (ERCP) is currently used more for the treatment than the diagnosis of biliary tract disease. The first choice of treatment for biliary calculus, postoperative bile leak and benign or malignant biliary stenosis is endoscopic, i.e., therapeutic ERCP. A preliminary condition to successful therapeutic ERCP is selective biliary cannulation. Deep biliary cannulation performed by an experienced endoscopist using standard cannulation techniques is successful in approximately 90% of cases[1]. Biliary cannulation may become particularly difficult in the 10%-15% of patients with an anatomical problem or a disease such as fibrosis, tumor of the sphincter of Oddi, periampullary diverticuli, calculus impaction in the sphincter, or status post Billroth I - Ⅱ. Such situations are also risk factors for complications of ERCP[2,3]. In a retrospective review of 502 patients, Fukatsu *et al*[4] were not successful in cannulating the biliary tract within the first 20 min using standard methods in 80 of these patients (16%) for one of the following reasons: female sex, post-hepatectomy

hypertrophy of the left lobe, status post Billroth I gastric resection with reconstruction, and malignant stenosis. In such patients, deep biliary cannulation fails often enough, notwithstanding the use of various instruments such as a tapered or standard-tip catheter, a sphincterotome, a guide wire, a metal-tip catheter, *etc.* Alternatively, the pancreatic duct ends up being cannulated. Every new attempt to cannulate through the papilla, or repeated cannulation of the pancreatic duct, inevitably increases the risk of post-ERCP pancreatitis (PEP)[5]. Published reports indicate that ten or more transpapillary cannulation attempts or more than five cannulations of the pancreatic duct are independent risk factors for PEP[2,3,5].

The most popular method to address a difficult cannulation is needle-knife (NK) precut sphincterotomy, which has been in use since the 1980s. The term precut was first used by Siegel[6] and later popularized by Huibregtse *et al*[7]. A more recent technique for difficult cases is transpancreatic precut sphincterotomy (TPS), which was described after 1990[8]. Although precut (access) sphincterotomy ensures over 90% success of biliary cannulation[9], it has been characterized as an independent risk factor for PEP[10,11]. Patients in whom the precut technique is applied are, however, already at a high risk for PEP; they have, as a rule, already undergone mechanical trauma due to previous attempts at transpapillary cannulation. Both situations are well known as PEP risk factors. Therefore, a timely use of the precut technique could reduce the risk of PEP. The complications of the precut technique are not limited to PEP. Two more important ones, bleeding and perforation, are reported in some publications as being observed more frequently than during standard sphincterotomy. It is also indicated that precut sphincterotomy increases morbidity when performed in patients who have no dilatation of their biliary tract. This paper discusses the technical details, timing, efficacy and complications of precut sphincterotomy.

TECHNICAL DETAILS

Precut sphincterotomy is an endoscopic technique used for deep biliary cannulation, and also for reaching the pancreatic duct in rare cases. It consists of an incision in the papilla in the absence of free biliary cannulation or a guide wire. The incision is performed most frequently with a needle-tip sphincterotome, and the technique is referred to as "NK precutting". Depending on the point of origin of the incision, the technique is designated as "standard" or "modified".

Standard technique

The incision starts at the orifice and proceeds upwards, in the direction of 11 h on a clock dial, until it is past the papillary protuberance. The length of the cut is determined by the intraduodenal part of the common bile duct: it stops at the upper limit of the duodenal bulge of the common bile duct.

Modified technique

The incision starts where the lower two thirds of the papillary protuberance meet the upper third, and it proceeds downwards on the left of the median line of the papilla and stops before reaching the papillary orifice. The incision depth is obtained with 2-3 pushes on the NK in a straight plane, while exerting sufficient pressure from the surface of the ampulla towards the catheter.

Transpancreatic precut sphincterotomy or transpancreatic papillary septotomy

This technique provides bile duct cannulation by entering the common duct or pancreatic duct through the papillary orifice and then cutting the septum that separates the pancreatic duct from the bile duct. Transpancreatic papillary septotomy can be either wire-guided after introducing a guide wire in the pancreatic duct, or it can be performed without a guide. The wire-guided method consists of introducing a soft guide in the pancreatic duct, then directing a papillotome, usually of the standard type, towards the bile duct to perform the cut. The catheter is introduced into the bile duct after its ostium has become visible. The unguided technique first introduces the sphincterotome into the common duct; the septum between the pancreatic and the bile ducts is incised, and the biliary and pancreatic orifices are made visible separately. The pancreatic orifice is generally lower than the central position, to the right of the papillary median line, and the bile duct orifice is to its left in the middle. The latter presents a velvety appearance with an off-yellow color. After visualizing the biliary orifice, deep biliary cannulation is performed, and the sphincterotomy operation is completed.

Different sphincterotomes have been developed for precutting and NK[12] (Table 1). The catheters may possess a single, double or triple lumen Iso-Tome. An isolated-tip papillotome to prevent energy leakage from the needle point has also been developed, as well as a coated-tip NK aimed toward preventing perforation. The Erlangen-type papillotome is one of these. It is similar to the standard traction-type sphincterotome, with a 5 mm monofilament cutting wire and a catheter tip measuring less than 1 mm. Other modifications of the short-tip or noseless sphincterotome are also used for precutting. They are available with insulated or non-insulated cutting wires. Yet another type of device used to perform a papillotomy to permit access is that of a catheter equipped with a small scissor at the tip[13]. The lower blade of the scissor is introduced into the papillary orifice, and the scissor blades close to obtain a cut.

How effective is a precut sphincterotomy in achieving deep biliary cannulation? Does it increase post-ERCP complications?

Standard cannulation techniques achieve deep biliary cannulation in approximately 90% of cases, while 10%-15% of cases present difficulties. Patients experiencing such difficulties generally have a disease such as

Table 1 Precut sphincterotomy devices

Product	Distal tip OD (F)	Tip length (mm)	Cut-wire length (mm)	Recommended guidewire size (inch)	Comments
Boston Scientific (United States)					
Needle knife RX	5	NA	5	0.035	Triple lumen, shortwire
Micro knife XL	5	NA	5	0.035	Triple lumen, precut
Mediglobe (Tempe, Arizona)					
Precut sphincterotome	5 or 6	NA	20 or 30	0.021-0.035	Double or tripleumen, noseless, erlange n-typesphincterotome
Needle knife	5 or 6	NA	0-15	NA	Single lumen
Olympus (Tokyo, Japan)					
Triple lumen needle with clever coating	5	NA	5	0.035	Precut device
Triple lumen needle knife	5	NA	5	0.035	Precut device
Cook Medical (Winston-Salem, NC)					
Huibregtse triple lumen needle knife	5	NA	4	0.035	Triple lumen, precut
Zimmon needle knife	5	NA	7	0.035	Precut device
Fusion needle knife	6	NA	4	0.035	Short-wire system

OD: Outside diameter; NA : Not available.

a stenosis or a tumor of the sphincter of Oddi, periampullar diverticula, status post-Billroth Ⅱ, *etc.*, or an anatomic abnormality. Cannulation difficulties lead to multiple attempts. The resulting trauma and edema create a favorable ground for PEP. Precut papillotomy is an invasive procedure to help achieve deep biliary cannulation. Studies and discussion of precut papillotomy have been ongoing since the early 1980s, when this technique was first used. Rabenstein *et al*[14] reviewed the patients on whom they had performed precut papillotomy and ERCP, dividing them in two groups based on the year of the operation, 1981-1987 and 1988-1993. In both periods, approximately one-third of the patients needed a precut papillotomy to achieve deep biliary cannulation, and precutting was successful in 85% of these cases. The complication rates of primary endoscopic sphincterotomy (6.8%) and precutting (7.3%) were similar. Foutch *et al*[15] succeeded in the deep cannulation of 414 of 456 patients (91%); endoscopic sphincterotomy was successful in 395 patients (87%), and 40 patients (9.7%) experienced complications, 21 (5.1%) of which were serious. Precutting, which was used in 52 patients because of insufficient deep cannulation or endoscopic sphincterotomy (EST), was successful in 47 patients (90%), 3 (5.8%) of whom experienced complications, and these complications were serious in 2 (3.8%). No mortality related to EST or precutting was observed. Binmoeller *et al*[16] used precutting with an Erlangen-type instrument in 123 (38%) of 327 patients. Selective biliary cannulation was successful in all patients. Hyperamylasemia, observed in half of the patients who underwent precutting, was present in 27% of the standard sphincterotomy group ($P < 0.001$), while clinical PEP and the bleeding frequency were similar in both groups (2.7% *vs* 2.4% and 1.6% *vs* 3.9%, respectively). Palm *et al*[17] compared their results in 121 patients on whom they had performed precutting with the same type of papillotome with the result of procedures performed in 481 other patients who had received standard sphincterotomy (a precut ratio of 25%). As in the earlier cited study, hyperamylasemia was more frequent in the patients who underwent precutting than in those with EST (31.3% *vs* 13.3%), while the rates of other complications were similar (7.18% *vs* 8.3%). Precutting resulted in a success rate of 98.2% for deep biliary cannulation. In a study comparing primary and secondary precutting, de Weerth *et al*[18] reported a 100% success rate of biliary cannulation with a cannulation time of 6.9 ± 1.8 min in the primary precutting. In 146 patients with secondary precutting who had previously undergone attempts at standard cannulation and sphincterotomy, 104 had a successful cannulation within 8.3 ± 2.1 min ($P < 0.001$), and 42 had to repeat the secondary precutting. PEP was observed with the same frequency in both groups (2.1% *vs* 2.9%), and 1 patient in the secondary precut group experienced light bleeding. The authors conclude that primary precutting is safer, more efficient and faster. Kasmin *et al*[19] achieved a successful biliary cannulation at the first attempt in 50 (67%) of 72 difficult patients, and 17 others were successful at the second attempt, for a total of 67 (93%) successful procedures with NK sphincterotomy. Complications were observed in eight (11%) patients, including guide wire-related perforation, bleeding from precutting and pancreatitis. A narrow common bile duct diameter was determined to be an independent risk factor for complications in patients undergoing precutting. In a study of the correlation of NK precut and PEP, Testoni *et al*[10] performed NK precutting in 12.8% of their 732 patients with naive papilla, with an 85% success rate. The PEP frequency was 14.9% in the precut group *vs* 6.1% in the non-precut group. Female sex, stenosis of the sphincter of Oddi, partial pancreatic drainage and more than 15 previous cannulation attempts were independent risk factors for PEP, while NK precutting was not. A stent was implanted in the pancreatic duct in 22 patients, and five of these experienced pancreatitis. The authors indicate that the pancreatic stent provides no protection from PEP to patients at risk. Perforation, bleeding or

Table 2 Precut admission, success and complication rates (%)

Ref.	The need for precutting	Deep biliary cannulation	Precut-associated complication	Standard sphincterotomy-associated complication
Bailey *et al*[10]	12.8	85	14.9	6.1
Rabenstein *et al*[14]	30	85	7.3	6.8
Foutch *et al*[15]	9	90	9.8	9.7
Binmoeller *et al*[16]	38	100	5.5	5.1
Palm *et al*[17]	25	98.2	8.3	7.18
Cennamo *et al*[20]	20	92	8	
deWeerth *et al*[18]	30	99	2.9	2.1

severe pancreatitis were not observed. As for Cennamo *et al*[20], they applied criteria somewhat differently from the previous studies, performing precutting if the standard attempt failed within 5 min or after three repeated cannulations of the pancreatic duct; they compared this group of patients to those who had undergone late precutting after failed cannulation within 20 min. In the early precut group, cannulation was successful in 32 (92%) of 36 patients at the first attempt and 100% at the second. In 78 of the 110 late precut patients, biliary cannulation was achieved within an average of 7.6 min by standard methods, while precutting was performed in the first 20 min in the remaining patients. Deep biliary cannulation was successful at the first attempt in 26 (81%) of these patients and at the second in the remaining four. The complication rate was 8% in the early precut group and 6% in the late precut ($P = 0.7$). Patients with periampullary diverticuli, gastric surgery and papillary neoplasia were excluded from this study. Oddi's sphincter stenosis was not an exclusion criterion, but it was not found in any of the patients during the procedure. The authors concluded that there was no difference between the early and late precut patient groups with regard to biliary cannulation success rates and complication rates. Considering, however, the early decision regarding the failure of standard cannulation in the early precut group, it can be speculated that biliary cannulation could still have been achieved by standard methods with a longer trial in some of these patients. Another question is the exclusion of patients with important PEP risk factors and the absence of cases of stenosis of the sphincter of Oddi, which both may have contributed to the absence of a difference in terms of complications. Additionally, an apparent difference in the first-pass success rates in favor of the early precut patients, 92% *vs* 81%, was not discussed in the conclusion. A review of these studies (Table 2) shows that precutting is successful in achieving deep biliary cannulation in more than 95% of the cases, without causing a substantial increase in the complication rate.

The timing of the precutting intervention is important for reducing the complications. The number of transpapillary cannulation attempts and that of pancreatic duct cannulations are two of the most important factors contributing to PEP. Published reports indicate that ten or more transpapillary cannulation attempts or more than five cannulations of the pancreatic duct are independent risk factors for PEP. Precutting before reaching such a number of attempts would not constitute an additional PEP risk factor. In a meta-analysis by Cennamo *et al*[21] of six controlled, randomized studies, pancreatitis was found in 2.5% of patients undergoing early precutting *vs* 5.3% in those in whom the unsuccessful attempts continued (odds ratio 0.47, 95% confidence interval: 0.24-0.91); early precutting was shown to lower the risk of PEP. In another study, Tang *et al*[22] found fewer cases of PEP in patients subject to precutting before reaching 10 unsuccessful cannulation attempts through the papilla as opposed to those with 10 or more attempts. When comparing the patients who underwent early precutting with those in whom the endoscopists persisted with difficult cannulation, the success rates were, respectively, 75% and 73%, and the complication rates were 4% and 9%; the differences were not significant. Kaffes *et al*[23] performed early NK precut sphincterotomy in 70 (20%) patients out of a population of 346 undergoing therapeutic ERCP. The operation was performed in patients in whom selective biliary cannulation was not successful within the first ten min or those with more than five cannulations of the pancreatic duct. Successful biliary cannulation was achieved at the first attempt in 58 (83%) patients, and at the second attempt 24-48 h later, it was successful in 7 more patients. Precutting was unsuccessful in 5 patients. Thus the total success rate for biliary cannulation was 95%; six patients had light bleeding, and one had light pancreatitis. It was concluded that early precutting does not add to the PEP risk. Bruins Slot *et al*[24], reporting a success rate of 99% for precut sphincterotomy in achieving biliary cannulation with a complication rate of 12%, interestingly found a 0.1% PEP rate but observed relatively frequent perforation (3%) compared to other reports, where perforation is rare, and bleeding was reported in 5.5% of patients. The bleeding reported with the NK precutting procedure is characterized as mild, but it is generally more frequent than with standard sphincterotomy. As a result of all these reviewed studies, it may be said that, in addition to not representing a risk factor for developing PEP, early precut sphincterotomy does not cause complications in addition to those of the ERCP or standard sphincterotomy methods, and it is safe.

Should a transpancreatic septotomy and NK precut method be used, and what are their respective advantages and complications? To answer these questions, the results of several studies should be considered. In a paper by Goff[25], who also first described TPS, on the long-term comparative results of this technique *vs* those obtained with standard sphincterotomy, 51 (25%) patients of a total of 200 were subjected to precutting. Of these, 49 (94%) had a successful biliary cannulation at the first attempt; the complication rate was 2.1% for standard and 1.96% for precut sphincterotomy. Catalano *et al*[26] performed TPS in 29 patients and NK precutting in 34. The cannulation success rate was 100% in the former and 77% in

Table 3 The comparison of needle knife papillotomy and transpancreatic precut sphincterotomy (%)

Ref.	Transpancreatic precut	Needle knife precut	*P* value
Catalano *et al*[26]			
Successful biliary cannulation	100	77	0.01
Complications	3.5	17.7	NS
Wang *et al*[27]			
Successful biliary cannulation	90	90.8	NS
Complications	14.9	18.4	NS
Halttunen *et al*[30]			
Successful biliary cannulation	97.3	7.3	< 0.001
Complications	9.3	8.2	NS

NS: Not significant.

the latter group (P = 0.1). Complications were observed in one TPS patient (3,5%) and in six (17.7%) who had NK. Wang *et al*[27] applied TPS in 140 patients and NK in 76, with similar rates of biliary cannulation success, PEP, and other complications (90% *vs* 90.8%, 11.4% *vs* 11.8% and 14.9% *vs* 18.4%, respectively). Akashi *et al*[28] performed TPS without using a guide wire in 172 patients, with 163 (95%) successful biliary cannulations and a 10% complication rate. They note in their conclusions that the general complication rate of ERCP was low at 0.8%, and that of TPS was relatively elevated (P < 0.001). Kahaleh *et al*[29] obtained a deep biliary cannulation success rate of 85% without guide wire in 116 patients; three patients (2.6%) experienced bleeding, 9 (8%) had pancreatitis, and 2 (1.7%) had a perforation. The authors reported that these frequencies were no different than those of the standard sphincterotomy patients. Halttunen *et al*[30] used precutting in 588 (9%) of 6209 ERCP patients due to cannulation difficulties and compared TPS to NK. Precutting was successful in 529 of the 588 patients. Of these, 351 had TPS and 178 had NK. The success rate for biliary cannulation was 97.3% in the TPS group and 71.3% with NK (P < 0.001); the frequency of PEP was 8.8% in the TPS group and 5.1% in the NK patients (P = 0.16). The authors characterized TPS as a substantially efficient and safe technique (Table 3). Weber *et al*[31] inserted a soft guide wire in the pancreatic duct to perform TPS in 108 patients; 94 (87%) of these were successfully cannulated at the first attempt, and nine others were successful at a second session 1-4 d later, for a total success of 103 (95.4%) patients. PEP was observed in six (5.6%) cases, four of which were mild, and bleeding was similarly observed in six (5.6%) patients, four of whom required transfusion. There was no perforation and no mortality. The bleeding frequency was higher than usual in the last study; this may be related to the reported use of a pure cutting current. As summarized above, the results of TPS with or without the placement of a guide in the pancreatic duct seemed to be similar to each other with regard to the rates of biliary cannulation success and complications. Although there is thus no definite evidence as to the superiority of either method, the macroscopic appearance of the papilla and the presence or absence of bile duct dilatation may be decisive. Patients in whom there is no stenosis of the sphincter of Oddi and the bile duct, and thus no bulging in the duodenum of the duodenal portion of the bile duct, are not good candidates for fistulotomy or the modified technique of NK precut. The perforation risk due to precutting was elevated in such patients; TPS is more adapted to these cases. Patients with a stenosis of the sphincter of Oddi are at high risk for PEP, and biliary cannulation by TPS following the placement of a stent in the pancreatic duct is the procedure of choice. As for patients with an impacted calculus in the orifice, or those with a papillary orifice displaced downwards due to a tumor of the sphincter of Oddi, fistulotomy or NK precut by the modified technique are appropriate.

Stent placement in the pancreatic duct has also been attempted to prevent PEP. Goldberg *et al*[32] were successful in the deep cannulation of 38 (97.4%) out of 39 patients who presented cannulation difficulties, using TPS after having implanted a stent in the pancreatic duct. Two (5.1%) of these patients had mild pancreatitis. Performing TPS after pancreatic duct stent implantation seemed to be as successful in cannulation as other methods, while the lower pancreatitis frequency was doubtful. This was a retrospective study with few patients. Madácsy *et al*[33] implanted a pancreatic duct stent in 22 patients with a dysfunction of the sphincter of Oddi if cannulation had failed within the first ten min or if the pancreatic duct had been entered five times before performing NK precutting. Biliary cannulation was successful in 20 patients, and none developed PEP, which was present in 14 of the 35 control group patients. A reduction of the risk of PEP due to the use of a pancreatic stent in patients at high risk is a possibility; prospective studies with more patients are needed to resolve this issue. The stent mostly falls off spontaneously within 3-5 d from the time of implantation because its distal notch is generally left outside the duct. If the stent is still in place on the follow-up abdominal x-ray at one week, it must be pulled out by esophagogastroduodenoscopy.

In a study comparing the standard technique to the modified technique for NK[22], three patients in each group had bleeding, and one in the modified technique group had pancreatitis. No difference was detected among the two techniques regarding their complications. It is assumed that the pancreatic duct orifice is protected by the incision in the modified technique, which starts at the summit of the papillary protuberance and ends before reaching the orifice, and PEP will not develop after its use. The only patient who experienced pancreatitis in the cited study was, however, in the group that received the modified technique. It may be that edema due to temperature elevation in the tissues obstructs the pancreatic duct orifice. The emptying of the pancreatic duct is easier with the standard technique or TPS due to the incision of the common duct or even the pancreatic duct starting from the papillary orifice.

Articles on precut sphincterotomy generally start with the following disclaimer: "this method is efficient

and safe if performed by experienced endoscopists". Sphincterotomy and precutting are the most critical therapeutic operations during ERCP, and they necessitate a certain amount of experience. A study regarding this by Rabenstein *et al*[34] showed a complication frequency of 5.6% with endoscopists who treated 40 or more cases per year *vs* 9.3% for those with fewer cases ($P < 0.05$). In their study addressing the endoscopist's experience in precut fistulotomy, Lee *et al*[35] used this technique in 23% of cases, achieving 88.7% biliary cannulation success in the first session and 93.7% by the second session. PEP developed in 5.7% of cases. More than 15 cannulation attempts before fistulotomy was defined as being a PEP risk factor. The study determined that the operation duration decreased in an inverse relationship to the number of interventions practiced by the endoscopist, thus reducing the operative complications. Sufficient experience is of crucial importance not only for precutting but also for ERCP, especially for procedures of therapeutic nature. Experience is acquired by treating a large number of cases, following what is being performed, and continuously updating knowledge. The endoscopist will be entirely familiar with papillary anatomy after having observed many cases. The person performing the endoscopy must know what will be observed, what is being sought and what will be found in every millimeter that is being cut.

Is precutting the ultimate solution to difficult biliary cannulation? There is no doubt that it is not. A new technique is that of endoscopic ultrasound (EUS) rendezvous for common bile duct cannulation. Dhir *et al*[36] have compared precutting to the rendezvous technique with EUS in a non-randomized, retrospective study. Successful common bile duct cannulation was achieved in 90% of patients with precutting and 98% with EUS ($P = 0.03$); the complication rates were 6.9% and 3.4%, respectively ($P = 0.27$). The authors reported that the EUS rendezvous technique is more successful than precutting for difficult biliary cannulation. It would be wrong, however, to accept these results from a retrospective, non-randomized study as unbiased; additionally, conventional cannulation was considered to have failed after five attempts and EUS was initiated, where one may speculate that extending this limit to ten or another number could have allowed it to succeed. Without a doubt, the EUS rendezvous technique represents an important step to be used as a salvage procedure when precutting is insufficient.

CONCLUSION

Precutting is necessary in roughly 10% of patients with biliary obstruction. Precut sphincterotomy ensures a rate of 90%-100% successful deep biliary cannulation in patients in whom such cannulation is difficult. The frequency of side effects, varying between 5% and 10%, is not different from that of a standard sphincterotomy. Even with these efficacy and safety ratios, the method should remain reserved for patients who need a therapeutic intervention.

REFERENCES

1 **Freeman ML**, Guda NM. ERCP cannulation: a review of reported techniques. *Gastrointest Endosc* 2005; **61**: 112-125 [PMID: 15672074]
2 **Faber HR**, Matthews BW. A mutant T4 lysozyme displays five different crystal conformations. *Nature* 1990; **348**: 263-266 [PMID: 2234094]
3 **Freeman ML**. Adverse outcomes of endoscopic retrograde cholangiopancreatography: avoidance and management. *Gastrointest Endosc Clin N Am* 2003; **13**: 775-798 [PMID: 14986798]
4 **Fukatsu H**, Kawamoto H, Kato H, Hirao K, Kurihara N, Nakanishi T, Mizuno O, Okamoto Y, Ogawa T, Ishida E, Okada H, Sakaguchi K. Evaluation of needle-knife precut papillotomy after unsuccessful biliary cannulation, especially with regard to postoperative anatomic factors. *Surg Endosc* 2008; **22**: 717-723 [PMID: 17704885]
5 **Freeman ML**, DiSario JA, Nelson DB, Fennerty MB, Lee JG, Bjorkman DJ, Overby CS, Aas J, Ryan ME, Bochna GS, Shaw MJ, Snady HW, Erickson RV, Moore JP, Roel JP. Risk factors for post-ERCP pancreatitis: a prospective, multicenter study. *Gastrointest Endosc* 2001; **54**: 425-434 [PMID: 11577302]
6 **Siegel JH**. Precut papillotomy: a method to improve success of ERCP and papillotomy. *Endoscopy* 1980; **12**: 130-133 [PMID: 7379762]
7 **Huibregtse K**, Katon RM, Tytgat GN. Precut papillotomy via fine-needle knife papillotome: a safe and effective technique. *Gastrointest Endosc* 1986; **32**: 403-405 [PMID: 3803839]
8 **Goff JS**. Common bile duct pre-cut sphincterotomy: transpancreatic sphincter approach. *Gastrointest Endosc* 1995; **41**: 502-505 [PMID: 7615231]
9 **Baillie J**. Needle-knife papillotomy revisited. *Gastrointest Endosc* 1997; **46**: 282-284 [PMID: 9378222]
10 **Testoni PA**, Giussani A, Vailati C, Testoni S, Di Leo M, Mariani A. Precut sphincterotomy, repeated cannulation and post-ERCP pancreatitis in patients with bile duct stone disease. *Dig Liver Dis* 2011; **43**: 792-796 [PMID: 21733768]
11 **Bailey AA**, Bourke MJ, Kaffes AJ, Byth K, Lee EY, Williams SJ. Needle-knife sphincterotomy: factors predicting its use and the relationship with post-ERCP pancreatitis (with video). *Gastrointest Endosc* 2010; **71**: 266-271 [PMID: 20003969]
12 **Kethu SR**, Adler DG, Conway JD, Diehl DL, Farraye FA, Kantsevoy SV, Kaul V, Kwon RS, Mamula P, Pedrosa MC, Rodriguez SA, Tierney WM. ERCP cannulation and sphincterotomy devices. *Gastrointest Endosc* 2010; **71**: 435-445 [PMID: 20189502]
13 **Heiss FW**, Cimis RS, MacMillan FP. Biliary sphincter scissor for pre-cut access: preliminary experience. *Gastrointest Endosc* 2002; **55**: 719-722 [PMID: 11979258]
14 **Rabenstein T**, Ruppert T, Schneider HT, Hahn EG, Ell C. Benefits and risks of needle-knife papillotomy. *Gastrointest Endosc* 1997; **46**: 207-211 [PMID: 9378205]
15 **Foutch PG**. A prospective assessment of results for needle-knife papillotomy and standard endoscopic sphincterotomy. *Gastrointest Endosc* 1995; **41**: 25-32 [PMID: 7698621]
16 **Binmoeller KF**, Seifert H, Gerke H, Seitz U, Portis M, Soehendra N. Papillary roof incision using the Erlangen-type pre-cut papillotome to achieve selective bile duct cannulation. *Gastrointest Endosc* 1996; **44**: 689-695 [PMID: 8979059]
17 **Palm J**, Saarela A, Mäkelä J. Safety of Erlangen precut papillotomy: an analysis of 1044 consecutive ERCP examinations in a single institution. *J Clin Gastroenterol* 2007; **41**: 528-533 [PMID: 17450039]
18 **de Weerth A**, Seitz U, Zhong Y, Groth S, Omar S, Papa-

georgiou C, Bohnacker S, Seewald S, Seifert H, Binmoeller KF, Thonke F, Soehendra N. Primary precutting versus conventional over-the-wire sphincterotomy for bile duct access: a prospective randomized study. *Endoscopy* 2006; **38**: 1235-1240 [PMID: 17163325]

19 **Kasmin FE**, Cohen D, Batra S, Cohen SA, Siegel JH. Needle-knife sphincterotomy in a tertiary referral center: efficacy and complications. *Gastrointest Endosc* 1996; **44**: 48-53 [PMID: 8836716]

20 **Cennamo V**, Fuccio L, Repici A, Fabbri C, Grilli D, Conio M, D'Imperio N, Bazzoli F. Timing of precut procedure does not influence success rate and complications of ERCP procedure: a prospective randomized comparative study. *Gastrointest Endosc* 2009; **69**: 473-479 [PMID: 19231488]

21 **Cennamo V**, Fuccio L, Zagari RM, Eusebi LH, Ceroni L, Laterza L, Fabbri C, Bazzoli F. Can early precut implementation reduce endoscopic retrograde cholangiopancreatography-related complication risk? Meta-analysis of randomized controlled trials. *Endoscopy* 2010; **42**: 381-388 [PMID: 20306386]

22 **Tang SJ**, Haber GB, Kortan P, Zanati S, Cirocco M, Ennis M, Elfant A, Scheider D, Ter H, Dorais J. Precut papillotomy versus persistence in difficult biliary cannulation: a prospective randomized trial. *Endoscopy* 2005; **37**: 58-65 [PMID: 15657860]

23 **Kaffes AJ**, Sriram PV, Rao GV, Santosh D, Reddy DN. Early institution of pre-cutting for difficult biliary cannulation: a prospective study comparing conventional vs. a modified technique. *Gastrointest Endosc* 2005; **62**: 669-674 [PMID: 16246677]

24 **Bruins Slot W**, Schoeman MN, Disario JA, Wolters F, Tytgat GN, Huibregtse K. Needle-knife sphincterotomy as a precut procedure: a retrospective evaluation of efficacy and complications. *Endoscopy* 1996; **28**: 334-339 [PMID: 8813498]

25 **Goff JS**. Long-term experience with the transpancreatic sphincter pre-cut approach to biliary sphincterotomy. *Gastrointest Endosc* 1999; **50**: 642-645 [PMID: 10536319]

26 **Catalano MF**, Linder JD, Geenen JE. Endoscopic transpancreatic papillary septotomy for inaccessible obstructed bile ducts: Comparison with standard pre-cut papillotomy. *Gastrointest Endosc* 2004; **60**: 557-561 [PMID: 15472678]

27 **Wang P**, Zhang W, Liu F, Li ZS, Ren X, Fan ZN, Zhang X, Lu NH, Sun WS, Shi RH, Li YQ, Zhao Q. Success and complication rates of two precut techniques, transpancreatic sphincterotomy and needle-knife sphincterotomy for bile duct cannulation. *J Gastrointest Surg* 2010; **14**: 697-704 [PMID: 20054659]

28 **Akashi R**, Kiyozumi T, Jinnouchi K, Yoshida M, Adachi Y, Sagara K. Pancreatic sphincter precutting to gain selective access to the common bile duct: a series of 172 patients. *Endoscopy* 2004; **36**: 405-410 [PMID: 15100948]

29 **Kahaleh M**, Tokar J, Mullick T, Bickston SJ, Yeaton P. Prospective evaluation of pancreatic sphincterotomy as a precut technique for biliary cannulation. *Clin Gastroenterol Hepatol* 2004; **2**: 971-977 [PMID: 15551249]

30 **Halttunen J**, Keränen I, Udd M, Kylänpää L. Pancreatic sphincterotomy versus needle knife precut in difficult biliary cannulation. *Surg Endosc* 2009; **23**: 745-749 [PMID: 18649101]

31 **Weber A**, Roesch T, Pointner S, Born P, Neu B, Meining A, Schmid RM, Prinz C. Transpancreatic precut sphincterotomy for cannulation of inaccessible common bile duct: a safe and successful technique. *Pancreas* 2008; **36**: 187-191 [PMID: 18376311]

32 **Goldberg E**, Titus M, Haluszka O, Darwin P. Pancreatic-duct stent placement facilitates difficult common bile duct cannulation. *Gastrointest Endosc* 2005; **62**: 592-596 [PMID: 16185975]

33 **Madácsy L**, Kurucsai G, Fejes R, Székely A, Székely I. Prophylactic pancreas stenting followed by needle-knife fistulotomy in patients with sphincter of Oddi dysfunction and difficult cannulation: new method to prevent post-ERCP pancreatitis. *Dig Endosc* 2009; **21**: 8-13 [PMID: 19691794]

34 **Rabenstein T**, Schneider HT, Nicklas M, Ruppert T, Katalinic A, Hahn EG, Ell C. Impact of skill and experience of the endoscopist on the outcome of endoscopic sphincterotomy techniques. *Gastrointest Endosc* 1999; **50**: 628-636 [PMID: 10536317]

35 **Lee TH**, Bang BW, Park SH, Jeong S, Lee DH, Kim SJ. Precut fistulotomy for difficult biliary cannulation: is it a risky preference in relation to the experience of an endoscopist? *Dig Dis Sci* 2011; **56**: 1896-1903 [PMID: 21082346]

36 **Dhir V**, Bhandari S, Bapat M, Maydeo A. Comparison of EUS-guided rendezvous and precut papillotomy techniques for biliary access (with videos). *Gastrointest Endosc* 2012; **75**: 354-359 [PMID: 22248603]

P- Reviewers Tekin A, Ustundag Y **S- Editor** Gou SX
L- Editor A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i1.8

World J Gastroenterol 2013 January 7; 19(1): 8-11
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

Tumor-infiltrating B cells come into vogue

Michael Linnebacher

Michael Linnebacher, Division of Molecular Oncology and Immunotherapy, Department of General Surgery, University of Rostock, D-18057 Rostock, Germany
Author contributions: Linnebacher M designed, drafted, revised and finally approved this paper.
Correspondence to: Michael Linnebacher, PhD, Division of Molecular Oncology and Immunotherapy, Department of General Surgery, University of Rostock, Schillingallee 35, D-18057 Rostock, Germany. michael.linnebacher@med.uni-rostock.de
Telephone: +49-381-4946043 Fax: +49-381-4946002
Received: October 16, 2012 Revised: November 8, 2012
Accepted: November 14, 2012
Published online: January 7, 2013

Abstract

Lymphocyte infiltration into solid tumors has been recognized as a main determinator of positive prognosis. For the most part this is attributed to cytotoxic T cells capable of directly destroying malignant cells. However, when considering the complex composition of the human immune system, recent findings of Nielsen *et al* on a potentially central role of tumor-infiltrating B cells is not really surprising. In this commentary article, I want to highlight the enormous potential impact of this observation for basic and translational research, prognostic procedures and ultimately for the development of future therapeutic concepts.

© 2013 Baishideng. All rights reserved.

Key words: $CD20^+$ tumor-infiltrating lymphocytes; Tumor-infiltrating B cells; Prognosis; Immunotherapy; Functional immunology

Linnebacher M. Tumor-infiltrating B cells come into vogue. *World J Gastroenterol* 2013; 19(1): 8-11 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i1/8.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i1.8

INVITED COMMENTARY ON HOT ARTICLES

Most previous studies on the infiltration of lymphocytes into human malignant tumors focused on $CD8^+$ T cells, the cell type with best antitumoral potential. And for many tumors, the degrees as well as the composition of tumor-infiltrating lymphocytes (TIL) have strong positive prognostic relevance[1]. However, recent findings established tumor-infiltrating B cells as next-best predictor of a positive patient outcome[2-4]. In their very recent publication, Nielsen *et al*[5] analyzed $CD20^+$ lymphocytes infiltrating into ovarian cancer. It is one of the very few detailed and comprehensive analysis of tumor-infiltrating B cells and moreover, it raises the question of functionality. The authors end up not only with confirmation of a strong association of $CD20^+$ TILs' presence and prolonged survival but with several findings shedding light into the true function of B cells in the immune systems' fight against cancer.

Major findings

First, they confirm earlier work demonstrating antigen-maturation and an activated phenotype of $CD20^+$ TIL [immunoglobulin (Ig)D^-, IgM^-, IgG^+][6-8]. Sequencing of the immunoglobulin genes consequently reveals high levels of somatic hypermutation in TIL B cells. This has also frequently been described for mammary carcinoma[6,7,9-12]. Thus the authors conclude that $CD20^+$ TILs have undergone activation, Ig class switching, somatic hypermutation, and clonal expansion; all of which are hallmarks of antigen exposure.

Furthermore, detailed analysis of the B cells' phenotype shows that naïve B cells were absent and only marginal amounts of germinal center B cells and plasmablasts ($CD38^+$ but $CD138^-$) were present. The vast majority of $CD20^+$ TILs were memory B cells (IgD^-, $CD38^{-/low}$) of an atypical type since they did not express the canonical memory marker CD27. The authors explain this finding

with either recent activation of B cells, since exposure of B cells to $CD70^+$ T cells leads to rapid CD27 down-regulation. Alternatively, the lack of CD27 expression on $CD20^+$ TIL may allow foregoing helper T cell-induced antibody production by ligation of CD27 with CD70. They conclude that $CD20^+$ TILs may thus directly contribute to cellular immunity. Other markers were expressed as expected from B cells: human leukocyte antigen (HLA)-ABC, HLA-DR, CD40 as well as moderate CD80 and CD86. This finding was interpreted as prerequisite for and hint towards functional antigen-presentation of the $CD20^+$ TIL to T cells in the tumor environment.

Finally, Nielsen *et al* observed an activated effector phenotype ($HLA\text{-}DR^+$, $CD45RO^+$, $CD45RA^-$, $CD62L^-$) of tumor-infiltrating $CD4^+$ and $CD8^+$ T cells. This finding is again consistent with the literature[13]. Moreover, they describe frequent co-localization of $CD8^+$ and $CD20^+$ lymphocytes both in the tumor stroma and epithelium. Clinical relevance comes from the fact that patients' survival is best when both $CD8^+$ and $CD20^+$ lymphocytes are present. The authors conclude that $CD20^+$ TIL most likely have a function as professional antigen presenting cells (APC) to support the T cells in the immune systems' fight against cancer. The latter is of extreme interest in the context of the development of novel immunotherapies. Even though dendritic cells (DCs) are established as the most potent and thus possibly also most important professional antigen presenting cell type, antigen-specific B cells may be similar if not more important in the body's fight against cancer cells. This assumption may seem a bit provocative, but Nielsen *et al*[5] hint towards the unique potential of B cells to concentrate the level of an antigen presented to T cells by membrane-IgG-guided antigen capture. Since the levels of tumor-released antigens are virtually highest in the tumor bed, it seems only logical for the $CD20^+$ antitumoral B cell to "work there".

Impact on future basic and translational research

The authors arrive at the conclusion that it will be necessary to identify the cognate antigens of $CD20^+$ TILs and to assess recognition by $CD4^+$ and $CD8^+$ TILs in order to definitively show that $CD20^+$ TILs serve as APC in the tumor environment. It will be a demanding task demonstrating simultaneous recognition of a given tumor antigen at the tumor site by all the cellular players involved: $CD4^+$, $CD8^+$ and $CD20^+$ TILs. Technically, this can be solved either by recombinant molecular biological tools, i.e., presence of T and B cell receptors. However, this approach is complicated by the fact that the exact receptor rearrangements should be known in advance. Alternatively, functional cellular tests using $CD20^+$ TIL B cells as APC to stimulate $CD4^+$ as well as $CD8^+$ TILs may also lead to the identification of antigens relevant for the immunological control of a given tumor. In our recent study on tumor-infiltrating B cells of colorectal cancer[14], we presented a simple method to immortalize and clone tumor-infiltrating B cells. The potential of these clones to functionally influence tumor growth either directly or in mutual interaction with T lymphocytes will be an interesting field of research[15]. Beside antigen identification, *in vitro* analysis using cell-cell interaction assays and even functional *in vivo* tests will be possible when using matched tumor cell lines or established tissue lines of primary tumor pieces engrafted in immunodeficient animals[16].

Another interesting aspect lies in the mere frequency of $CD20^+$ TILs in comparison to DCs; the latter are much less prominent in ovarian cancer. Many of the studies on DCs infiltrating tumor tissues used CD83 as a typical DC marker[17]. We frequently observed CD83-expression on the above mentioned *ex vivo* cloned and immortalized B cells[14]. Simultaneously, many of these clones totally or at least partially failed to express the classical B cell markers CD19 and CD20[14]. Although the marker expression may be influenced by the *in vitro* culture, one may still speculate on tumor-infiltrating B cells falsely counted as DCs - at least when CD83 was used as DC marker. Multi-color stainings on cryosections will help to clarify this question. Similarly, Nielsen *et al* shed light into another open question: which functional subtypes of B cells infiltrate tumor tissues? To definitely answer this, our above mentioned *ex vivo* cloning strategy will, together with classical analyses of different surface markers by immunohistochemical staining, possibly be very helpful.

Impact on future diagnostic procedures and prognosis

The contribution of B cells present in tumor tissues on the outcome of the disease is still largely cryptic. The data of Nielsen *et al*[5] clearly support a positive prognostic value of $CD20^+$ TIL in ovarian cancer. Together with recent similar findings of other groups on head and neck cancer[18], cutaneous melanoma[19] and breast cancer[20], it is likely that the presence of $CD20^+$ TIL may be a good prognosticator in general. Moreover, all the above mentioned analyses clearly focus on the B cell marker CD20 and broader analyses using B cell subset markers are definitely warranted in order to allow for diagnostic values of B cell subtypes infiltrating different cancers.

Contrary to these findings, it has been shown that B cells' presence in tumor draining lymph nodes is associated with lymphangiogenesis in a mouse model, which subsequently increases metastasis[21]. In human cancer, a metastasis-promoting role is discussed for B1 lymphocytes in melanoma[22] and B cell-secreted lymphotoxin can promote castration-resistant prostate cancer[23]. Taken together, these findings clearly imply that B lymphocytes' subtypes and most likely additional tissue context parameters will be predictive for either antitumoral or tumor-promoting activity of tumor-infiltrating B cells.

Impact on future therapeutic concepts

In my opinion, the most interesting argument brought forward by Nielsen *et al*[5] is the following: during pro-

longed immune responses as observed in autoimmunity, allograft rejection as well as chronic infection, B cells and T cells form so-called tertiary lymphoid structures. Thus, both cell types maintain a strong immune response in cooperative interactions at the affected site. The authors suggest that future antitumoral immunotherapies should be designed as to mimic these chronic immune reactions: T-cell responses are sustained over many months or years by direct interactions with co-localized B cells. Thus, it may be possible to generate more potent, sustained T-cell responses in the tumor environment by promoting the infiltration of tumor-reactive B cells. This line of argumentation is well taken, since in some colorectal cancers tumor-infiltrating B cells typically reside in tertiary lymphoid structures together with other tumor-infiltrating leukocyte populations thus permitting anti-tumor effects. This so-called "Crohn's like" reaction has repeatedly been associated with very good prognosis for colorectal cancer[24] and is especially associated with the molecular subtype with best prognostic behaviour; i.e., microsatellite-instability[25].

Another very promising strategy may base on the above mentioned unique potential of B cells to specifically concentrate antigens *via* their membrane-bound Ig molecules. I would like to speculate that tumor-antigen-specific B cells will have an enormous T cell stimulatory potential. Thus, cellular therapies can be envisioned basing on isolation and expansion of tumor-infiltrating and antigen-specific B cells.

In summary, improved understanding of the functional phenotype of tumor-infiltrating B cells ($CD20^{+}$ but possibly also $CD20^{neg}$ B cells), their target antigens, and their mechanism of recruitment to target tissues may not only help to understand the role of B cells in the natural curse of tumor diseases but also will have potentially strong impact on patients' diagnosis and last but not least will facilitate the design of more effective immunotherapies for the treatment of cancer.

REFERENCES

1 **Galon J**, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, Tosolini M, Camus M, Berger A, Wind P, Zinzindohoué F, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Pagès F. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* 2006; **313**: 1960-1964 [PMID: 17008531 DOI: 10.1126/science.112913]

2 **Erdag G**, Schaefer JT, Smolkin ME, Deacon DH, Shea SM, Dengel LT, Patterson JW, Slingluff CL. Immunotype and immunohistologic characteristics of tumor-infiltrating immune cells are associated with clinical outcome in metastatic melanoma. *Cancer Res* 2012; **72**: 1070-1080 [PMID: 22266112 DOI: 10.1158/0008-5472.CAN-11-3218]

3 **Nelson BH**. CD20+ B cells: the other tumor-infiltrating lymphocytes. *J Immunol* 2010; **185**: 4977-4982 [PMID: 20962266 DOI: 10.4049/jimmunol.1001323]

4 **Schmidt M**, Böhm D, von Törne C, Steiner E, Puhl A, Pilch H, Lehr HA, Hengstler JG, Kölbl H, Gehrmann M. The humoral immune system has a key prognostic impact in node-negative breast cancer. *Cancer Res* 2008; **68**: 5405-5413 [PMID: 18593943 DOI: 10.1158/0008-5472.CAN-07-5206]

5 **Nielsen JS**, Sahota RA, Milne K, Kost SE, Nesslinger NJ, Watson PH, Nelson BH. CD20+ tumor-infiltrating lymphocytes have an atypical CD27- memory phenotype and together with CD8+ T cells promote favorable prognosis in ovarian cancer. *Clin Cancer Res* 2012; **18**: 3281-3292 [PMID: 22553348 DOI: 10.1158/1078-0432.CCR-12-0234]

6 **Coronella JA**, Telleman P, Kingsbury GA, Truong TD, Hays S, Junghans RP. Evidence for an antigen-driven humoral immune response in medullary ductal breast cancer. *Cancer Res* 2001; **61**: 7889-7899 [PMID: 11691809]

7 **Hansen MH**, Nielsen HV, Ditzel HJ. Translocation of an intracellular antigen to the surface of medullary breast cancer cells early in apoptosis allows for an antigen-driven antibody response elicited by tumor-infiltrating B cells. *J Immunol* 2002; **169**: 2701-2711 [PMID: 12193744]

8 **Nzula S**, Going JJ, Stott DI. Antigen-driven clonal proliferation, somatic hypermutation, and selection of B lymphocytes infiltrating human ductal breast carcinomas. *Cancer Res* 2003; **63**: 3275-3280 [PMID: 12810659]

9 **Coronella JA**, Spier C, Welch M, Trevor KT, Stopeck AT, Villar H, Hersh EM. Antigen-driven oligoclonal expansion of tumor-infiltrating B cells in infiltrating ductal carcinoma of the breast. *J Immunol* 2002; **169**: 1829-1836 [PMID: 12165506]

10 **Simsa P**, Teillaud JL, Stott DI, Tóth J, Kotlan B. Tumor-infiltrating B cell immunoglobulin variable region gene usage in invasive ductal breast carcinoma. *Pathol Oncol Res* 2005; **11**: 92-97 [PMID: 15999153]

11 **Kotlan B**, Simsa P, Teillaud JL, Fridman WH, Toth J, McKnight M, Glassy MC. Novel ganglioside antigen identified by B cells in human medullary breast carcinomas: the proof of principle concerning the tumor-infiltrating B lymphocytes. *J Immunol* 2005; **175**: 2278-2285 [PMID: 16081796]

12 **Pavoni E**, Monteriù G, Santapaola D, Petronzelli F, Anastasi AM, Pelliccia A, D'Alessio V, De Santis R, Minenkova O. Tumor-infiltrating B lymphocytes as an efficient source of highly specific immunoglobulins recognizing tumor cells. *BMC Biotechnol* 2007; **7:** 70 [PMID: 17945015 DOI: 10.1186/1472-6750-7-70]

13 **Milne K**, Barnes RO, Girardin A, Mawer MA, Nesslinger NJ, Ng A, Nielsen JS, Sahota R, Tran E, Webb JR, Wong MQ, Wick DA, Wray A, McMurtrie E, Köbel M, Kalloger SE, Gilks CB, Watson PH, Nelson BH. Tumor-infiltrating T cells correlate with NY-ESO-1-specific autoantibodies in ovarian cancer. *PLoS One* 2008; **3:** e3409 [PMID: 18923710 DOI: 10.1371/journal.pone.0003409]

14 **Maletzki C**, Jahnke A, Ostwald C, Klar E, Prall F, Linnebacher M. Ex-vivo clonally expanded B lymphocytes infiltrating colorectal carcinoma are of mature immunophenotype and produce functional IgG. *PLoS One* 2012; **7**: e32639 [PMID: 22393427 DOI: 10.1371/journal.pone.0032639]

15 **Linnebacher M**, Maletzki C. Tumor-infiltrating B cells: The ignored players in tumor immunology. *Oncoimmunology* 2012; **1**: 1186-1188 [PMID: 23170274]

16 **Linnebacher M**, Maletzki C, Ostwald C, Klier U, Krohn M, Klar E, Prall F. Cryopreservation of human colorectal carcinomas prior to xenografting. *BMC Cancer* 2010; **10**: 362 [PMID: 20615215 DOI: 10.1186/1471-2407-10-362]

17 **Gulubova MV**, Ananiev JR, Vlaykova TI, Yovchev Y, Tsoneva V, Manolova IM. Role of dendritic cells in progression and clinical outcome of colon cancer. *Int J Colorectal Dis* 2012; **27**: 159-169 [PMID: 22065108 DOI: 10.1007/s00384-011-1334-1]

18 **Pretscher D**, Distel LV, Grabenbauer GG, Wittlinger M, Buettner M, Niedobitek G. Distribution of immune cells in head and neck cancer: CD8+ T-cells and CD20+ B-cells in metastatic lymph nodes are associated with favourable outcome in patients with oro- and hypopharyngeal carcinoma. *BMC Cancer* 2009; **9**: 292 [PMID: 19698134 DOI: 10.1186/1471-2407-9-292]

19 **Ladányi A**, Kiss J, Mohos A, Somlai B, Liszkay G, Gilde K, Fejös Z, Gaudi I, Dobos J, Tímár J. Prognostic impact of B-cell

density in cutaneous melanoma. *Cancer Immunol Immunother* 2011; **60**: 1729-1738 [PMID: 21779876 DOI: 10.1007/s00262-011-1071-x]

20 **Yamaguchi R**, Tanaka M, Yano A, Tse GM, Yamaguchi M, Koura K, Kanomata N, Kawaguchi A, Akiba J, Naito Y, Ohshima K, Yano H. Tumor-infiltrating lymphocytes are important pathologic predictors for neoadjuvant chemotherapy in patients with breast cancer. *Hum Pathol* 2012; **43**: 1688-1694 [PMID: 22516244 DOI: 10.1016/j.humpath.2011.12.013]

21 **Ruddell A**, Harrell MI, Furuya M, Kirschbaum SB, Iritani BM. B lymphocytes promote lymphogenous metastasis of lymphoma and melanoma. *Neoplasia* 2011; **13**: 748-757 [PMID: 21847366]

22 **Staquicini FI**, Tandle A, Libutti SK, Sun J, Zigler M, Bar-Eli M, Aliperti F, Pérez EC, Gershenwald JE, Mariano M, Pasqualini R, Arap W, Lopes JD. A subset of host B lymphocytes controls melanoma metastasis through a melanoma cell adhesion molecule/MUC18-dependent interaction: evidence from mice and humans. *Cancer Res* 2008; **68**: 8419-8428 [PMID: 18922915 DOI: 10.1158/0008-5472.CAN-08-1242]

23 **Ammirante M**, Luo JL, Grivennikov S, Nedospasov S, Karin M. B-cell-derived lymphotoxin promotes castration-resistant prostate cancer. *Nature* 2010; **464**: 302-305 [PMID: 20220849 DOI: 10.1038/nature08782]

24 **Ogino S**, Nosho K, Irahara N, Meyerhardt JA, Baba Y, Shima K, Glickman JN, Ferrone CR, Mino-Kenudson M, Tanaka N, Dranoff G, Giovannucci EL, Fuchs CS. Lymphocytic reaction to colorectal cancer is associated with longer survival, independent of lymph node count, microsatellite instability, and CpG island methylator phenotype. *Clin Cancer Res* 2009; **15**: 6412-6420 [PMID: 19825961 DOI: 10.1158/1078-0432.CCR-09-1438]

25 **Buckowitz A**, Knaebel HP, Benner A, Bläker H, Gebert J, Kienle P, von Knebel Doeberitz M, Kloor M. Microsatellite instability in colorectal cancer is associated with local lymphocyte infiltration and low frequency of distant metastases. *Br J Cancer* 2005; **92**: 1746-1753 [PMID: 15856045 DOI: 10.1038/sj.bjc.6602534]

P- Reviewer Glassy MC **S- Editor** Gou SX
L- Editor A **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i1.12

World J Gastroenterol 2013 January 7; 19(1): 12-16
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

Is endoscopic therapy the treatment of choice in all patients with chronic pancreatitis?

Beata Jabłońska

Beata Jabłońska, Department of Digestive Tract Surgery, University Hospital of the Medical University of Silesia, 40-752 Katowice, Poland
Author contributions: Jabłońska B wrote this paper.
Correspondence to: Beata Jabłońska, MD, PhD, Department of Digestive Tract Surgery, University Hospital of the Medical University of Silesia, Medyków 14 Street, 40-752 Katowice, Poland. bjablonska@poczta.onet.pl
Telephone: +48-32-7894251 Fax: +48-32-7894251
Received: September 4, 2012 Revised: October 29, 2012
Accepted: November 11, 2012
Published online: January 7, 2013

Abstract

Chronic pancreatitis (CP) is a progressive inflammatory disease of the pancreas characterized by destruction of the pancreatic parenchyma with subsequent fibrosis that leads to pancreatic exocrine and endocrine insufficiency. Abdominal pain and local complications (bile duct or duodenal stenosis and pancreatic tumor) secondary to CP are indications for therapy. At the beginning, medical therapy is used. More invasive treatment is recommended for patients with pancreatic duct stones (PDS) and pancreatic obstruction in whom standard medical therapy is not sufficient. Recently, Clarke *et al* assessed the long-term effectiveness of endoscopic therapy (ET) in CP patients. The authors compared ET with medical treatment. They reported that ET was clinically successful in 50% of patients with symptomatic CP. In this commentary, current CP treatment, including indications for ET and surgery in CP patients, is discussed. Recommendations for endoscopic treatment of CP according to the European Society of Gastrointestinal Endoscopy Clinical Guidelines are reviewed. Different surgical methods used in the treatment of CP patients are also discussed. ET is the most useful in patients with large PDS, pancreatic duct obstruction and dilation. It should be the first-line option because it is less invasive than surgery. Surgery should be the first-line option in patients in whom ET has failed or in those with a pancreatic mass with suspicion of malignancy. ET is a very effective and less invasive procedure, but it cannot be recommended as the treatment of choice in all CP patients.

© 2013 Baishideng. All rights reserved.

Key words: Chronic pancreatitis; Endoscopic therapy; Surgery; Endoscopic retrograde cholangiopancreatography; Endoscopy

Jabłońska B. Is endoscopic therapy the treatment of choice in all patients with chronic pancreatitis? *World J Gastroenterol* 2013; 19(1): 12-16 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i1/12.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i1.12

INVITED COMMENTARY ON HOT ARTICLES

I have read with great interest the recent article by Clarke *et al*[1] describing the endoscopic treatment in patients with chronic pancreatitis (CP). I would strongly recommend this article to the readers of *World Journal of Gastroenterology*. CP is a progressive inflammatory disease of the pancreas characterized by destruction of the pancreatic parenchyma with subsequent fibrosis that leads to pancreatic exocrine and endocrine insufficiency[2,3].

We can distinguish two stages in the natural CP course. At the beginning, recurrent acute pancreatitis is observed in patients. In the later stage, pancreatic exocrine and endocrine insufficiency manifested by maldigestion and malabsorption, loss of weight, steatorrhea and diabetes are reported[4].

Abdominal pain is the most common clinical symptom requiring therapy in patients with CP. It is caused by obstruction of the pancreatic duct either by stones

or stricture with increasing intraductal pressure and parenchymal ischemia[5]. Therefore, the main aim of CP treatment is to decompress the main pancreatic duct by pancreatic stone removal and pancreatic duct dilation. Different conservative and invasive methods are used in the treatment of CP. At the beginning, medical therapy with analgesics, oral pancreatic enzyme supplements, insulin and a low fat diet is used[6]. More invasive treatment is recommended for patients with pancreatic duct stones (PDS) and pancreatic obstruction in whom standard medical therapy is not sufficient. Local complications such as bile duct stenosis and pancreatic tumor are also indications for invasive treatment. Currently, two main invasive (endoscopic and surgical) methods are used in CP treatment. It should be emphasized that surgery is an older treatment method than endoscopic therapy (ET). The first case of surgical removal of PDS was reported in 1883[7]. ET as a less invasive treatment has become popular recently. Currently, both treatment methods are used in CP patients[8-26].

In their study, Clarke *et al*[1] analyzed the use and long-term effectiveness of ET in CP patients. The authors compared ET with medical treatment. They reported that ET was clinically successful in 50% of patients with symptomatic CP. When ET was not successful, surgery had successful outcomes in 50% of patients. Medical therapy was successful only in 31% of symptomatic patients. Based on their study, the authors concluded that ET was an effective treatment method in CP patients. This is a significant study and I agree with the authors' opinion. However, I would like to precisely define the use of endoscopic methods in CP patients. It is important to know which patients are good candidates for ET. Can we recommend this treatment method for all CP patients? In order to answer this question I would like to cite the most recent (2012) recommendations of the European Society of Gastrointestinal Endoscopy (ESGE) regarding therapeutic intervention in CP patients[8].

The ESGE recommends extracorporeal shock wave lithotripsy (ESWL) and endoscopic retrograde cholangiopancreatography (ERCP) as the first-line treatment method. Surgery should be considered in patients with a predicted poor outcome following ET. In patients with stones $\geqslant$ 5 mm obstructing the main pancreatic duct, the ESGE recommends ESWL as a first step, combined or not with endoscopic extraction of stone fragments depending on the expertise of the center. In CP patients with a main pancreatic duct stricture, the ESGE recommends placement of a single 10-Fr plastic stent, with stent exchange planned within 1 year. If ductal strictures persist after 12 mo of single plastic stenting, the ESGE recommends that endoscopic placement of multiple pancreatic stents or surgery should be discussed by a multidisciplinary team. The ESGE recommends ET as the first-line interventional option in pancreatic pseudocysts in CP patients. In CP-related biliary strictures, endoscopy and surgery should be considered depending on local expertise, patient co-morbidities, and expected patient compliance with repeat endoscopic procedures [8].

Some authors[9] recommend surgery as an initial treatment in CP patients with pancreatic and bile duct strictures with upstream dilation, and ET as a second-line option in high-risk surgical candidates.

Clarke *et al*[1] analyzed a cohort of 146 patients with CP, including 71 (49%) patients receiving ET. The authors analyzed patients following pancreatic and biliary sphincterotomy, pancreatic duct dilation, stone extraction, ESWL pancreatic and bile duct stenting, and transgastric pseudocyst drainage. The pancreatic duct stenting was the most frequent endoscopic procedure. The interesting aspect of this study is the analysis of 8 (11%) so-called bridge patients, in whom ET as the first step was followed by surgery. Another interesting aspect is comparison of the endoscopic with medical treatment.

There are a number of publications comparing endoscopy with surgery in CP patients in the literature. ET is less invasive than surgery. However, according to most authors, surgery is more effective compared to endoscopy[24,25]. Hirota *et al*[26] compared costs of these two treatment methods. Duration and frequency of hospitalizations, and medical costs were similar between the short-period endoscopic treatment group and surgery group. On the other hand, patients in the long-period endoscopic treatment group required significantly longer and more frequent hospitalizations, and had higher medical costs than both the short-period endoscopic treatment group and the surgery group. According to the authors' analysis, patients who underwent serial endoscopic stenting for more than 1 year showed no benefit compared with surgical treatment in terms of the frequency of hospital stays and medical costs.

In addition, I would like to describe the role of surgery in CP patients. I agree with Clarke *et al*[1] that ET is effective in CP patients, but I would not recommend this treatment method in all patients. In my opinion, patients with multiple strictures and dilation within the pancreatic duct can have more benefits following surgery. It is my belief that patients with a pancreatic mass with suspicion of malignancy and duodenal stricture secondary to CP should also undergo surgery. In the most frequently encountered situation, PDS are treated endoscopically, but in some cases surgery is necessary. In the study of Liu *et al*[5], indications for ET were $\leqslant$ 3 stones, stones confined to the pancreatic head and body, absence of restricted pancreatic duct, PDS diameter $\leqslant$ 10 mm, and noncompacted stone(s). Surgery was indicated for patients who needed treatment but did not meet the above-mentioned indications, or for whom conservative therapy had failed.

We can distinguish the following types of surgical procedures in CP patients: drainage operations (Puestow, Partington-Rochelle, Duval procedures), resectional operations (partial and subtotal or total pancreatectomies), resections with extended drainage (Beger, Frey procedures) and neurolysis (celiac plexus block). The most frequently performed surgical procedures are presented in Figure 1. Total pancreatectomy with islet autotransplan-

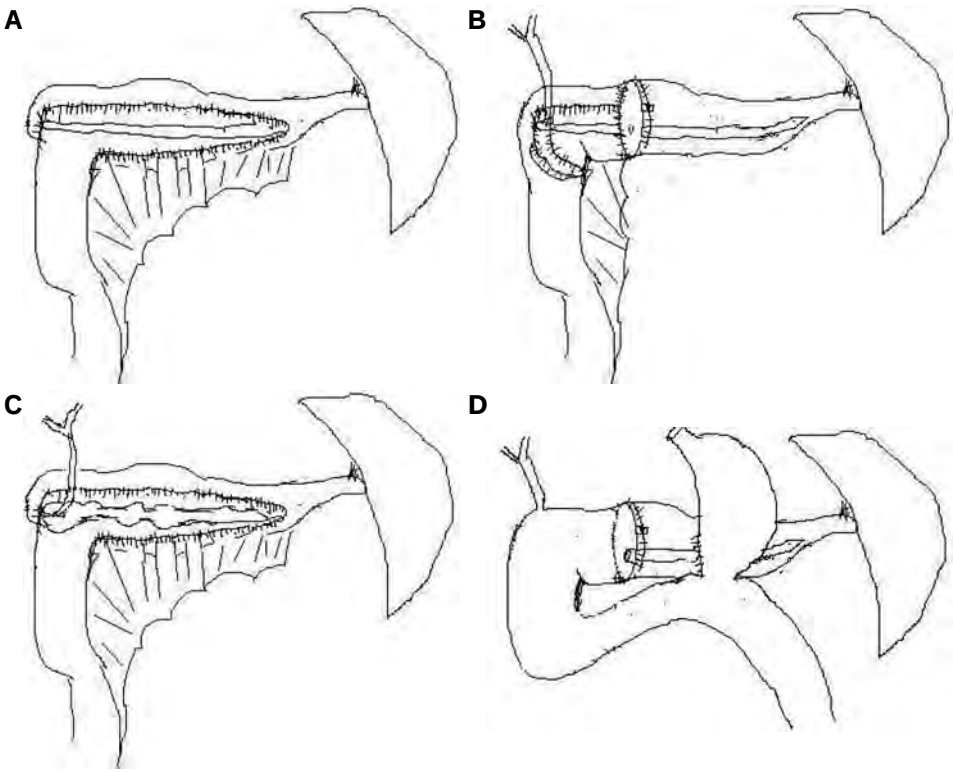


Figure 1 The most frequently performed surgical procedures. A: Partington-Rochelle procedure involving lateral (longitudinal) pancreaticojejunostomy (anastomosis between the longitudinally incised main pancreatic duct and Roux-Y jejunal loop); B: Beger procedure involving resection of the pancreatic head preserving the duodenum. The pancreas is transected at a border between the pancreatic head and body, leaving a thin pancreatic disc between the common bile duct and duodenum. The pancreatic body is drained by end-to-end pancreaticojejunostomy and pancreatic head disc is drained by side-to-side pancreaticojejunostomy using a Roux-Y jejunal loop; C: Frey procedure involving coring out of the pancreatic head overlying the main and accessory pancreatic ducts and uncinate process, keeping at least 5 mm pancreatic tissue posteriorly and medially along with opening the main duct in body and tail. The cored head and opened main duct are drained by lateral pancreaticojejunostomy using a Roux-Y jejunal loop; D: Whipple procedure involving pancreaticoduodenectomy with reconstruction by pancreaticojejunostomy, hepaticojejunostomy and gastrojejunostomy.

tation is an alternative surgical option for CP patients. It is important that this procedure can relieve pain and preserve β-cells in patients when other therapies fail[19,20]. Sutherland *et al*[20] from the University of Minnessota reported a > 30-year single-center series with good treatment results.

The Frey procedure is a safe and effective procedure with low morbidity and mortality rates in CP patients. It allows effective pain relief in about 90% of patients[27]. It is alternative and effective surgery for CP patients with a pancreatic inflammatory mass within the pancreatic head without malignancy[28].

According to Palanivelu *et al*[23], the Partington procedure is still the procedure of choice for patients with a dilated main pancreatic duct but without an inflammatory pancreatic head mass because it is simple and associated with a low postoperative morbidity and mortality, preserving pancreatic exocrine and endocrine function. Frey and Beger operations as duodenum-preserving pancreatic head resections (DPPHRs) with extended pancreatic duct drainage are recommended for patients with an inflammatory pancreatic head mass[29]. Furthermore, long-term results of two (Beger and Frey) DPPHRs in CP were compared by Keck *et al*[30]. This study revealed a better pain relief following the Frey procedure compared to the Beger operation.

There are some studies comparing DPPHRs and pancreaticoduodenectomy (PD) in treatment of CP patients. According to Zheng *et al*[18], both procedures are effective in relieving pain, but DPPHR is associated with less postoperative morbidity, including pancreatic exocrine and endocrine insufficiency, and better quality of life in patients following operation. Möbius *et al*[31] demonstrated a better quality of life and comparable pain relief and exocrine and endocrine pancreatic function in patients following DPPHR (Beger procedure) compared to patients following PD (Whipple procedure). Also, Witzigmann *et al*[32] revealed a better quality of life in CP patients following DPPHR compared to that experienced by patients following PD.

According to another recent study by Keck *et al*[33], both DPPHR and PD allow the achievement of effective pain relief and good quality of life without differences in endocrine or exocrine function in CP patients. According to Hildebrand *et al*[34], the Frey procedure is an advantageous alternative to the Whipple operation in CP patients. It is comparable to PD in pain relief and is less invasive (with lower morbidity and mortality rates, and a lower rate of newly occurring diabetes mellitus) than PD.

Similarly, McClaine *et al*[35] reported that DPPHR was equally as effective as PD in relieving pain and improving quality of life in CP patients; DPPHR was associated with a shorter hospital stay and less blood loss.

The choice of optimal surgery depends on size, number and location of PDS or presence of other complications (pancreatic mass, duodenal or biliary stenosis). In patients with pancreatic ductal stones, the PDS type should firstly be identified. Depending on location, four types of PDS have been established: type I in the head of the pancreas, type II in the body, type III in the tail, and type IV in the whole pancreas. Drainage by pancreatic ductotomy is generally recommended for patients with a dilated pancreatic duct (≥ 6 mm). The Beger procedure and PD are suitable for type I PDS; however, distal pancreatectomy is useful for type III PDS. For patients with type II PDS, the Partington-Rochelle procedure or segmental resection of the pancreas is recommended. For patients with type IV PDS, an alternative to drainage is a Child procedure (subtotal pancreatectomy)[4,5].

In conclusion, all methods including medical, endoscopic and surgical treatment are used in CP patients. Medical therapy is suitable in patients without complications at the outset of CP. ET is the most useful in patients with large PDS, pancreatic duct obstruction and dilation. It should be the first-line option because it is less invasive than surgery. The method of ET (pancreatic sphincterotomy or ESWL followed by pancreatic duct stenting) depends on the stone location and the pancreatic duct diameter. Surgery should be the first-line option in patients in whom ET failed or those with pancreatic mass with suspicion of malignancy. Therefore, although ET is a very effective and less invasive procedure, it cannot be recommended as the treatment of choice in all CP patients. In my opinion, surgical treatment without endoscopic treatment should be recommended for patients with duodenal stricture and pancreatic mass with suspicion of malignancy. In these patients, endoscopy should be used only in the diagnosis.

REFERENCES

1 **Clarke B**, Slivka A, Tomizawa Y, Sanders M, Papachristou GI, Whitcomb DC, Yadav D. Endoscopic therapy is effective for patients with chronic pancreatitis. *Clin Gastroenterol Hepatol* 2012; **10**: 795-802 [PMID: 22245964]

2 **Delhaye M**, Arvanitakis M, Bali M, Matos C, Devière J. Endoscopic therapy for chronic pancreatitis. *Scand J Surg* 2005; **94**: 143-153 [PMID: 16111097]

3 **Etemad B**, Whitcomb DC. Chronic pancreatitis: diagnosis, classification, and new genetic developments. *Gastroenterology* 2001; **120**: 682-707 [PMID: 11179244 DOI: 10.1053/gast.2001.22586]

4 **Han SL**, Chen J, Zhou HZ, Lan SH, Zhang PC, Zhu GB. Indications and surgical treatment of chronic pancreatitis. *Hepatobiliary Pancreat Dis Int* 2008; **7**: 638-642 [PMID: 19073411]

5 **Liu BN**, Zhang TP, Zhao YP, Liao Q, Dai MH, Zhan HX. Pancreatic duct stones in patients with chronic pancreatitis: surgical outcomes. *Hepatobiliary Pancreat Dis Int* 2010; **9**: 423-427 [PMID: 20688608]

6 **Choi EK**, Lehman GA. Update on endoscopic management of main pancreatic duct stones in chronic calcific pancreatitis. *Korean J Intern Med* 2012; **27**: 20-29 [PMID: 22403495 DOI: 10.3904/kjim.2012.27.1.20]

7 **Haggard WD**, Kirtley JA. Pancreatic calculi: a review of sixty-five operative and one hundred thirty-nine non-operative cases. *Ann Surg* 1939; **109**: 809-826 [PMID: 17857364]

8 **Dumonceau JM**, Delhaye M, Tringali A, Dominguez-Munoz JE, Poley JW, Arvanitaki M, Costamagna G, Costea F, Devière J, Eisendrath P, Lakhtakia S, Reddy N, Fockens P, Ponchon T, Bruno M. Endoscopic treatment of chronic pancreatitis: European Society of Gastrointestinal Endoscopy (ESGE) Clinical Guideline. *Endoscopy* 2012; **44**: 784-800 [PMID: 22752888 DOI: 10.1055/s-0032-1309840]

9 **Ito K**, Fujita N, Noda Y, Kobayashi G, Horaguchi J. Endoscopic treatment for biliary stricture secondary to chronic pancreatitis. *Dig Endosc* 2012; **24** Suppl 1: 17-21 [PMID: 22533746 DOI: 10.1111/j.1443-1661.2012.01250.x]

10 **Giacino C**, Grandval P, Laugier R. Fully covered self-expanding metal stents for refractory pancreatic duct strictures in chronic pancreatitis. *Endoscopy* 2012; **44**: 874-877 [PMID: 22826158 DOI: 10.1055/s-0032-1309774]

11 **Bartoli E**, Delcenserie R, Yzet T, Brazier F, Geslin G, Regimbeau JM, Dupas JL. Endoscopic treatment of chronic pancreatitis. *Gastroenterol Clin Biol* 2005; **29**: 515-521 [PMID: 15980744 DOI: 10.1016/S0399-8320(05)82122-0]

12 **Tringali A**, Boskoski I, Costamagna G. The role of endoscopy in the therapy of chronic pancreatitis. *Best Pract Res Clin Gastroenterol* 2008; **22**: 145-165 [PMID: 18206819 DOI: 10.1016/j.bpg.2007.10.021]

13 **Seven G**, Schreiner MA, Ross AS, Lin OS, Gluck M, Gan SI, Irani S, Brandabur JJ, Patterson D, Kuhr C, Kozarek R. Long-term outcomes associated with pancreatic extracorporeal shock wave lithotripsy for chronic calcific pancreatitis. *Gastrointest Endosc* 2012; **75**: 997-1004.e1 [PMID: 22401819 DOI: 10.1016/j.gie.2012.01.014]

14 **Seza K**, Yamaguchi T, Ishihara T, Tadenema H, Tawada K, Saisho H, Yokosuka O. A long-term controlled trial of endoscopic pancreatic stenting for treatment of main pancreatic duct stricture in chronic pancreatitis. *Hepatogastroenterology* 2011; **58**: 2128-2131 [PMID: 22234084 DOI: 10.5754/hge09346]

15 **Prochorov AV**, Oldhafer KJ, Tretyak SI, Rashchynski SM, Donati M, Rashchynskaya NT, Audzevich DA. Surgical treatment of pain in patients with chronic pancreatitis. *Hepatogastroenterology* 2012; **59**: 1265-1269 [PMID: 22580679 DOI: 10.5754/hge10218]

16 **van der Gaag NA**, van Gulik TM, Busch OR, Sprangers MA, Bruno MJ, Zevenbergen C, Gouma DJ, Boermeester MA. Functional and medical outcomes after tailored surgery for pain due to chronic pancreatitis. *Ann Surg* 2012; **255**: 763-770 [PMID: 22418009 DOI: 10.1097/SLA.0b013e31824b7697]

17 **Riediger H**, Adam U, Fischer E, Keck T, Pfeffer F, Hopt UT, Makowiec F. Long-term outcome after resection for chronic pancreatitis in 224 patients. *J Gastrointest Surg* 2007; **11**: 949-959; discussion 959-960 [PMID: 17534689 DOI: 10.1007/s11605-007-0155-6]

18 **Zheng Z**, Xiang G, Tan C, Zhang H, Liu B, Gong J, Mai G, Liu X. Pancreaticoduodenectomy versus duodenum-preserving pancreatic head resection for the treatment of chronic pancreatitis. *Pancreas* 2012; **41**: 147-152 [PMID: 21775913 DOI: 10.1097/MPA.0b013e318221c91b]

19 **Bramis K**, Gordon-Weeks AN, Friend PJ, Bastin E, Burls A, Silva MA, Dennison AR. Systematic review of total pancreatectomy and islet autotransplantation for chronic pancreatitis. *Br J Surg* 2012; **99**: 761-766 [PMID: 22434330 DOI: 10.1002/bjs.8713]

20 **Sutherland DE**, Radosevich DM, Bellin MD, Hering BJ, Beilman GJ, Dunn TB, Chinnakotla S, Vickers SM, Bland B, Balamurugan AN, Freeman ML, Pruett TL. Total pancreatectomy and islet autotransplantation for chronic pan-

creatitis. *J Am Coll Surg* 2012; **214**: 409-24; discussion 424-6 [PMID: 22397977 DOI: 10.1016/j.jamcollsurg.2011.12.040]
21 **Schnelldorfer T**, Lewin DN, Adams DB. Operative management of chronic pancreatitis: longterm results in 372 patients. *J Am Coll Surg* 2007; **204**: 1039-1045; discussion 1045-1047 [PMID: 17481536 DOI: 10.1016/j.jamcollsurg.2006.12.045]
22 **Regimbeau JM**, Fuks D, Bartoli E, Fumery M, Hanes A, Yzet T, Delcenserie R. A comparative study of surgery and endoscopy for the treatment of bile duct stricture in patients with chronic pancreatitis. *Surg Endosc* 2012; **26**: 2902-2908 [PMID: 22580872]
23 **Palanivelu C**, Ahluwalia JS, Parthasarathi R, Palanisami S, Vaithiswaran V, Rajapandian S, Palanivelu P. Laparoendoscopic single-site lateral pancreaticojejunostomy. *Pancreatology* 2011; **11**: 500-505 [PMID: 22042294 DOI: 10.1159/000331461]
24 **Cahen DL**, Gouma DJ, Nio Y, Rauws EA, Boermeester MA, Busch OR, Stoker J, Laméris JS, Dijkgraaf MG, Huibregtse K, Bruno MJ. Endoscopic versus surgical drainage of the pancreatic duct in chronic pancreatitis. *N Engl J Med* 2007; **356**: 676-684 [PMID: 17301298 DOI: 10.1056/NEJMoa060610]
25 **Ahmed Ali U**, Pahlplatz JM, Nealon WH, van Goor H, Gooszen HG, Boermeester MA. Endoscopic or surgical intervention for painful obstructive chronic pancreatitis. *Cochrane Database Syst Rev* 2012; **1**: CD007884 [PMID: 22258975]
26 **Hirota M**, Asakura T, Kanno A, Kikuta K, Kume K, Hamada S, Unno J, Ito H, Ariga H, Masamune A, Satoh K, Motoi F, Egawa S, Unno M, Shimosegawa T. Long-period pancreatic stenting for painful chronic calcified pancreatitis required higher medical costs and frequent hospitalizations compared with surgery. *Pancreas* 2011; **40**: 946-950 [PMID: 21747315]
27 **Pessaux P**, Kianmanesh R, Regimbeau JM, Sastre B, Delcenserie R, Sielezneff I, Arnaud JP, Sauvanet A. Frey procedure in the treatment of chronic pancreatitis: short-term results. *Pancreas* 2006; **33**: 354-358 [PMID: 17079939]
28 **Roch AM**, Brachet D, Lermite E, Pessaux P, Arnaud JP. Frey procedure in patients with chronic pancreatitis: short and long-term outcome from a prospective study. *J Gastrointest Surg* 2012; **16**: 1362-1369 [PMID: 22580839 DOI: 10.1007/s11605-012-1904-8]
29 **Isaji S**. Has the Partington procedure for chronic pancreatitis become a thing of the past? A review of the evidence. *J Hepatobiliary Pancreat Sci* 2010; **17**: 763-769 [PMID: 19779664 DOI: 10.1007/s00534-009-0181-8]
30 **Keck T**, Wellner UF, Riediger H, Adam U, Sick O, Hopt UT, Makowiec F. Long-term outcome after 92 duodenum-preserving pancreatic head resections for chronic pancreatitis: comparison of Beger and Frey procedures. *J Gastrointest Surg* 2010; **14**: 549-556 [PMID: 20033344 DOI: 10.1007/s11605-009-1119-9]
31 **Möbius C**, Max D, Uhlmann D, Gumpp K, Behrbohm J, Horvath K, Hauss J, Witzigmann H. Five-year follow-up of a prospective non-randomised study comparing duodenum-preserving pancreatic head resection with classic Whipple procedure in the treatment of chronic pancreatitis. *Langenbecks Arch Surg* 2007; **392**: 359-364 [PMID: 17375317 DOI: 10.1007/s00423-007-0175-4]
32 **Witzigmann H**, Max D, Uhlmann D, Geissler F, Ludwig S, Schwarz R, Krauss O, Lohmann T, Keim V, Hauss J. Quality of life in chronic pancreatitis: a prospective trial comparing classical whipple procedure and duodenum-preserving pancreatic head resection. *J Gastrointest Surg* 2002; **6**: 173-179; discussion 179-180 [PMID: 11992802 DOI: 10.1016/S1091-255X(01)00023-3]
33 **Keck T**, Adam U, Makowiec F, Riediger H, Wellner U, Tittelbach-Helmrich D, Hopt UT. Short- and long-term results of duodenum preservation versus resection for the management of chronic pancreatitis: a prospective, randomized study. *Surgery* 2012; **152**: S95-S102 [PMID: 22906892 DOI: 10.1016/j.surg.2012.05.016]
34 **Hildebrand P**, Dudertadt S, Czymek R, Bader FG, Roblick UJ, Bruch HP, Jungbluth T. Different surgical strategies for chronic pancreatitis significantly improve long-term outcome: a comparative single center study. *Eur J Med Res* 2010; **15**: 351-356 [PMID: 20947472 DOI: 10.1186/2047-783X-15-8-351]
35 **McClaine RJ**, Lowy AM, Matthews JB, Schmulewitz N, Sussman JJ, Ingraham AM, Ahmad SA. A comparison of pancreaticoduodenectomy and duodenum-preserving head resection for the treatment of chronic pancreatitis. *HPB* (Oxford) 2009; **11**: 677-683 [PMID: 20495636 DOI: 10.1111/j.1477-2574.2009.00118.x]

P- Reviewers Kleeff J, Kalaitzakis E, Sakata N
S- Editor Huang XZ **L- Editor** Logan S **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i1.17

World J Gastroenterol 2013 January 7; 19(1): 17-25
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

TOPIC HIGHLIGHT

Juan-Ramón Larrubia, PhD, *Series Editor*

Clinical significance of cytomegalovirus infection in patients with inflammatory bowel disease

Elena Garrido, Elisa Carrera, Rebeca Manzano, Antonio Lopez-Sanroman

Elena Garrido, Antonio Lopez-Sanroman, Inflammatory Bowel Disease Clinic, Department of Gastroenterology, University Hospital Ramón y Cajal, 28034 Madrid, Spain
Elisa Carrera, Department of Gastroenterology, University Hospital of Guadalajara, 19002 Guadalajara, Spain
Rebeca Manzano, Department of Gastroenterology, Hospital Sureste, 28500 Madrid, Spain
Author contributions: Garrido E, Carrera E, Manzano R and Lopez-Sanroman A contributed equally to this work; and all authors have approved the final version of the manuscript.
Correspondence to: Dr. Elena Garrido, Inflammatory Bowel Disease Clinic, Department of Gastroenterology, University Hospital Ramón y Cajal, Carretera Colmenar K9.100, 28034 Madrid, Spain. elenagarridogomez@hotmail.com
Telephone: +34-913-368305 Fax: +34-913-368354
Received: May 29, 2012 Revised: August 3, 2012
Accepted: August 14, 2012
Published online: January 7, 2013

Abstract

Cytomegalovirus (CMV) infection is common in humans. The virus then enters a "latency phase" and can reactivate to different stimuli such as immunosuppression. The clinical significance of CMV infection in inflammatory bowel disease is different in Crohn's disease (CD) and ulcerative colitis (UC). CMV does not interfere in the clinical course of CD. However, CMV reactivation is frequent in severe or steroid-resistant UC. It is not known whether the virus exacerbates the disease or simply appears as a bystander of a severe disease. Different methods are used to diagnose CMV colitis. Diagnosis is classically based on histopathological identification of viral-infected cells or CMV antigens in biopsied tissues using haematoxylin-eosin or immunohistochemistry, other tests on blood or tissue samples are currently being investigated. Polymerase chain reaction performed in colonic mucosa has a high sensitivity and a positive result could be associated with a worse prognosis disease; further studies are needed to determine the most appropriate strategy with positive CMV-DNA in colonic mucosa. Specific endoscopic features have not been described in active UC and CMV infection. CMV colitis is usually treated with ganciclovir for several weeks, there are different opinions about whether or not to stop immunosuppressive therapy. Other antiviral drugs may be used. Multicenter controlled studies would needed to determine which subgroup of UC patients would benefit from early antiviral treatment.

© 2013 Baishideng. All rights reserved.

Key words: Cytomegalovirus; Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Hematoxylin and eosin; Immunohistochemical; Polymerase chain reaction; Ganciclovir; Infectious colitis

Garrido E, Carrera E, Manzano R, Lopez-Sanroman A. Clinical significance of cytomegalovirus infection in patients with inflammatory bowel disease. *World J Gastroenterol* 2013; 19(1): 17-25 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i1/17.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i1.17

NATURAL HISTORY OF CYTOMEGALOVIRUS INFECTION

Cytomegalovirus (CMV) is a member of the *Herpesviridae* family which contains a double-stranded DNA. It often causes primary infection in humans, and later persists lifelong in a latent stage. In different situations of immunosuppression [birth, human immunodeficiency virus (HIV) infection, use of immunosuppressant therapy or chemotherapy] the virus can reactivate[1,2] and cause disease, but this may also occur in immunocompetent hosts.

In CMV infection, CMV antigens or antibodies can be detected in blood, whereas CMV disease symptoms

generally appear in a target organ[3]. The prevalence of CMV infection is high, ranging from 30%-100%[4-7], depending on age and race of population tested[8-10].

Primary CMV infection in immunocompetent individuals is usually asymptomatic, and rarely manifests itself as a mononucleosis-type disease similar to that caused by Epstein-Barr virus, consisting of fever, fatigue and cervical lymphadenopathy. Subsequently, the virus enters a latency phase in endothelial cells, macrophages or granulocyte stem cells[11-13]. Virus can reactivate in the healthy adult, this new activation being typically asymptomatic; however, this delicate balance can be disrupted in patients whose immune response is compromised, which may lead to development of symptoms in different organs, such as CMV colitis[13]. The use of highly active antiretroviral therapy in patients with HIV has decreased significantly CMV disease in these patients[14]; on the other hand, induced immunodeficiency in situations such as solid organ transplant or inflammatory bowel disease (IBD) sometimes allows CMV disease to develop, with significant morbidity and even mortality[15].

Antibody response to CMV infection reflects this relapsing-remitting pattern. In primary infection, an early increase of specific IgM antibodies occurs, which can be detected in the first week of infection[16], showing a sensitivity of 100% and a specificity of 99%[17]. Over the next 3-6 mo immunoglobulin M (IgM) antibodies fall to undetectable levels, although some may persist for up to 12-24 mo. Persistence of IgM antibodies could be related to concomitant immunosuppression[18]. Reactivation can cause again a rising IgM titers[17,19]. Immunoglobulin G (IgG) antibody production occurs within a few weeks of IgM increase[16]. When patients develop specific CMV IgG antibodies, they are considered "seropositives". In the reactivation of CMV, IgG antibody levels do not change, so that this situation can only be differentiated by the detection of an increase in IgM antibody titers that does not always occur. Thus, serology has a limited value in the diagnosis of reactivation[10]. Seronegative patients are not at risk for CMV disease, unless primoinfection occurs.

Gastrointestinal tract is a common site of CMV disease, with preference the esophagus and the colonic mucosa (especially at the right colon). The pouch mucosa behaves in a similar way to colon mucosa[20-22]. Clinical presentation of CMV colitis begins with watery diarrhoea that may be progress to rectal bleeding, abdominal pain, fatigue and fever. Other presentations such as fever of unknown origin, megacolon or digestive hemorrhage, are also possible.

It is unknown whether the virus remains in colon after a primary infection or if it disappears spontaneously[23]. A recent study[24] demonstrated persistence of colonic CMV [using polymerase chain reaction (PCR) method], following an acute ulcerative colitis (UC) flare-up despite remission of symptoms. The virus may disappear or not from the colon after an acute UC flare-up, but if it persists is detected only by PCR DNA, without displaying inflammatory changes in colonic mucosa or CMV antigens in infected colonic cells.

There have been reports of CMV colitis in immunocompetent hosts[25,26], especially in elderly patients with comorbidities in whom the disease followed a severe course with high mortality rate. Also, rare cases of CMV colitis preceding IBD have been reported[27].

RELATIONSHIP BETWEEN CMV AND IBD

The association between CMV and IBD was described long ago. The first case report dates from 1961, when Powell *et al*[28] described a patient with UC and cytomegalic inclusion disease. Since then, questions remain about the role of CMV in these patients: does CMV reactivation exacerbate the disease in patients with established IBD or is reactivation a consequence of IBD activity and its treatment with CMV acting as an innocent bystander[29-31]? Interpretation of existing results is limited because most studies are small and retrospective, different diagnostic methods of CMV detection are available and even different classifications are used for the severity of concomitant IBD[3].

CMV colitis occurs in "seropositive" patients with IBD. CMV does not appear to interfere with the clinical evolution of Crohn's disease (CD), and its involvement in UC is debated, especially in severe flare-ups.

DIAGNOSTIC TECHNIQUES

Diagnostic techniques for CMV infection

Serology: Serology is useful to determine previous viral exposure and identify patients at risk, as only seropositive patients (positive IgG antibodies in blood) may develop CMV disease[30,31].

Antigenemia assay: It detects viral protein pp65 produced in peripheral blood polymorphonuclear leukocytes. It is a simple and rapid technique, with a sensitivity of 60%-100% and a specificity of 83%-100%[32,33]. However, it does not differentiate between latent infection and active disease[23], no association exists with virus reactivation in the intestinal mucosa[31,34], and false negatives can occur in neutropenic patients[35].

PCR DNA amplification assay in blood: This test is supplanting antigenemia. Sensitivity ranges 65%-100% and specificity ranges 40%-92%[32,36,37]. This diagnostic method cannot differentiate between latency and activity states, so it is necessary to determine a cut-off above which active infection is diagnosed in patients with IBD[30,35], as well as in solid organ transplantation, where a viremia > 1000 copies/100 000 leukocytes indicates symptomatic CMV infection[38]. This method can be used both to detect disease and to monitor treatment response; a slow or absent decline in DNA levels after treatment could be an early indicator of drug resistance[39], and positive result after therapy indicates continuing treatment[40]. Most studies in patients with IBD have reported a correlation between

identification of CMV by PCR in blood and detection colonic CMV by haematoxylin and eosin (HE) or immunohistochemistry (IHC)[34,41-43].

PCR assay in stool: A qualitative and quantitative PCR assay for CMV DNA has been performed on human faecal specimens from immunocompromised patients[44,45].

Diagnostic techniques for CMV colitis

Histological diagnosis is considered the "gold standard" for diagnosing CMV disease in the gastrointestinal tract[46-51]. Several methods are available.

Histology by HE: Typically reveals cytomegalic cells that are 2 or 4 fold larger than normal cells, containing basophilic intranuclear inclusions in eccentric location surrounded by a clear halo, giving it an "owl's eye" appearence. Cells show a thickened nuclear membrane and smaller granular intracytoplasmic inclusions. Colonic biopsies should be taken from inflamed mucosa near or within the ulcer. This method has very high specificity (92%-100%), but its main problem is a poor sensitivity (10%-87%), making the diagnosis requires many samples and a trained pathologist[46,47]. Anecdotally cytomegalic cells have been described in colon biopsies from normal mucosa in healthy individuals[48,49].

IHC in colon biopsies: It involves the identification of CMV antigens in infected cells. It has a higher sensitivity than HE (78%-93%)[50,51]. Other techniques, however, do not rely on histology.

PCR DNA amplification assay in colon mucosa: PCR DNA amplification assay in colon mucosa has the greatest accuracy for virus detection[29,52,53] and may be used as a qualitative or quantitative method. PCR DNA levels in the colon are not related to viremia levels measured in blood[31,43,54,55]. Some studies detected prevalences greater than 30% in IBD patients[56], although the significance of a positive result in absence of histological signs of CMV infection is unclear. Very few studies have shown a correlation between histology (HE/IHC) and PCR results[31,43]. This suggests that detection of low DNA levels could determine latent infection and requires a cut-off level of viremia to distinguish infection from disease[30]. In other words, a positive result in colon does not necessarily reflect the involvement of CMV in UC flare-ups. A recent study from France suggests a cut-off of > 250 copies/mg for CMV disease[54] with a sensitivity of 100% and a specificity of 66%. To avoid false positives, and awaiting further studies, this determination should be performed only in patients with active UC refractory to conventional treatment.

Viral culture: Viral culture was previously regarded as the gold standard in CMV detection. Culture has a sensitivity of 45%-78% and a very high specificity (89%-100%). The virus is placed in a fibroblast tissue culture and diagnosis is made once the virus causes cytopathic changes in fibroblasts. The problem is that the result takes days to weeks[10], so this method is not used in clinical practice[57].

Typical endoscopic findings of CMV colitis: Typical endoscopic findings of CMV colitis are microerosions, deep ulcers and pseudotumoral lesions[58]. Most studies in patients with IBD, specifically in active UC, have not found specific endoscopic features[43,54,59,60]. Anecdotal studies describe some characteristic endoscopic (large, irregular, punched-out or longitudinal ulcers) with virus[61]. Discrepancy can be explained by the different criteria used to define CMV infection or disease.

In conclusion, different methods of detection of CMV have different sensitivities and specificities. In the setting of a severe UC flare-up refractory to conventional treatment, colonic IHC or PCR in blood should be performed, PCR presents a good correlation with the results of HE and IHC[30]. Serology and antigenemia only are useful as negative predictors for CMV infection, since they do not correlate with actual CMV colitis. The role of PCR positivity in colon remains to be determined, it is not known how to interpret its positivity in the absence of histological changes. However, recent studies imply positivity of this method in the disease prognosis[54]. Current European guidelines[62] recommend the use of tissue PCR or IHC to detect CMV in patients with UC resistant to immunomodulators, whereas older American guidelines[63] recommend performing sigmoidoscopic biopsy and viral culture in refractory cases of UC.

CMV PREVALENCE IN IBD

Overall prevalence of CMV is unknown in IBD patients. Most of studies have been carried out using a selected patient group and using different diagnostic methods, so that the available data include a wide range of prevalences.

CMV prevalence in CD

Seropositivity in CD patients is as high as in other populations (70%)[64,65], however CMV disease is rare in CD, making the virus an unlikely etiological factor in the novo development of IBD[27,64,66,67].

In most surveys, IHC did not detect CMV in CD patients, and PCR in tissue or stool samples found a very small frequency (< 5% of patients)[27,66-69], except in one study that used a highly sensitive PCR (detection < 10 copies of DNA) which detected CMV in 66% of individuals with CD and in 29% of controls, showing no association between CMV DNA and disease activity, suggesting the authors that small quantities of viral DNA are not clinically relevant[29].

Recently some theories have been published that explain these findings based on small studies[70], so results should be interpreted with caution. Tumor necrosis factor-α (TNFα) would be significantly associated with CMV infection or reactivation in IBD. However interferon-γ (IFNγ), which is produced from CD4 + T

cells[71], could suppress CMV reactivation. CD is considered a Th1-type inflammatory process with high expression of IFNγ, which does no help the virus reactivation and may explain the different prevalence of CMV disease in UC and CD[70].

CMV prevalence in UC

Prevalence of CMV infection is about 70%[31], a similar percentage to that of the general population.

Patients with inactive or mild-moderate UC did not show an increased risk of CMV colitis, using HE and IHC[27,31,41] or IHC in colectomy patients undergoing surgery for dysplasia or cancer[72].

The most extensive literature is on severe and/or steroid-refractory colitis. These two terms are used interchangeably in studies and are not defined clearly, so the results are difficult to interpret[30].

Severe colitis: The combination of serological tests and rectal biopsies found a CMV disease prevalence of around 20%[42,73]. Risk factors that have been identified are female gender, older age, pancolonic disease with active inflammation at histology and azathioprine therapy[60,72,73]. According only to antigenemia, prevalence increased to 34%[60], while using HE or IHC in colonic mucosa prevalence decreased to 3%[74].

Severe steroid-resistant colitis: In retrospective studies the prevalence according to HE was 0.5%[75]. The prevalence of CMV increased by combining HE and IHC with antigenemia (20%-40%)[31,41,72,76,77]. The prevalence using blood PCR was 60%[43], whereas PCR results in colon were lower (38%)[78]. There is a poor correlation between peripheral and colonic viral load, this can be explained by theory proposed by Criscuoli *et al*[24,42] about a specific genotype, which possibly has a particular colonic tropism and pathogenic character, whilst other genotypes are not pathogenic.

Urgent colectomy for colitis: A higher prevalence of CMV is expected in these patients because the greater severity of their disease, but the prevalence using HE or IHC ranged between 11.5% and 27%[77,79-82], which is similar to the previous groups.

Experimental studies have identified three factors that influence the reactivation of CMV infection in active colitis: (1) increased cell proliferation in inflamed tissue with ulcers that attract CMV; (2) inherent impaired natural killer cell activity presented by patients with IBD[83,84]; and (3) use of immunosuppressive drugs[31,42,69]. The third factor is controversial[73-75,80,85], since use of steroids may be either a risk factor or a surrogate marker of severity disease[41]. *In vitro* data suggest that steroids and cyclosporine could support the replication of CMV[76,86,87]. Infliximab treatment has not been associated with increased risk of CMV reactivation in IBD patients[66]. Moreover, TNFα has been identified as an activator of CMV, and thus infliximab (IFX) could promote viral latency, by lowering the levels of this cytokine[88,89]. This suggests that IFX could be used in patients with severe UC flare and CMV reactivation. However, some cases of tacrolimus-refractory UC that were treated with IFX did not have a favourable outcome[90].

CMV reaches the mucosa by way of monocytes and then colonize the colonic cells, acquiring particular affinity for the inflammatory sites, probably due to the presence of pro-inflammatory cytokines such as IFNγ and TNFα produced by macrophages and T-cells in active UC[91-94]. CMV avoids immune recognition of macrophages and uses immune specific functions to reactivate and spread[95]. Recent studies suggested CMV can appear only in inflamed tissue and is not found in healthy tissue[54].

Hommes *et al*[94] proposed the following sequence to explain the pathophysiology of CMV disease in patients with active UC: (1) initiation phase, mucosal inflammatory response induces expression of cytokines and chemokines which activates latently infected cells and the migration of monocytes and dendritic cells into the inflamed mucosa; (2) reactivation phase, in which infected monocytes differentiate into tissue macrophages and dendritic cells; and (3) consolidation phase, during which the virus causes an active replication predominantly in endothelial cells that likely exacerbates inflammation. The virus in the first and second phase has not led to histological damage and can only be detected by PCR-DNA. It would be appropriate to detect CMV in these phases, so the optimal treatment would be administered at an early stage before complicating the disease. They do not consider CMV as an innocent bystander but rather involved in resistance to immunosuppressive treatment.

In the same line, other authors suggest that the positivity of CMV DNA in the colonic mucosa in patients with refractory UC indicates uncontrolled intestinal inflammation, suggesting a change in immunosuppressive therapy[96].

Coincidental detection of primary CMV infection at the first appearance of IBD has been reported before[66,97], primary CMV infection in IBD patients without immunosupression[98,99], and even disseminated CMV infection in CD[100].

PROGNOSIS OF CMV COLITIS IN IBD

As discussed above, it is unclear whether CMV superinfection leads to a more aggressive course of pre-existing inflammatory disease[29,30].

Referring only to UC, case-reports and retrospective series[29,42,55,68,69] suggested that CMV may trigger a steroid-refractory flare-up and worsen disease prognosis[76,77,80,85,101], associated increased risk of toxic megacolon[81] and surgical intervention[31,77,82,102,103]. This hypothesis is supported by two prospective studies[41,54]. The second, a recent French study, relates positivity of CMV (PCR in colon > 250 copies/mg) to treatment resistance (steroids and other three line of treatment), demonstrating that early initiation of antiviral therapy in these cases delays treatment resistance

and improves prognosis[54].

However, other studies[3,27,34,43,72] that analyzed prognosis in patients with severe UC refractory to conventional treatments did not find increased disease severity nor increased rate of colectomy in CMV positive patients.

These conclusions are limited by differences in quality of existing studies.

TREATMENT OF CMV COLITIS IN IBD

It is difficult to draw conclusions about the role of antiviral therapy based on the available evidence[23], which is of varying quality.

Most authors recommend the use of antivirals[30,31,41,43,75] in steroid-refractory UC flare-up and CMV positive patients. Treatment can significantly decrease the mortality rate and need for surgery. However, some studies show that treatment of CMV does not alter disease course[80], and has even been shown the transience of positivity of serological tests (antigenemia, PCR) in patients with severe UC, but which as discussed earlier has little correlation with PCR determination in colon[34,94].

As CMV reactivation impact in clinical outcome is not yet elucidated, a rational approach would be to determine the CMV serology (IgG) when prescribing steroids in UC[31]. If steroid-refractoriness develops, rectal biopsies should be conducted only in seropositive patients, who are the ones who are at risk of developing CMV disease[31]. A detection of CMV (HE, IHC or PCR) in the inflamed mucosa should imply that standard therapies alone will not control intestinal inflammation, and make mandatory to consider alternative therapies[43], including CMV treatment[30]. If detection methods are not readily available, empiric therapy of IgG positive individuals with steroid-refractory disease is probably justified.

An original study recommended only antiviral treatment in severe UC with large ulcers (> 5 mm) and positive PCR in colon, all patients with positive PCR and no large ulcers in endoscopy responded to conventional therapy[104].

CMV colitis is typically treated with ganciclovir, although other antivirals have been used as foscarnet, valganciclovir and cidofovir[105-107]. Ganciclovir has a poor oral bioavailability so initially it has to be administered intravenously[105,106]. The recommended dosage is 5 mg/kg twice daily for at least 3 wk[107]. After some days of initial treatment the patient can switched to oral ganciclovir (1 g three times daily), although its value is uncertain. Treatment benefits must outweigh the risks associated with the medications used, as this drug can trigger significant adverse effects such as bone marrow suppression, particularly neutropenia[105]. Other side effects include headaches, somnolence, psychosis, elevated transaminases, fever and rash[105]. Foscarnet can be used when ganciclovir is contraindicated (90 mg/kg intravenously twice daily for 3-6 wk)[107]. Its main adverse effect is nephrotoxicity.

Response rate to antiviral therapy is 72% (range 50% to 83%)[31,41,43,75], but these data should be interpreted with caution because "response" was not uniformly defined in these studies and patients were treated concomitantly with immunosuppressants[30], although some authors decreased the dose of immunosuppressive drug[75]. Other authors consider to increase immunosuppressive therapies (other than corticosteroids) if CMV-DNA in colonic mucosa is detected, because a positive finding of CMV-DNA in inflamed colonic mucosa of patients with refractory UC suggests uncontrolled intestinal inflammation[96]. Antiviral treatment should be considerate in disseminated disease[108] which has very poor prognosis.

ECCO guides[62] and American College of Gastroenterology[63] recommend treatment when CMV is detected in colon tissue. Immunosuppressive therapy is stopped only in case of severe systemic CMV reactivation[62].

A multicenter randomized controlled trial with a large number of patients, using homogeneous diagnostic methods, including endoscopic evaluation, would be required to determine in which subgroup of patients early antiviral treatment would be useful[23,96].

REFERENCES

1 **Alford CA**, Stagno S, Pass RF, Britt WJ. Congenital and perinatal cytomegalovirus infections. *Rev Infect Dis* 1993; **12** Suppl 7: S745-S753 [PMID: 2173104 DOI: 10.1093/clinids/12.Supplement_7.S745]

2 **Nelson MR**, Erskine D, Hawkins DA, Gazzard BG. Treatment with corticosteroids--a risk factor for the development of clinical cytomegalovirus disease in AIDS. *AIDS* 1993; **7**: 375-378 [PMID: 8097096 DOI: d10.1097/00002030-199303000-00011]

3 **Ayre K**, Warren BF, Jeffery K, Travis SP. The role of CMV in steroid-resistant ulcerative colitis: A systematic review. *J Crohns Colitis* 2009; **3**: 141-148 [PMID: 21172262 DOI: 10.1016/j.crohns.2009.03.002]

4 **Krech U**. Complement-fixing antibodies against cytomegalovirus in different parts of the world. *Bull World Health Organ* 1973; **49**: 103-106 [PMID: 4363395]

5 **Staras SA**, Dollard SC, Radford KW, Flanders WD, Pass RF, Cannon MJ. Seroprevalence of cytomegalovirus infection in the United States, 1988-1994. *Clin Infect Dis* 2006; **43**: 1143-1151 [PMID: 17029132 DOI: 10.1086/508173]

6 **Zhang LJ**, Hanff P, Rutherford C, Churchill WH, Crumpacker CS. Detection of human cytomegalovirus DNA, RNA, and antibody in normal donor blood. *J Infect Dis* 1995; **171**: 1002-1006 [PMID: 7706776 DOI: 10.1093/infdis/171.4.1002]

7 **Colugnati FA**, Staras SA, Dollard SC, Cannon MJ. Incidence of cytomegalovirus infection among the general population and pregnant women in the United States. *BMC Infect Dis* 2007; **7**: 71 [PMID: 17605813 DOI: 10.1186/1471-2334-7-71]

8 **Mcgavran MH**, Smith MG. Ultrastructural, cytochemical, and microchemical observations on cytomegalovirus (salivary gland virus) infection of human cells in tissue culture. *Exp Mol Pathol* 1965; **76**: 1-10 [PMID: 14297550 DOI: 10.1016/0014-4800(65)90019-5]

9 **Pass RF**. Epidemiology and transmission of cytomegalovirus. *J Infect Dis* 1985; **152**: 243-248 [PMID: 2993429 DOI: 10.1093/infdis/152.2.243]

10 **de la Hoz RE**, Stephens G, Sherlock C. Diagnosis and treatment approaches of CMV infections in adult patients. *J Clin Virol* 2002; **25** Suppl 2: S1-12 [PMID: 12361752 DOI: 10.1016/S1386-6532(02)00091-4]

11 **Crough T**, Khanna R. Immunobiology of human cytomegalovirus: from bench to bedside. *Clin Microbiol Rev* 2009; **22**: 76-98 [PMID: 19136435 DOI: 10.1128/CMR.00034-08]

12 **Grefte A**, van der Giessen M, van Son W, The TH. Circulating cytomegalovirus (CMV)-infected endothelial cells in patients with an active CMV infection. *J Infect Dis* 1993; **167**: 270-277 [PMID: 8380609 DOI: 10.1093/infdis/167.2.270]

13 **Jacobson MA**, Mills J. Serious cytomegalovirus disease in the acquired immunodeficiency syndrome (AIDS). Clinical findings, diagnosis, and treatment. *Ann Intern Med* 1988; **108**: 585-594 [PMID: 2831765 DOI: 10.7326/0003-4819-108-4-585]

14 **Macdonald JC**, Torriani FJ, Morse LS, Karavellas MP, Reed JB, Freeman WR. Lack of reactivation of cytomegalovirus (CMV) retinitis after stopping CMV maintenance therapy in AIDS patients with sustained elevations in CD4 T cells in response to highly active antiretroviral therapy. *J Infect Dis* 1998; **177**: 1182-1187 [PMID: 9593001 DOI: 10.1086/515281]

15 **Ho M**. Virus infections after transplantation in man. Brief review. *Arch Virol* 1977; **55**: 1-24 [PMID: 200197 DOI: 10.1007/BF01314475]

16 **Van der Giessen M**, van den Berg AP, van der Bij W, Postma S, van Son WJ, The TH. Quantitative measurement of cytomegalovirus-specific IgG and IgM antibodies in relation to cytomegalovirus antigenaemia and disease activity in kidney recipients with an active cytomegalovirus infection. *Clin Exp Immunol* 1990; **80**: 56-61 [PMID: 2157567 DOI: 10.1111/j.1365-2249.1990.tb06441.x]

17 **Revello MG**, Zavattoni M, Furione M, Lilleri D, Gorini G, Gerna G. Diagnosis and outcome of preconceptional and periconceptional primary human cytomegalovirus infections. *J Infect Dis* 2002; **186**: 553-557 [PMID: 12195384 DOI: 10.1086/341831]

18 **Nagington J**. Cytomegalovirus antibody production in renal transplant patients. *J Hyg* (Lond) 1971; **69**: 645-660 [PMID: 4332511 DOI: 10.1017/S0022172400021926]

19 **Rawlinson WD**. Broadsheet. Number 50: Diagnosis of human cytomegalovirus infection and disease. *Pathology* 1999; **31**: 109-115 [PMID: 10399165 DOI: 10.1080/003130299105287]

20 **Moonka D**, Furth EE, MacDermott RP, Lichtenstein GR. Pouchitis associated with primary cytomegalovirus infection. *Am J Gastroenterol* 1998; **93**: 264-266 [PMID: 9468256 DOI: 10.1016/S0002-9270(97)00068-3]

21 **Casadesus D**, Tani T, Wakai T, Maruyama S, Iiai T, Okamoto H, Hatakeyama K. Possible role of human cytomegalovirus in pouchitis after proctocolectomy with ileal pouch-anal anastomosis in patients with ulcerative colitis. *World J Gastroenterol* 2007; **13**: 1085-1089 [PMID: 17373744 DOI: 1007-9327/13/1085.asp]

22 **Muñoz-Juarez M**, Pemberton JH, Sandborn WJ, Tremaine WJ, Dozois RR. Misdiagnosis of specific cytomegalovirus infection of the ileoanal pouch as refractory idiopathic chronic pouchitis: report of two cases. *Dis Colon Rectum* 1999; **42**: 117-120 [PMID: 10211531 DOI: 10.1007/BF02235196]

23 **Criscuoli V**, Rizzuto MR, Cottone M. Cytomegalovirus and inflammatory bowel disease: is there a link? *World J Gastroenterol* 2006; **12**: 4813-4818 [PMID: 16937462 DOI: 1007-9327/12/4813.asp]

24 **Criscuoli V**, Rizzuto MR, Montalbano L, Gallo E, Cottone M. Natural history of cytomegalovirus infection in a series of patients diagnosed with moderate-severe ulcerative colitis. *World J Gastroenterol* 2011; **17**: 633-638 [PMID: 21350712 DOI: 10.3748/wjg.v17.i5.633]

25 **Galiatsatos P**, Shrier I, Lamoureux E, Szilagyi A. Meta-analysis of outcome of cytomegalovirus colitis in immunocompetent hosts. *Dig Dis Sci* 2005; **50**: 609-616 [PMID: 15844689 DOI: 10.1007/s10620-005-2544-6]

26 **Ng FH**, Chau TN, Cheung TC, Kng C, Wong SY, Ng WF, Lee KC, Chan E, Lai ST, Yuen WC, Chang CM. Cytomegalovirus colitis in individuals without apparent cause of immunodeficiency. *Dig Dis Sci* 1999; **44**: 945-952 [PMID: 10235602]

27 **Kim CH**, Bahng S, Kang KJ, Ku BH, Jo YC, Kim JY, Chang DK, Son HJ, Rhee PL, Kim JJ, Rhee JC, Kim YH. Cytomegalovirus colitis in patients without inflammatory bowel disease: a single center study. *Scand J Gastroenterol* 2010; **45**: 1295-1301 [PMID: 20568970]

28 **Powell RD**, Warner NE, Levine RS, Kirsner JB. Cytomegalic inclusion disease and ulcerative colitis; report of a case in a young adult. *Am J Med* 1961; **30**: 334-340 [PMID: 13737621 DOI: 10.1016/0002-9343(61)90105-X]

29 **Wakefield AJ**, Fox JD, Sawyerr AM, Taylor JE, Sweenie CH, Smith M, Emery VC, Hudson M, Tedder RS, Pounder RE. Detection of herpesvirus DNA in the large intestine of patients with ulcerative colitis and Crohn's disease using the nested polymerase chain reaction. *J Med Virol* 1992; **38**: 183-190 [PMID: 1287131 DOI: 10.1002/jmv.1890380306]

30 **Lawlor G**, Moss AC. Cytomegalovirus in inflammatory bowel disease: pathogen or innocent bystander? *Inflamm Bowel Dis* 2010; **16**: 1620-1627 [PMID: 20232408 DOI: 10.1002/ibd.21275]

31 **Domènech E**, Vega R, Ojanguren I, Hernández A, Garcia-Planella E, Bernal I, Rosinach M, Boix J, Cabré E, Gassull MA. Cytomegalovirus infection in ulcerative colitis: a prospective, comparative study on prevalence and diagnostic strategy. *Inflamm Bowel Dis* 2008; **14**: 1373-1379 [PMID: 18452205 DOI: 10.1002/ibd.20498]

32 **Boivin G**, Handfield J, Toma E, Murray G, Lalonde R, Tevere VJ, Sun R, Bergeron MG. Evaluation of the AMPLICOR cytomegalovirus test with specimens from human immunodeficiency virus-infected subjects. *J Clin Microbiol* 1998; **36**: 2509-2513 [PMID: 9705384]

33 **Lazzarotto T**, Dal Monte P, Landini MP. Recent advances in the diagnosis of cytomegalovirus infection. *Ann Biol Clin* (Paris) 1996; **54**: 259-265 [PMID: 8949423 DOI: 10.1016/j.humimm.2004.02.006]

34 **Matsuoka K**, Iwao Y, Mori T, Sakuraba A, Yajima T, Hisamatsu T, Okamoto S, Morohoshi Y, Izumiya M, Ichikawa H, Sato T, Inoue N, Ogata H, Hibi T. Cytomegalovirus is frequently reactivated and disappears without antiviral agents in ulcerative colitis patients. *Am J Gastroenterol* 2007; **102**: 331-337 [PMID: 17156136 DOI: 10.1111/j.1572-0241.2006.00989.x]

35 **Nakase H**, Matsumura K, Yoshino T, Chiba T. Systematic review: cytomegalovirus infection in inflammatory bowel disease. *J Gastroenterol* 2008; **43**: 735-740 [PMID: 18958541 DOI: 10.1007/s00535-008-2246-x]

36 **Michaelides A**, Liolios L, Glare EM, Spelman DW, Bailey MJ, Walters EH, Williams TJ, Snell GI, Kotsimbos TC. Increased human cytomegalovirus (HCMV) DNA load in peripheral blood leukocytes after lung transplantation correlates with HCMV pneumonitis. *Transplantation* 2001; **72**: 141-147 [PMID: 11468549]

37 **Rowshani AT**, Bemelman FJ, van Leeuwen EM, van Lier RA, ten Berge IJ. Clinical and immunologic aspects of cytomegalovirus infection in solid organ transplant recipients. *Transplantation* 2005; **79**: 381-386 [PMID: 15729162 DOI: 10.1097/01.TP.0000148239.00384.F0]

38 **Gerna G**, Lilleri D. Monitoring transplant patients for human cytomegalovirus: Diagnostic update. *Herpes* 2006; **13**: 4-11 [PMID: 16732996 DOI: 10.1111/j.1600-6143.2006.01488.x]

39 **Roberts TC**, Brennan DC, Buller RS, Gaudreault-Keener M, Schnitzler MA, Sternhell KE, Garlock KA, Singer GG, Storch GA. Quantitative polymerase chain reaction to predict occurrence of symptomatic cytomegalovirus infection and assess response to ganciclovir therapy in renal transplant recipients. *J Infect Dis* 1998; **178**: 626-635 [PMID: 9728529 DOI: 10.1086/515383]

40 **Einsele H**, Ehninger G, Steidle M, Vallbracht A, Müller M, Schmidt H, Saal JG, Waller HD, Müller CA. Polymerase chain reaction to evaluate antiviral therapy for cytomegalovirus disease. *Lancet* 1991; **338**: 1170-1172 [PMID: 1682592 DOI: 10.1016/0140-6736(91)92032-W]

41 **Cottone M**, Pietrosi G, Martorana G, Casà A, Pecoraro G,

Oliva L, Orlando A, Rosselli M, Rizzo A, Pagliaro L. Prevalence of cytomegalovirus infection in severe refractory ulcerative and Crohn's colitis. *Am J Gastroenterol* 2001; **96**: 773-775 [PMID: 11280549 DOI: 10.1016/S0002-9270(00)02413-8]

42 **Criscuoli V**, Casà A, Orlando A, Pecoraro G, Oliva L, Traina M, Rizzo A, Cottone M. Severe acute colitis associated with CMV: a prevalence study. *Dig Liver Dis* 2004; **36**: 818-820 [PMID: 15646428 DOI: 10.1016/j.dld.2004.05.013]

43 **Yoshino T**, Nakase H, Ueno S, Uza N, Inoue S, Mikami S, Matsuura M, Ohmori K, Sakurai T, Nagayama S, Hasegawa S, Sakai Y, Chiba T. Usefulness of quantitative real-time PCR assay for early detection of cytomegalovirus infection in patients with ulcerative colitis refractory to immunosuppressive therapies. *Inflamm Bowel Dis* 2007; **13**: 1516-1521 [PMID: 17828781 DOI: 10.1002/ibd.20253]

44 **Boom R**, Sol C, Weel J, Lettinga K, Gerrits Y, van Breda A, Wertheim-Van Dillen P. Detection and quantitation of human cytomegalovirus DNA in faeces. *J Virol Methods* 2000; **84**: 1-14 [PMID: 10644082 DOI: 10.1016/S0166-0934(99)00127-5]

45 **Michel D**, Marre E, Hampl W, Roczkos J, Müller S, Hertenstein B, Kern P, Heymer B, Salzberger B, Arasteh K. Intestinal cytomegalovirus disease in immunocompromised patients may be ruled out by search for cytomegalovirus DNA in stool samples. *J Clin Microbiol* 1995; **33**: 3064-3067 [PMID: 8576379]

46 **Wu GD**, Shintaku IP, Chien K, Geller SA. A comparison of routine light microscopy, immunohistochemistry, and in situ hybridization for the detection of cytomegalovirus in gastrointestinal biopsies. *Am J Gastroenterol* 1989; **84**: 1517-1520 [PMID: 2556912]

47 **Cotte L**, Drouet E, Bissuel F, Denoyel GA, Trepo C. Diagnostic value of amplification of human cytomegalovirus DNA from gastrointestinal biopsies from human immunodeficiency virus-infected patients. *J Clin Microbiol* 1993; **31**: 2066-2069 [PMID: 8396587]

48 **Dieterich DT**, Rahmin M. Cytomegalovirus colitis in AIDS: presentation in 44 patients and a review of the literature. *J Acquir Immune Defic Syndr* 1991; **4** Suppl 1: S29-S35 [PMID: 1848619]

49 **Franzin G**, Muolo A, Griminelli T. Cytomegalovirus inclusions in the gastroduodenal mucosa of patients after renal transplantation. *Gut* 1981; **22**: 698-701 [PMID: 6271652 DOI: 10.1136/gut.22.9.698]

50 **Beaugerie L**, Cywiner-Golenzer C, Monfort L, Girard PM, Carbonnel F, Ngô Y, Cosnes J, Rozenbaum W, Nicolas JC, Châtelet FP, Gendre JP. Definition and diagnosis of cytomegalovirus colitis in patients infected by human immunodeficiency virus. *J Acquir Immune Defic Syndr Hum Retrovirol* 1997; **14**: 423-429 [PMID: 9170416 DOI: 10.1097/00042560-199704150-00005]

51 **Kandiel A**, Lashner B. Cytomegalovirus colitis complicating inflammatory bowel disease. *Am J Gastroenterol* 2006; **101**: 2857-2865 [PMID: 17026558 DOI: 10.1111/j.1572-0241.2006.00869.x]

52 **Rahbar A**, Boström L, Lagerstedt U, Magnusson I, Söderberg-Naucler C, Sundqvist VA. Evidence of active cytomegalovirus infection and increased production of IL-6 in tissue specimens obtained from patients with inflammatory bowel diseases. *Inflamm Bowel Dis* 2003; **9**: 154-161 [PMID: 12792220 DOI: 10.1097/00054725-200305000-00002]

53 **Robey SS**, Gage WR, Kuhajda FP. Comparison of immunoperoxidase and DNA in situ hybridization techniques in the diagnosis of cytomegalovirus colitis. *Am J Clin Pathol* 1988; **89**: 666-671 [PMID: 2833851]

54 **Roblin X**, Pillet S, Oussalah A, Berthelot P, Del Tedesco E, Phelip JM, Chambonnière ML, Garraud O, Peyrin-Biroulet L, Pozzetto B. Cytomegalovirus load in inflamed intestinal tissue is predictive of resistance to immunosuppressive therapy in ulcerative colitis. *Am J Gastroenterol* 2011; **106**: 2001-2008 [PMID: 21788989 DOI: 10.1038/ajg.2011.202]

55 **Kou T**, Nakase H, Tamaki H, Kudo T, Nishio A, Chiba T. Cytomegalovirus infection in patients with ulcerative colitis diagnosed by quantitative real-time PCR analysis. *Dig Dis Sci* 2006; **51**: 1052-1055 [PMID: 16865568 DOI: 10.1007/s10620-006-8006-y]

56 **Dimitroulia E**, Spanakis N, Konstantinidou AE, Legakis NJ, Tsakris A. Frequent detection of cytomegalovirus in the intestine of patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2006; **12**: 879-884 [PMID: 16954807 DOI: 10.1097/01.mib.0000231576.11678.57]

57 **Landry ML**, Ferguson D. Comparison of quantitative cytomegalovirus antigenemia assay with culture methods and correlation with clinical disease. *J Clin Microbiol* 1993; **31**: 2851-2856 [PMID: 8263166]

58 **Ljungman P**, Griffiths P, Paya C. Definitions of cytomegalovirus infection and disease in transplant recipients. *Clin Infect Dis* 2002; **34**: 1094-1097 [PMID: 11914998 DOI: 10.1086/339329]

59 **Sakamoto I**, Shirai T, Kamide T, Igarashi M, Koike J, Ito A, Takagi A, Miwa T, Kajiwara H. Cytomegalovirus enterocolitis in an immunocompetent individual. *J Clin Gastroenterol* 2002; **34**: 243-246 [PMID: 11873105 DOI: 10.1097/00004836-200203000-00010]

60 **Wada Y**, Matsui T, Matake H, Sakurai T, Yamamoto J, Kikuchi Y, Yorioka M, Tsuda S, Yao T, Yao S, Haraoka S, Iwashita A. Intractable ulcerative colitis caused by cytomegalovirus infection: a prospective study on prevalence, diagnosis, and treatment. *Dis Colon Rectum* 2003; **46**: S59-S65 [PMID: 14530660 DOI: 10.1097/01.DCR.0000087486.21981.C6]

61 **Suzuki H**, Kato J, Kuriyama M, Hiraoka S, Kuwaki K, Yamamoto K. Specific endoscopic features of ulcerative colitis complicated by cytomegalovirus infection. *World J Gastroenterol* 2010; **16**: 1245-1251 [PMID: 20222169 DOI: 10.3748/wjg.v16.i10.1245]

62 **Rahier JF**, Ben-Horin S, Chowers Y, Conlon C, De Munter P, D'Haens G, Domènech E, Eliakim R, Eser A, Frater J, Gassull M, Giladi M, Kaser A, Lémann M, Moreels T, Moschen A, Pollok R, Reinisch W, Schunter M, Stange EF, Tilg H, Van Assche G, Viget N, Vucelic B, Walsh A, Weiss G, Yazdanpanah Y, Zabana Y, Travis SP, Colombel JF. European evidence-based Consensus on the prevention, diagnosis and management of opportunistic infections in inflammatory bowel disease. *J Crohns Colitis* 2009; **3**: 47-91 [PMID: 21172250 DOI: 10.1016/j.crohns.2009.02.010]

63 **Kornbluth A**, Sachar DB. Ulcerative colitis practice guidelines in adults (update): American College of Gastroenterology, Practice Parameters Committee. *Am J Gastroenterol* 2004; **99**: 1371-1385 [PMID: 15233681 DOI: 10.1111/j.1572-0241.2004.40036.x]

64 **Hommes DW**, Sterringa G, Boom R. Incidence and outcome of cytomegalovirus infection in patients with inflammatory bowel disease. *Gastroenterology* 2002; **122**: A1287

65 **D'Ovidio V**, Vernia P, Gentile G, Capobianchi A, Marcheggiano A, Viscido A, Martino P, Caprilli R. Cytomegalovirus infection in inflammatory bowel disease patients undergoing anti-TNFalpha therapy. *J Clin Virol* 2008; **43**: 180-183 [PMID: 18614396 DOI: 10.1016/j.jcv.2008.06.002]

66 **Orvar K**, Murray J, Carmen G, Conklin J. Cytomegalovirus infection associated with onset of inflammatory bowel disease. *Dig Dis Sci* 1993; **38**: 2307-2310 [PMID: 8261839 DOI: 10.1007/BF01299914]

67 **Knösel T**, Schewe C, Petersen N, Dietel M, Petersen I. Prevalence of infectious pathogens in Crohn's disease. *Pathol Res Pract* 2009; **205**: 223-230 [PMID: 19186006 DOI: 10.1016/j.prp.2008.04.018]

68 **Takahashi Y**, Tange T. Prevalence of cytomegalovirus infection in inflammatory bowel disease patients. *Dis Colon Rectum* 2004; **47**: 722-726 [PMID: 15037934 DOI: 10.1007/

s10350-003-0117-3]
69 **Kaufman HS**, Kahn AC, Iacobuzio-Donahue C, Talamini MA, Lillemoe KD, Hamilton SR. Cytomegaloviral enterocolitis: clinical associations and outcome. *Dis Colon Rectum* 1999; **42**: 24-30 [PMID: 10211516 DOI: 10.1007/BF02235178]
70 **Nakase H**, Yoshino T, Honzawa Y, Chiba T. Low prevalence of CMV infection in patients with Crohn's disease in comparison with ulcerative colitis: effect of different immune response on prevalence of CMV infection. *Dig Dis Sci* 2010; **55**: 1498-1499 [PMID: 20198427 DOI: 10.1007/s10620-010-1162-0]
71 **Fuss IJ**, Neurath M, Boirivant M, Klein JS, de la Motte C, Strong SA, Fiocchi C, Strober W. Disparate CD4+ lamina propria (LP) lymphokine secretion profiles in inflammatory bowel disease. Crohn's disease LP cells manifest increased secretion of IFN-gamma, whereas ulcerative colitis LP cells manifest increased secretion of IL-5. *J Immunol* 1996; **157**: 1261-1270 [PMID: 8757634]
72 **Kojima T**, Watanabe T, Hata K, Shinozaki M, Yokoyama T, Nagawa H. Cytomegalovirus infection in ulcerative colitis. *Scand J Gastroenterol* 2006; **41**: 706-711 [PMID: 16716970 DOI: 10.1080/00365520500408584]
73 **Kishore J**, Ghoshal U, Ghoshal UC, Krishnani N, Kumar S, Singh M, Ayyagari A. Infection with cytomegalovirus in patients with inflammatory bowel disease: prevalence, clinical significance and outcome. *J Med Microbiol* 2004; **53**: 1155-1160 [PMID: 15496396 DOI: 10.1099/jmm.0.45629-0]
74 **Vega R**, Bertrán X, Menacho M, Domènech E, Moreno de Vega V, Hombrados M, Cabré E, Ojanguren I, Gassull MA. Cytomegalovirus infection in patients with inflammatory bowel disease. *Am J Gastroenterol* 1999; **94**: 1053-1056 [PMID: 10201482 DOI: 10.1016/S0002-9270(99)00068-4]
75 **Papadakis KA**, Tung JK, Binder SW, Kam LY, Abreu MT, Targan SR, Vasiliauskas EA. Outcome of cytomegalovirus infections in patients with inflammatory bowel disease. *Am J Gastroenterol* 2001; **96**: 2137-2142 [PMID: 11467645 DOI: 10.1016/S0002-9270(01)02512-6]
76 **Kambham N**, Vij R, Cartwright CA, Longacre T. Cytomegalovirus infection in steroid-refractory ulcerative colitis: a case-control study. *Am J Surg Pathol* 2004; **28**: 365-373 [PMID: 15104299 DOI: 10.1097/00000478-200403000-00009]
77 **Maconi G**, Colombo E, Zerbi P, Sampietro GM, Fociani P, Bosani M, Cassinotti A, Casini V, Russo A, Ardizzone S, Porta M, Bianchi Porro G. Prevalence, detection rate and outcome of cytomegalovirus infection in ulcerative colitis patients requiring colonic resection. *Dig Liver Dis* 2005; **37**: 418-423 [PMID: 15893280 DOI: 10.1016/j.dld.2005.01.011]
78 **Pofelski J**, Heluwaert F, Roblin X, Morand P, Gratacap B, Germain E, Brion JP, Salon C, Bonaz B. Cytomegalovirus and cryptogenic inflammatory bowel disease. *Gastroenterol Clin Biol* 2007; **31**: 292-296 [PMID: 17396088 DOI: GCB-03-2007-31-3-0399-8320-101019-200520007]
79 **Mendez JC**, Espy MJ, Smith TF, Wilson JA, Paya CV. Evaluation of PCR primers for early diagnosis of cytomegalovirus infection following liver transplantation. *J Clin Microbiol* 1998; **36**: 526-530 [PMID: 9466770 DOI: 0095-1137/98/$04.0010]
80 **Eyre-Brook IA**, Dundas S. Incidence and clinical significance of colonic cytomegalovirus infection in idiopathic inflammatory bowel disease requiring colectomy. *Gut* 1986; **27**: 1419-1425 [PMID: 3026932 DOI: 10.1136/gut.27.12.1419]
81 **Cooper HS**, Raffensperger EC, Jonas L, Fitts WT. Cytomegalovirus inclusions in patients with ulcerative colitis and toxic dilation requiring colonic resection. *Gastroenterology* 1977; **72**: 1253-1256 [PMID: 192627]
82 **Alcalá MJ**, Casellas F, Pallarés J, de Torres I, Malagelada JR. Infection by cytomegalovirus in patients with ulcerative colitis requiring colonic resection. *Med Clin* (Barc) 2000; **114**: 201-204 [PMID: 10757099]
83 **Targan SR**, Karp LC. Defects in mucosal immunity leading to ulcerative colitis. *Immunol Rev* 2005; **206**: 296-305 [PMID: 16048556 DOI: 10.1111/j.0105-2896.2005.00286.x]
84 **Ginsburg CH**, Dambrauskas JT, Ault KA, Falchuk ZM. Impaired natural killer cell activity in patients with inflammatory bowel disease: evidence for a qualitative defect. *Gastroenterology* 1983; **85**: 846-851 [PMID: 6884709]
85 **de Saussure P**, Lavergne-Slove A, Mazeron MC, Alain S, Matuchansky C, Bouhnik Y. A prospective assessment of cytomegalovirus infection in active inflammatory bowel disease. *Aliment Pharmacol Ther* 2004; **20**: 1323-1327 [PMID: 15606394 DOI: 10.1111/j.1365-2036.2004.02273.x]
86 **Widmann T**, Sester U, Gärtner BC, Schubert J, Pfreundschuh M, Köhler H, Sester M. Levels of CMV specific CD4 T cells are dynamic and correlate with CMV viremia after allogeneic stem cell transplantation. *PLoS One* 2008; **3**: e3634 [PMID: 18982061 DOI: 10.1371/journal.pone.0003634]
87 **Forbes BA**, Bonville CA, Dock NL. The effects of a promoter of cell differentiation and selected hormones on human cytomegalovirus infection using an in vitro cell system. *J Infect Dis* 1990; **162**: 39-45 [PMID: 1693943 DOI: 10.1093/infdis/162.1.39]
88 **Lavagna A**, Bergallo M, Daperno M, Sostegni R, Costa C, Leto R, Crocellà L, Molinaro G, Rocca R, Cavallo R, Pera A. Infliximab and the risk of latent viruses reactivation in active Crohn's disease. *Inflamm Bowel Dis* 2007; **13**: 896-902 [PMID: 17345605 DOI: 10.1002/ibd.20131]
89 **Adler SP**, Hempfling SH, Starr SE, Plotkin SA, Riddell S. Safety and immunogenicity of the Towne strain cytomegalovirus vaccine. *Pediatr Infect Dis J* 1998; **17**: 200-206 [PMID: 9535246 DOI: 10.1097/00006454-199803000-00006]
90 **Nakase H**, Yamamoto S, Matsuura M, Honzawa Y, Chiba T. Cytomegalovirus affects clinical outcome of infliximab in ulcerative colitis refractory to tacrolimus. *Aliment Pharmacol Ther* 2010; **32**: 510-511 [PMID: 20636704 DOI: 10.1111/j.1365-2036.2010.04372.x]
91 **Hahn G**, Jores R, Mocarski ES. Cytomegalovirus remains latent in a common precursor of dendritic and myeloid cells. *Proc Natl Acad Sci USA* 1998; **95**: 3937-3942 [PMID: 9520471 DOI: 10.1073/pnas.95.7.3937]
92 **Söderberg-Nauclér C**, Fish KN, Nelson JA. Interferon-gamma and tumor necrosis factor-alpha specifically induce formation of cytomegalovirus-permissive monocyte-derived macrophages that are refractory to the antiviral activity of these cytokines. *J Clin Invest* 1997; **100**: 3154-3163 [PMID: 9399963 DOI: 10.1172/JCI119871]
93 **Simon CO**, Seckert CK, Dreis D, Reddehase MJ, Grzimek NK. Role for tumor necrosis factor alpha in murine cytomegalovirus transcriptional reactivation in latently infected lungs. *J Virol* 2005; **79**: 326-340 [PMID: 15596827 DOI: 10.1128/JVI.79.1.326-340.2005]
94 **Hommes DW**, Sterringa G, van Deventer SJ, Tytgat GN, Weel J. The pathogenicity of cytomegalovirus in inflammatory bowel disease: a systematic review and evidence-based recommendations for future research. *Inflamm Bowel Dis* 2004; **10**: 245-250 [PMID: 15290919 DOI: 10.1097/00054725-200405000-00011]
95 **Söderberg-Nauclér C**, Nelson JY. Human cytomegalovirus latency and reactivation - a delicate balance between the virus and its host's immune system. *Intervirology* 1999; **42**: 314-321 [PMID: 10702712]
96 **Nakase H**, Yoshino T, Matumura K, Honzawa Y, Yamamoto S, Matsuura M, Chiba T. Positive finding of colonic polymerase chain reaction for cytomegalovirus DNA is not false positive but a warning for treating patients with ulcerative colitis refractory to immunosuppressive therapies. *Inflamm Bowel Dis* 2011; **17**: E13-E14 [PMID: 20629104 DOI: 10.1002/ibd.21401]
97 **Diepersloot RJ**, Kroes AC, Visser W, Jiwa NM, Rothbarth PH. Acute ulcerative proctocolitis associated with primary

cytomegalovirus infection. *Arch Intern Med* 1990; **150**: 1749-1751 [PMID: 2166491 DOI: 10.1001/archinte.150.8.1749]
98 **Rachima C**, Maoz E, Apter S, Thaler M, Grossman E, Rosenthal T. Cytomegalovirus infection associated with ulcerative colitis in immunocompetent individuals. *Postgrad Med J* 1998; **74**: 486-489 [PMID: 9926125 DOI: 10.1136/pgmj.74.874.486]
99 **Streetz KL**, Buhr T, Wedemeyer H, Bleck J, Schedel I, Manns MP, Göke MN. Acute CMV-colitis in a patient with a history of ulcerative colitis. *Scand J Gastroenterol* 2003; **38**: 119-122 [PMID: 12608474 DOI: 10.1080/00365520310000546]
100 **Helbling D**, Breitbach TH, Krause M. Disseminated cytomegalovirus infection in Crohn's disease following antitumour necrosis factor therapy. *Eur J Gastroenterol Hepatol* 2002; **14**: 1393-1395 [PMID: 12468964 DOI: 10.1097/00042737-200212000-00018]
101 **Farmer GW**, Vincent MM, Fuccillo DA, Horta-Barbosa L, Ritman S, Sever JL, Gitnick GL. Viral investigations in ulcerative colitis and regional enteritis. *Gastroenterology* 1973; **65**: 8-18 [PMID: 4352745]
102 **Minami M**, Ohta M, Ohkura T, Ando T, Ohmiya N, Niwa Y, Goto H. Cytomegalovirus infection in severe ulcerative colitis patients undergoing continuous intravenous cyclosporine treatment in Japan. *World J Gastroenterol* 2007; **13**: 754-760 [PMID: 17278199]
103 **Goodrich J**, Khardori N. Cytomegalovirus: the taming of the beast? *Lancet* 1997; **350**: 1718-1719 [PMID: 9413457 DOI: 10.1016/S0140-6736(97)22050-7]
104 **Omiya M**, Matsushita M, Tanaka T, Kawamata S, Okazaki K. The absence of large ulcer predicts latent cytomegalovirus infection in ulcerative colitis with positive mucosal viral assay. *Intern Med* 2010; **49**: 2277-2282 [PMID: 21048360 DOI: 10.2169/internalmedicine.49.3657]
105 **Faulds D**, Heel RC. Ganciclovir. A review of its antiviral activity, pharmacokinetic properties and therapeutic efficacy in cytomegalovirus infections. *Drugs* 1990; **39**: 597-638 [PMID: 2161731 DOI: 10.2165/00003495-199039040-00008]
106 **Markham A**, Faulds D. Ganciclovir. An update of its therapeutic use in cytomegalovirus infection. *Drugs* 1994; **48**: 455-484 [PMID: 7527763 DOI: 10.2165/00003495-199448030-00009]
107 **Heuman DM**. Cytomegalovirus colitis. Available from: URL: http://emedicine.medscape.com/article/173151-overview
108 **Eddleston M**, Peacock S, Juniper M, Warrell DA. Severe cytomegalovirus infection in immunocompetent patients. *Clin Infect Dis* 1997; **24**: 52-56 [PMID: 8994755 DOI: 10.1093/clinids/24.1.52]

P- Reviewers Naito Y, Abraham P **S- Editor** Gou SX
L- Editor A **E- Editor** Li JY



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i1.26

World J Gastroenterol 2013 January 7; 19(1): 26-34
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

REVIEW

Current pathogenetic aspects of hepatic encephalopathy and noncirrhotic hyperammonemic encephalopathy

Halina Cichoż-Lach, Agata Michalak

Halina Cichoż-Lach, Department of Gastroenterology, Medical University of Lublin, 20094 Lublin, Poland
Agata Michalak, 2nd Faculty of Medicine, Medical University of Lublin, 20059 Lublin, Poland
Author contributions: Cichoż-Lach H and Michalak A contributed equally to this work, they both made substantial contributions to the study conception and design, and the text of the paper.
Correspondence to: Halina Cichoż-Lach, Professor, Department of Gastroenterology, Medical University of Lublin, Jaczewski Str. 8, 20094 Lublin, Poland. lach.halina@wp.pl
Telephone: +48-60-1377656 Fax: +48-81-4796135
Received: May 11, 2012 Revised: July 25, 2012
Accepted: July 28, 2012
Published online: January 7, 2013

Abstract

Hepatic encephalopathy is a medical phenomenon that is described as a neuropsychiatric manifestation of chronic or acute liver disease that is characterized by psychomotor, intellectual and cognitive abnormalities with emotional/affective and behavioral disturbances. This article focuses on the underlying mechanisms of the condition and the differences between hepatic encephalopathy and noncirrhotic hyperammonemic encephalopathy. Hepatic encephalopathy is a serious condition that can cause neurological death with brain edema and intracranial hypertension. It is assumed that approximately 60%-80% of patients with liver cirrhosis develop hepatic encephalopathy. This review explores the complex mechanisms that lead to hepatic encephalopathy. However, noncirrhotic hyperammonemic encephalopathy is not associated with hepatic diseases and has a completely different etiology. Noncirrhotic hyperammonemic encephalopathy is a severe occurrence that is connected with multiple pathogeneses.

© 2013 Baishideng. All rights reserved.

Key words: Hepatic encephalopathy; Noncirrhotic hyperammonemic encephalopathy; Pathogeneses; Ammonia; Astrocyte; Oxidative stress

Cichoż-Lach H, Michalak A. Current pathogenetic aspects of hepatic encephalopathy and noncirrhotic hyperammonemic encephalopathy. *World J Gastroenterol* 2013; 19(1): 26-34 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i1/26.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i1.26

HEPATIC ENCEPHALOPATHY

Introduction and division

Hepatic encephalopathy (HE) continues to be a major clinical problem. In subjects suffering from acute/chronic liver failure, HE can lead to coma and death. This medical state causes a broad range of neuropsychiatric disturbances with variable intensities, including poor concentration, psychomotor retardation, decreased reaction time, short-term memory impairment and sensory dysfunction[1,2]. According to the newest data, HE occurs as one of four types. Therefore, this encephalopathy syndrome might be classified into four groups: A, B, C and D[3-5]. Type A HE is inseparably associated with acute liver failure resulting from severe inflammatory and/or necrotic rapid onset liver disease (e.g., paracetamol overdose, excessive alcohol intake or acute fatty liver caused by pregnancy or Reye's syndrome). Type B HE is associated with portosystemic bypass in the absence of parenchymal liver disorders and is mainly connected with congenital abnormalities. Type C HE accompanies chronic liver failure (cirrhosis) and portal hypertension with portosystemic shunts. Type C has three subcategories: episodic (precipitated, spontaneous, or recurrent), persistent (mild, severe, or treatment-dependent) and minimal. In 2009, the International Society for the Study of Hepatic Encephalopathy and Nitrogen Metabolism completed these classifications and added the experimental models

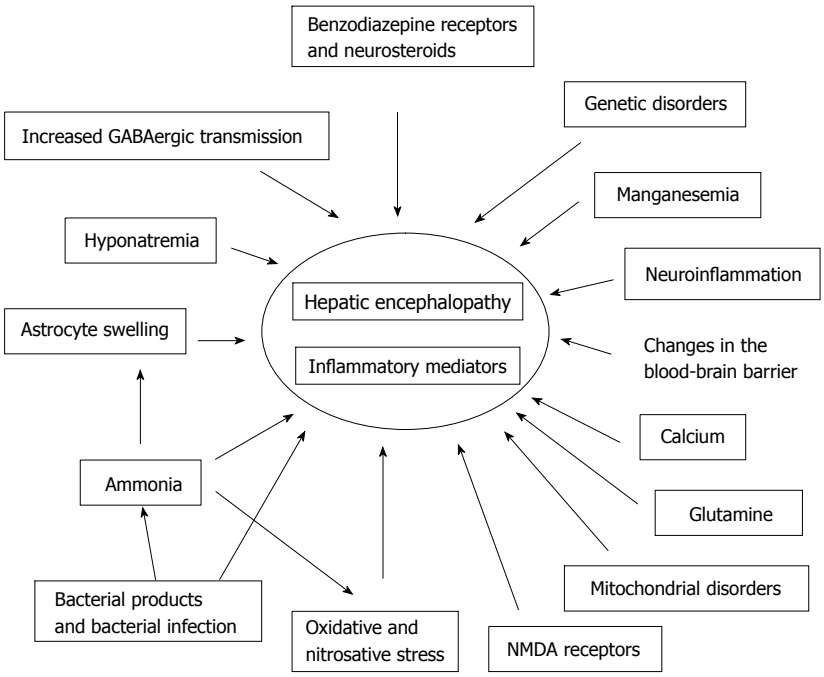


Figure 1 The different factors and mechanisms involved in the pathogenesis of hepatic encephalopathy. GABA: Gamma-aminobutyric acid; NMDA: N-methyl-D-aspartate.

associated with each type[6]. Finally, type D is associated with disorders of the urea cycle. Typically, these syndromes are rare hereditary disorders of urea biosynthesis caused by genetic defects in the urea cycle and have been described for each of the six enzymes that participate in urea formation. They occur mainly in neonates and more rarely in adults[7-16]. Alcohol, viral infections, drugs or toxins and biliary obstruction are several reasons for the development of cirrhosis. Portal hypertension resulting from cirrhosis stimulates the opening of embryonic venous channels. Portal-systemic shunts then allow intestinal toxins (ammonia, manganese and cytokines) to bypass the liver in the systemic circulation. A classification based on HE severity enables the division of HE into two types: (1) minimal hepatic encephalopathy (MHE); and (2) overt hepatic encephalopathy (OHE). MHE affects patients who have normal mental and neurological status; however, abnormalities appear in specific psychometric tests[17-20]. OHE is characterized by neurological and neuropsychiatric disruptions, which can be detected by bedside clinical tests[21].

Pathogenesis

Although the etiology of HE has not been conclusively established, it is widely agreed to be the result of numerous pathological processes. Research on the pathogenesis of HE is still being conducted, and several theories have been reported (Figure 1).

The ammonia hypothesis

The neurotoxicity of ammonia is well recognized and was first thought to play a significant role in the development of HE in the 1890s. In one study, creating a surgical shunt from the portal vein to the vena cava of dogs mitigated their aggression and irritability during the experiment[22]. Similar investigations led to the description of HE. In HE, complications from the hepatic detoxification of ammonia expose the brain to high ammonia concentrations. Consequently, these complications lead to neurotoxicity, which results in neuropsychiatric syndromes and abnormal cerebral blood flow[23,24]. The human body has several sources of ammonia. Primarily, ammonia is created in the colon as a byproduct of proteins and urea, which are metabolized by bacteria. Moreover, ammonia is produced in the small intestine as the result of the bacterial degradation of glutamine (an amino acid)[6,25]. Muscles and kidneys can also produce ammonia. However, ammonia appears to play a negligible role in organs that are beyond the gastrointestinal tract. In HE, the muscles and the kidneys are involved in the metabolism of ammonia to a greater extent than normal. In chronic liver failure, the skeletal muscle tissue (because of its large size) becomes a key organ that detoxifies ammonia in organisms. Liver disease often results in a condition requiring a hypoproteic diet. However, in the context of the role of muscles in hyperammonemia, this diet is not recommended for HE; a hypoproteic diet might decrease both muscle mass and ammonia detoxification. This proceeding is supported by the latest research proving that diet supplementation with branched-chain amino acids reduces MHE and improves muscle mass[26,27]. The kidneys also interact with the liver to metabolize ammonia. Under normal circumstances, 30% of produced ammonia is excreted in the urine. In liver disease, 70% of the synthesized ammonia is eliminated by this means. As a result, renal disturbanc-

es may intensify cognitive impairment during HE. Note that a healthy liver (periportal hepatocytes) protects the brain from ammonia by converting it into urea, which is excreted through the kidneys and into the colon. Ammonia is also detoxified by the formation of glutamine from glutamate, which occurs in the liver (the hepatocytes near the central vein) and the brain[28-30]. Therefore, HE, hepatocellular failure and portosystemic shunting disable the ability of the liver to neutralize ammonia, and the arterial levels of this compound become elevated. Because of the role it plays, ammonia is sometimes described as a key toxin in the etiology of HE. First, ammonia can induce astrocyte swelling as the result of osmotic imbalance. This phenomenon is thought to play a major role in the pathogenesis of HE. Astrocytes are the only brain cells that are able to metabolize ammonia. This metabolism occurs because of the presence of glutamine synthetase (a special enzyme) in the astrocytes, which transforms glutamate and ammonia into glutamine. Therefore, the intracellular level of glutamine rapidly increases in conjunction with the concentration of ammonia in liver disease. Glutamine is an osmocyte and causes the movement of water into the astrocytes. As the result, the astrocytes swell, and this process leads to cerebral edema and intracranial hypertension. In support of this theory, the administration of methionine sulfoximine (a glutamine synthetase inhibitor) prevents astrocytes from swelling in animal models[31-33]. Astrocytes that are exposed to ammonia for prolonged periods can undergo a morphologic transformation into Alzheimer's type Ⅱ astrocytes, which are defined by large nuclei, prominent nucleoli and marginated chromatin[34]. Hyperammonemia among neural cells also produces reactive oxygen species (ROS). These oxygen species are able to trigger astrocyte swelling and modulate Zn^{2+} mobilization, which results in the stimulation of the peripheral benzodiazepine receptor. Furthermore, ammonia increases the resting membrane potential in the brain, inactivates the neuronal chloride extrusion pumps, suppresses inhibitory postsynaptic potential formation and causes neuron depolarization[35]. This neuroinhibitory state is characteristic of HE. The molecular effects of ammonia reflect the behavioral changes in patients with hyperammonemia.

Bacterial products and bacterial infections in HE

Ammonia is an important factor in the pathogenesis of HE. However, approximately 10% of patients with significant encephalopathy have normal serum ammonia levels; this statistic supports the argument against the prevalence of the ammonia hypothesis. Furthermore, many cirrhosis patients have elevated ammonia levels and no evidence of encephalopathy. In addition to ammonia, other bacteria-produced substances may cause neurotoxicity in HE. Mercaptans exert their neurotoxic influence on human organisms as the result of the bacterial metabolism of amino acids. The phenols synthesized by bacteria from aromatic amino acids are also dangerous. Elevated levels of both mercaptans and phenols have been found in patients with chronic liver disease. These endogenous toxic substances, which are produced in the intestines, are absorbed into the portal venous flow. The toxins escape from liver catabolism because of the impaired liver function and the presence of portosystemic shunts. Finally, the toxins reach the brain through the blood-brain barrier (BBB) and cause cerebral function disorders[29]. Coexisting gastrointestinal bleeding enhances the flow of toxins into the systemic bloodstream. Bacterial flora might also be the source of benzodiazepine-like compounds, which are produced during the metabolism of amino acids. These compounds can impair the function of the astrocytes and disrupt gamma-aminobutyric acid (GABA)ergic neurotransmission. Similarly to bacterial flora, bacterial pathogens are also involved in the etiology of HE. Recent investigations underline the potential importance of acute infections caused by bacterial pathogens and the resulting inflammation in the neuropsychiatric symptoms of HE[36]. Moreover, the inflammatory response might modulate the reaction to hyperammonemia. In such a situation, cytokine-induced changes in ammonia diffusion can occur in the brain, and the expression of benzodiazepine receptors might increase.

The GABAergic neurotransmission theory

GABA is a principle inhibitory neurotransmitter. GABA-mediated transmission is based on the $GABA_A$/benzodiazepine receptor/chloride ionophore complex. This binding opens the ion channel and increases the amount of chloride in the neuron. Increased GABA-mediated neurotransmission contributes to motor function impairment and decreased consciousness, which are the main manifestations of HE[37,38]. Mechanisms leading to the increased GABAergic transmission in liver failure are not well known. Nevertheless, there is evidence suggesting that the amount of natural benzodiazepine receptor agonists in the liver increases with impaired function. Two observations confirm this theory. First, increased levels of these agonists have been found in the brain and body fluids of HE patients[3,21]. Furthermore, treatment based on flumazenil, a benzodiazepine receptor antagonist, ameliorates HE. The role of neurosteroids (e.g., tetrahydro-progesterone and tetrahydrodeoxycorticosterone) in the problem of GABAergic transmission should be noted[39]. Neurosteroids are potent agonists of the $GABA_A$ receptor, acting through a unique binding site to enhance GABAergic neurotransmission. Animal research revealed the ability of pregnenolone (an endogenous neurosteroid "antagonist") to decrease mortality in hyperammonemic models. The results demonstrate the important role of GABAergic enhancers in the pathology of HE. The enhancers are normally synthesized in astrocytes and appear in elevated concentrations in hyperammonemic mice or mice with acute liver failure. The elevated concentrations are caused by the glial cell

activity in ammonia-induced disorders. The specific path leading to neurosteroid synthesis is not yet clear; however, this phenomenon appears to be connected with ammonia-induced increases in the density of peripheral-type benzodiazepine receptors[40-42]. GABAergic neurotransmission might be enhanced by the increased access of GABA in the synaptic clefts. This phenomenon is caused by the loss of presynaptic feedback inhibition from the GABA release, which is the result of decreased $GABA_B$ receptor values. Finally, recent research suggests that ammonia itself might directly increase GABAergic transmission[43].

The idea of neuroinflammation

Ammonia dysmetabolism does not exist as the sole factor of the neurological changes observed in patients with HE. It is well known that sepsis is precipitating state that plays significant role in the decompensation of liver diseases[44-46]. The peripheral immune system communicates with the brain in response to infection and inflammation. This response results in neutrophil adhesion, migration across the BBB and release of chemokines, proinflammatory cytokines, proteases and ROS as well as inflammatory gene transcription[47]. Simultaneously, astrocytes and microglial cells release cytokines in response to injury or inflammation. Researchers have proven that the increased blood level of tumor necrosis factor (TNF), which occurs during inflammation, stimulates the glial cells to excrete the cytokines [interleukin (IL)-1, IL-6] and influences the permeability of the BBB[21,22,48-50]. In conclusion, the diffusion of ammonia into the astrocytes increases *in vitro*. Moreover, a recent study has revealed that TNF or IL-1 receptor gene deletion retards the process of encephalopathy and decreases the intensity of brain edema in mice with acute liver failure. In addition, treatment based on a TNF receptor antagonist (etanercept) similarly reduces the severity of encephalopathy and prevents brain edema[49]. The concept of neuroinflammation (microglial activation) is defined as a wide variety of neurological disorders including multiple sclerosis, Alzheimer's disease, stroke and acquired immune deficiency syndrome dementia complex. Microglial activation is followed by highly increased levels of proinflammatory cytokines (TNF, IL-1 and IL-6) in the brain[51,52]. This discovery revealed a new aspect of HE, provided a new look at the pathophysiology of HE and opened a new gate into the pharmacotherapy of HE. This neuroinflammatory concept is also supported by the therapeutic effect of mild hypothermia and indomethacin, which reduces the activation of microglial cells and simultaneously prevents the central proinflammatory process in mild HE[27,51,53,54]. Moreover, mild hypothermia remarkably decreases brain edema and the duration of HE. Intracranial pressure (ICP) may be reduced by hypothermia even in patients who are unresponsive to mannitol. The additional benefits of indomethacin depend on how it affects cognitive function *via* modulation of the glutamate-nitric oxide cyclic GMP pathway. Indomethacin might reduce ICP and cerebral edema independently of changing the cerebral blood flow (excepting the brain function modulation). Numerous studies have revealed the beneficial role of anti-inflammatory agents in the course of HE. Ibuprofen reportedly reduces locomotor disorders and increases the learning abilities of rats with portacaval shunts. Ibuprofen also normalizes blood and brain ammonia levels[46,55]. Consequently, anti-inflammatory therapy inhibits microglial activation during HE. The findings in different experimental models provide new therapeutic targets for HE and require further study.

Oxidative and nitrosative stress

The metabolism of astrocytes changes after exposure to ammonia, hyponatremia, benzodiazepines or inflammatory cytokines; under such circumstances, the production of reactive nitrogen species and ROS increases in the astrocytes. N-methyl-D-aspartate (NMDA) receptors play an important role in the production of free radicals. The stimulation of ammonia-induced NMDA receptors reduces antioxidant enzyme activity and increases the production of superoxide anions[56,57]. The presence of ammonia inside the astrocyte mitochondria (with the coexistence of calcium) initiates the process. Moreover, the ROS source (neutrophil metabolism) constitutes a systemic/local inflammatory state as well. Glutamine exposure is known to increase the oxidative stress among astrocytes (the "Trojan horse hypothesis")[32,58]. The other area of research interest involves RNA oxidation and the resulting impairment in protein synthesis. Molecular disorders lead to cognitive abnormalities in HE. ROS are involved in the nitration of tyrosine (the component of intracellular proteins). Nitrated tyrosine tends to selectively reduce the permeability of the BBB, which promotes astrocyte swelling and cerebral edema[59-61].

The 'two-hit hypothesis' is another pathological path leading to ROS output, and it is connected inseparably to the activity of ammonia. Liver disease followed by hyperammonemia constitutes a starting point (an 'initial hit') in this theory. Astrocyte swelling and oxidative stress appear next. Finally, there is a 'second hit', such as an ammonia load caused by an upper gastrointestinal bleed, dehydration, hyponatremia or infection. Astrocyte damage occurs, and the level of ROS increases further. This close connection between astrocyte swelling and oxidative stress generates an auto-amplifying signaling loop that results in the deterioration of neurocognitive abilities[22].

Changes in the blood-brain barrier

The BBB constitutes a highly specialized mechanism of brain microvasculature, and it is indispensable for the proper function of the central nervous system (CNS)[26]. The BBB consists of pericytes, capillary basement membranes, endothelial capillary cells and astrocytes, which encase the vessels[62]. There is evidence of BBB dysfunc-

tion in the pathogenesis of many CNS disorders (e.g., Alzheimer's disease and multiple sclerosis). The pathological appearance of HE is closely related to BBB changes. Swollen astrocytes constitute a well-known phenomenon in the course of HE[22,63]. The phenomenon is primarily caused by cellular and vasogenic edema and aquaporins (AQP). In CNS structures, elevated values of AQP 1, 4 and 9 are characteristic of brain edema[26]. The increased expression of these water channels serves an important role in the movement of water through the BBB and its pathological result. Moreover, AQPs have been reported to participate in apoptosis in the CNS[64,65]. Astrocyte disorders in HE result in changes to the BBB, reflected by the loss of ion homeostasis and the accumulation of intracellular water. Nevertheless, increased BBB ammonia permeability during hyperammonemia or HE is still a matter of controversy among scientists[66]. There are investigations that support and refute this theory. Several positron emission tomography studies have revealed elevated brain ammonia concentrations in HE. In addition, transmission electron microscopy demonstrated a disruption in the junction of BBB capillaries in the hippocampal area in a MHE model[26,35]. According to the presented data, the structure of the BBB and its connection to systemic hyperammonemia in HE require further exploration. The large number of studies conducted in this area indicates that it might be essential for the pathological appearance of HE.

Death of CNS cells

Brains exposed to high ammonia concentrations undergo several serious changes, including metabolic and neurophysiological disorders with pathological synaptic inhibition and excitation. As a result, alterations in cerebral energy metabolism appear and neurotransmitter-related processes begin to falter[59]. The mechanisms leading to cell death in the CNS are associated with changes in the neuronal mitochondria[67,68]. First, a reduction in the ATP concentration occurs in the course of HE. The two basic paths responsible for this modified energy metabolism are the NMDA receptors and the inhibition of the tricarboxylic acid cycle. The NMDA receptors are able to mediate mitochondrial swelling, neurotoxicity and neuron death during acute liver failure[57,69]. Furthermore, it is suggested that calcium plays a key role in the pathology of cell death in the CNS[22,26,70]. In HE, the mitochondrial calcium content becomes elevated because of acute ammonia intoxication. Calcium is able to enter the mitochondria *via* NMDA receptors[71,72]. This mechanism results in the release of glutamate and ROS and induces an apoptotic cascade among the neurons. Peripheral benzodiazepine receptors located in the mitochondrial membrane cells also cause the programmed death of neurons in association with calcium[21,40-42,73]. In conclusion, the loss of neuronal cells depends on the neurotoxicity of ammonia and mitochondrial dysfunction. The above-mentioned pathological mechanisms are reflected in the cerebral atrophy and mental retardation observed in patients suffering from HE[26].

Other pathogenic elements in HE

It is clear that the etiology of HE is complex and multifactorial. In addition, theories different from those presented above have been postulated to explain the pathogenesis of HE. The opioid, serotonergic and dopaminergic systems are likely involved. Furthermore, 'false' neurotransmitters (e.g., phenylethanolamine and octopamine) produced from aromatic amino acids are elevated in individuals with HE. These substances compete with real neurotransmitters and impair neurological functions[43]. The genetic background of HE is underlined. Genetic analyses showed that particular haplotypes in glutaminase (an enzyme that converts glutamine into glutamate) in patients suffering from cirrhosis affect the risk of HE. A characteristic section in the promoter region of the gene sequence for glutaminase has been detected that contains repeated base pairs. Studies have shown higher luciferase activity in the cells transfected with this doubled region. This research indicates that those repeated elements increase glutaminase transcriptional activity. As the result, genetic disorders appear to be responsible for the development of HE in patients with cirrhosis[74].

In addition to ammonia, manganese participates in the development of HE[75,76]. Manganese is an essential metal that is involved in the metabolism of carbohydrates, lipids and proteins. Manganese is an important cofactor of numerous enzymes. It is a neurotoxin that may be transported into the brain across the BBB and the brain-cerebrospinal fluid barriers. In such circumstances, it is preferentially accumulated in the basal ganglia. Manganese enhances neurosteroid synthesis *via* the activation of translocator proteins on astrocyte membranes[3]. Manganese affects the operations of certain proteins that interact with dopamine (a neurotransmitter), NMDA toxicity or oxidative and nitrosative stress. Three mechanisms of manganese neurotoxicity have been described[26,77,78]. The first is mitochondrial dysfunction and the disruption of energy metabolism; the second is connected with the inflammatory activation of the glial cells; and the third mechanism constitutes the disruption of synaptic transmission and neuroglial communication. This mechanism induces the phosphorylation of c-Jun and SEK1/MKK4 and the tyrosine phosphorylation of several proteins[79]. Manganese produces active oxygen species (superoxide hydrogen peroxide and hydroxyl radical) and toxic catecholamines (6-hydroxydopamine). Moreover, manganese is the first metal reported to induce apoptosis in the CNS[26]. Furthermore, manganese deposition has been detected by magnetic resonance imaging in the caudate nucleus and in the globus pallidus of cirrhotic patients. This detection might explain the presence of Parkinson symptoms (such as tremors) observed in certain patients with HE. Researchers have indicated that manganese enhances

neurosteroid synthesis and promotes the formation of Alzheimer's type II astrocytes[21].

The study conducted by Guevara *et al*[80] revealed that hyponatremia (serum sodium < 130 mEq/L) is an independent and serious predictor of overt HE. This hypothesis may open new strategies for HE treatment. The reduction of hyponatremia might be a novel therapeutic approach in preventing HE in liver cirrhosis[52].

Neuroendocrine correlations in the course of HE are not well recognized. Nevertheless, studies conducted on rats demonstrated decreased levels of prolactin in models suffering from HE. This disorder might be caused by a hyperammonemic state. Endocrinal curiosity reveals the necessity of further research in this area[26].

NONCIRRHOTIC HYPERAMMONEMIC ENCEPAHALOPATHY

Research shows that approximately 90% of adult hyperammonemia cases are caused by severe liver disease. A small number of cases have other causes and can be divided into two groups: (1) cases that are connected with increased ammonia production; and (2) cases that are caused by decreased ammonia elimination[81].

Increased ammonia production

Hyperammonemic encephalopathy may develop in patients with progressive multiple myeloma[82]. Several studies have demonstrated that malignant cells are able to produce excess ammonia as the result of increased amino acid metabolism. During intensive chemotherapy, leukemia patients tend to struggle with hyperammonemia[83]. Idiopathic hyperammonemia is also described among bone marrow recipients. The other reason for noncirrhotic hyperammonemic encephalopathy might be the presence of infections caused by urea-producing bacteria (*Proteus mirabilis*, *Escherichia coli*, *Klebsiella* and *Providencia rettgeri*). In extreme cases, the protein load and increased metabolism might cause the elevated ammonia level. Therefore, seizures, severe exercise, starvation, total parenteral nutrition or gastrointestinal bleeding can (rarely) result in encephalopathy.

Decreased ammonia elimination

Metabolic disorders and problems with ammonia elimination might be the source of encephalopathy. Such disorders include organic acidurias, urea-cycle disorders, dibasic aminoaciduria, impaired fatty acid oxidation and mistakes in pyruvate metabolism[7,11,12]. Noncirrhotic congenital portosystemic shunts can also cause an elevated ammonia level in the blood stream, and the intake of certain drugs (e.g., valproic acid, glycine, ribavirin and carbamazepine) may induce noncirrhotic hyperammonemic encephalopathy[84-87].

CONCLUSION

Encephalopathy can be caused by various factors. The most common causes are connected to liver disorders. The etiology is particularly complex, and researchers continue to explore the HE phenomenon. Nevertheless, hyperammonemia, inflammatory conception, oxidative stress and disrupted GABA-transmission appear to participate in the pathogenesis of HE. The pathophysiological mechanisms of noncirrhotic hyperammonemia encephalopathy are also multifactorial and include genetic disorders and side effects of drugs. Despite the rarity of this diagnosis, HE is a phenomenon worth examining because of its multifactorial character.

REFERENCES

1 **Av SP**. Hepatic encephalopathy: pathophysiology and advances in therapy. *Trop Gastroenterol* 2007; **28**: 4-10 [PMID: 17896602]

2 **Ferenci P**, Lockwood A, Mullen K, Tarter R, Weissenborn K, Blei AT. Hepatic encephalopathy--definition, nomenclature, diagnosis, and quantification: final report of the working party at the 11th World Congresses of Gastroenterology, Vienna, 1998. *Hepatology* 2002; **35**: 716-721 [PMID: 11870389 DOI: 10.1053/jhep.2002.31250]

3 **Wakim-Fleming J**. Hepatic encephalopathy: suspect it early in patients with cirrhosis. *Cleve Clin J Med* 2011; **78**: 597-605 [PMID: 21885692 DOI: 10.3949/ccjm.78a10117]

4 Quadrennial reviews and working party reports from the World Congress in Gastroenterology. February 24-March 1, 2002. Bangkok, Thailand. *J Gastroenterol Hepatol* 2002; **17** Suppl: S1-195 [PMID: 12227330]

5 **Mullen KD**. Review of the final report of the 1998 Working Party on definition, nomenclature and diagnosis of hepatic encephalopathy. *Aliment Pharmacol Ther* 2007; **25** Suppl 1: 11-16 [PMID: 17295847 DOI: 10.1111/j.1746-6342.2006.03216.x]

6 **Butterworth RF**, Norenberg MD, Felipo V, Ferenci P, Albrecht J, Blei AT. Experimental models of hepatic encephalopathy: ISHEN guidelines. *Liver Int* 2009; **29**: 783-788 [PMID: 19638106 DOI: 10.1111/j.1478-3231.2009.02034.x]

7 **Leonard JV**, Morris AA. Urea cycle disorders. *Semin Neonatol* 2002; **7**: 27-35 [PMID: 12069536 DOI: 10.1053/siny.2001.0085]

8 **Panlaqui OM**, Tran K, Johns A, McGill J, White H. Acute hyperammonemic encephalopathy in adult onset ornithine transcarbamylase deficiency. *Intensive Care Med* 2008; **34**: 1922-1924 [PMID: 18651132 DOI: 0.1007/s00134-008-1217-2]

9 **Ben-Ari Z**, Dalal A, Morry A, Pitlik S, Zinger P, Cohen J, Fattal I, Galili-Mosberg R, Tessler D, Baruch RG, Nuoffer JM, Largiader CR, Mandel H. Adult-onset ornithine transcarbamylase (OTC) deficiency unmasked by the Atkins' diet. *J Hepatol* 2010; **52**: 292-295 [PMID: 20031247 DOI: 10.1016/j.jhep.2009.11.014]

10 **Cohn RM**, Roth KS. Hyperammonemia, bane of the brain. *Clin Pediatr* (Phila) 2004; **43**: 683-689 [PMID: 15494874 DOI: 10.1177/000992280404300801]

11 **Maillot F**, Crenn P. [Urea cycle disorders in adult patients]. *Rev Neurol* (Paris) 2007; **163**: 897-903 [PMID: 18033025]

12 **Wasant P**, Srisomsap C, Liammongkolkul S, Svasti J. Urea cycle disorders in Thai infants: a report of 5 cases. *J Med Assoc Thai* 2002; **85** Suppl 2: S720-S731 [PMID: 12403252]

13 **Mak CM**, Siu TS, Lam CW, Chan GC, Poon GW, Wong KY, Low LC, Tang NL, Li SK, Lau KY, Kwong NS, Tam S. Complete recovery from acute encephalopathy of late-onset ornithine transcarbamylase deficiency in a 3-year-old boy. *J Inherit Metab Dis* 2007; **30**: 981 [PMID: 17922216 DOI: 10.1007/s10545-007-0692-x]

14 **Gulati S**, Menon S, Kabra M, Kalra V. Intermittent hyperammonemic encephalopathy in a child with ornithine trans-

carbamylase deficiency. *Indian J Pediatr* 2004; **71**: 645-647 [PMID: 15280615 DOI: 10.1007/BF02724127]
15 **Hawrot-Kawecka AM**, Kawecki GP, Duława J. [Hyperammonemia type II as an example of urea cycle disorder]. *Wiad Lek* 2006; **59**: 512-515 [PMID: 17209350]
16 **Bisanzi S**, Morrone A, Donati MA, Pasquini E, Spada M, Strisciuglio P, Parenti G, Parini R, Papadia F, Zammarchi E. Genetic analysis in nine unrelated Italian patients affected by OTC deficiency: detection of novel mutations in the OTC gene. *Mol Genet Metab* 2002; **76**: 137-144 [PMID: 12083811 DOI: 10.1016/S1096-7192(02)00028-8]
17 **Guerit JM**, Amantini A, Fischer C, Kaplan PW, Mecarelli O, Schnitzler A, Ubiali E, Amodio P. Neurophysiological investigations of hepatic encephalopathy: ISHEN practice guidelines. *Liver Int* 2009; **29**: 789-796 [PMID: 19638107 DOI: 10.1111/j.1478-3231.2009.02030.x]
18 **Rivera Ramos JF**, Rodríguez Leal C. Review of the final report of the 1998 Working Party on definition, nomenclature and diagnosis of hepatic encephalopathy. *Ann Hepatol* 2011; **10** Suppl 2: S36-S39 [PMID: 22228879]
19 **Dhiman RK**, Saraswat VA, Sharma BK, Sarin SK, Chawla YK, Butterworth R, Duseja A, Aggarwal R, Amarapurkar D, Sharma P, Madan K, Shah S, Seth AK, Gupta RK, Koshy A, Rai RR, Dilawari JB, Mishra SP, Acharya SK. Minimal hepatic encephalopathy: consensus statement of a working party of the Indian National Association for Study of the Liver. *J Gastroenterol Hepatol* 2010; **25**: 1029-1041 [PMID: 20594216 DOI: 10.1111/j.1440-1746.2010.06318.x]
20 **Dhiman RK**, Chawla YK. Minimal hepatic encephalopathy. *Indian J Gastroenterol* 2009; **28**: 5-16 [PMID: 19529896 DOI: 10.1007/s12664-009-0003-6]
21 **Prakash R**, Mullen KD. Mechanisms, diagnosis and management of hepatic encephalopathy. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 515-525 [PMID: 20703237 DOI: 10.1038/nrgastro.2010.116]
22 **Seyan AS**, Hughes RD, Shawcross DL. Changing face of hepatic encephalopathy: role of inflammation and oxidative stress. *World J Gastroenterol* 2010; **16**: 3347-3357 [PMID: 20632436 DOI: 10.3748/wjg.v16.i27.3347]
23 **Norenberg MD**, Rama Rao KV, Jayakumar AR. Signaling factors in the mechanism of ammonia neurotoxicity. *Metab Brain Dis* 2009; **24**: 103-117 [PMID: 19104923 DOI: 10.1007/s11011-008-9113-6]
24 **Detry O**, De Roover A, Honore P, Meurisse M. Brain edema and intracranial hypertension in fulminant hepatic failure: pathophysiology and management. *World J Gastroenterol* 2006; **12**: 7405-7412 [PMID: 17167826]
25 **Weber FL**, Friedman DW, Fresard KM. Ammonia production from intraluminal amino acids in canine jejunum. *Am J Physiol* 1988; **254**: G264-G268 [PMID: 3348378]
26 **Perazzo JC**, Tallis S, Delfante A, Souto PA, Lemberg A, Eizayaga FX, Romay S. Hepatic encephalopathy: An approach to its multiple pathophysiological features. *World J Hepatol* 2012; **4**: 50-65 [PMID: 22489256 DOI: 10.4254/wjh.v4.i3.50]
27 **Wright G**, Chattree A, Jalan R. Management of hepatic encephalopathy. *Int J Hepatol* 2011; **2011**: 841407 [PMID: 21994873 DOI: 10.4061/2011/841407]
28 **Patel D**, McPhail MJ, Cobbold JF, Taylor-Robinson SD. Hepatic encephalopathy. *Br J Hosp Med* (Lond) 2012; **73**: 79-85 [PMID: 22504749]
29 **Williams R**. Review article: bacterial flora and pathogenesis in hepatic encephalopathy. *Aliment Pharmacol Ther* 2007; **25** Suppl 1: 17-22 [PMID: 17295848 DOI: 10.1111/j.1746-6342.2006.03217.x]
30 **Olde Damink SW**, Jalan R, Dejong CH. Interorgan ammonia trafficking in liver disease. *Metab Brain Dis* 2009; **24**: 169-181 [PMID: 19067143]
31 **Caruana P,** Shah N. Hepatic Encephalopathy: Are NH_4 levels and protein restriction obsolete? *Pract Gastroenterol* 2011; **25:** 6-18
32 **Häussinger D**, Schliess F. Pathogenetic mechanisms of hepatic encephalopathy. *Gut* 2008; **57**: 1156-1165 [PMID: 18628377 DOI: 10.1136/gut.2007.122176]
33 **Tanigami H**, Rebel A, Martin LJ, Chen TY, Brusilow SW, Traystman RJ, Koehler RC. Effect of glutamine synthetase inhibition on astrocyte swelling and altered astroglial protein expression during hyperammonemia in rats. *Neuroscience* 2005; **131**: 437-449 [PMID: 15708485 DOI: 10.1016/j.neuroscience.2004.10.045]
34 **Butterworth RF.** Hepatic encephalopathy. *Alcohol Res Health* 2003; **27:** 240-246 [PMID: 15535452]
35 **Basile AS**, Jones EA. Ammonia and GABA-ergic neurotransmission: interrelated factors in the pathogenesis of hepatic encephalopathy. *Hepatology* 1997; **25**: 1303-1305 [PMID: 9185743]
36 **Bajaj JS**, Ridlon JM, Hylemon PB, Thacker LR, Heuman DM, Smith S, Sikaroodi M, Gillevet PM. Linkage of gut microbiome with cognition in hepatic encephalopathy. *Am J Physiol Gastrointest Liver Physiol* 2012; **302**: G168-G175 [PMID: 21940902 DOI: 10.1152/ajpgi.00190.2011]
37 **Jones EA**, Mullen KD. Theories of the pathogenesis of hepatic encephalopathy. *Clin Liver Dis* 2012; **16**: 7-26 [PMID: 22321462 DOI: 10.1016/j.cld.2011.12.010]
38 **Jones EA**. Ammonia, the GABA neurotransmitter system, and hepatic encephalopathy. *Metab Brain Dis* 2002; **17**: 275-281 [PMID: 12602504]
39 **Ahboucha S**, Butterworth RF. The neurosteroid system: implication in the pathophysiology of hepatic encephalopathy. *Neurochem Int* 2008; **52**: 575-587 [PMID: 17610999 DOI: 10.1016/j.neuint.2007.05.004]
40 **Parola AL**, Yamamura HI, Laird HE. Peripheral-type benzodiazepine receptors. *Life Sci* 1993; **52**: 1329-1342 [PMID: 8385255 DOI: 10.1016/0024-3205(93)90168-3]
41 **Casellas P**, Galiegue S, Basile AS. Peripheral benzodiazepine receptors and mitochondrial function. *Neurochem Int* 2002; **40**: 475-486 [PMID: 11850104 DOI: 10.1016/S0197-0186(01)00118-8]
42 **Azarashvili T**, Grachev D, Krestinina O, Evtodienko Y, Yurkov I, Papadopoulos V, Reiser G. The peripheral-type benzodiazepine receptor is involved in control of Ca2+-induced permeability transition pore opening in rat brain mitochondria. *Cell Calcium* 2007; **42**: 27-39 [PMID: 17174393 DOI: 10.1016/j.ceca.2006.11.004]
43 **Moriwaki H**, Shiraki M, Iwasa J, Terakura Y. Hepatic encephalopathy as a complication of liver cirrhosis: an Asian perspective. *J Gastroenterol Hepatol* 2010; **25**: 858-863 [PMID: 20546438 DOI: 10.1111/j.1440-1746.2009.06242.x]
44 **Vaquero J**, Polson J, Chung C, Helenowski I, Schiodt FV, Reisch J, Lee WM, Blei AT. Infection and the progression of hepatic encephalopathy in acute liver failure. *Gastroenterology* 2003; **125**: 755-764 [PMID: 12949721 DOI: 10.1016/S0016-5085(03)01051-5]
45 **Jalan R**, Olde Damink SW, Hayes PC, Deutz NE, Lee A. Pathogenesis of intracranial hypertension in acute liver failure: inflammation, ammonia and cerebral blood flow. *J Hepatol* 2004; **41**: 613-620 [PMID: 15464242 DOI: 10.1016/j.jhep.2004.06.011]
46 **Cauli O**, Rodrigo R, Piedrafita B, Boix J, Felipo V. Inflammation and hepatic encephalopathy: ibuprofen restores learning ability in rats with portacaval shunts. *Hepatology* 2007; **46**: 514-519 [PMID: 17659565 DOI: 10.1002/hep.21734]
47 **Shawcross DL**, Shabbir SS, Taylor NJ, Hughes RD. Ammonia and the neutrophil in the pathogenesis of hepatic encephalopathy in cirrhosis. *Hepatology* 2010; **51**: 1062-1069 [PMID: 19890967 DOI: 10.1002/hep.23367]
48 **Wright G**, Shawcross D, Olde Damink SW, Jalan R. Brain cytokine flux in acute liver failure and its relationship with

intracranial hypertension. *Metab Brain Dis* 2007; **22**: 375-388 [PMID: 17899343 DOI: 10.1007/s11011-007-9071-4]
49 **Jalan R**, Pollok A, Shah SH, Madhavan K, Simpson KJ. Liver derived pro-inflammatory cytokines may be important in producing intracranial hypertension in acute liver failure. *J Hepatol* 2002; **37**: 536-538 [PMID: 12217609 DOI: 10.1016/S0168-8278(02)00240-4]
50 **Montoliu C**, Piedrafita B, Serra MA, del Olmo JA, Urios A, Rodrigo JM, Felipo V. IL-6 and IL-18 in blood may discriminate cirrhotic patients with and without minimal hepatic encephalopathy. *J Clin Gastroenterol* 2009; **43**: 272-279 [PMID: 18562979 DOI: 10.1097/MCG.0b013e31815e7f58]
51 **Jiang W**, Desjardins P, Butterworth RF. Direct evidence for central proinflammatory mechanisms in rats with experimental acute liver failure: protective effect of hypothermia. *J Cereb Blood Flow Metab* 2009; **29**: 944-952 [PMID: 19259110 DOI: 10.1038/jcbfm.2009.18]
52 **Rodrigo R**, Cauli O, Gomez-Pinedo U, Agusti A, Hernandez-Rabaza V, Garcia-Verdugo JM, Felipo V. Hyperammonemia induces neuroinflammation that contributes to cognitive impairment in rats with hepatic encephalopathy. *Gastroenterology* 2010; **139**: 675-684 [PMID: 20303348 DOI: 10.1053/j.gastro.2010.03.040]
53 **Baine AM**, Hori T, Chen F, Gardner LB, Uemoto S, Nguyen JH. Fulminant liver failure models with subsequent encephalopathy in the mouse. *Hepatobiliary Pancreat Dis Int* 2011; **10**: 611-619 [PMID: 22146625 DOI: 10.1016/S1499-3872(11)60104-5]
54 **Jalan R**, Olde Damink SW, Deutz NE, Hayes PC, Lee A. Moderate hypothermia in patients with acute liver failure and uncontrolled intracranial hypertension. *Gastroenterology* 2004; **127**: 1338-1346 [PMID: 15521003 DOI: 10.1053/j.gastro.2004.08.005]
55 **Butterworth RF**. Hepatic encephalopathy: a central neuroinflammatory disorder? *Hepatology* 2011; **53**: 1372-1376 [PMID: 21480337 DOI: 10.1002/hep.24228]
56 **Hilgier W**, Anderzhanova E, Oja SS, Saransaari P, Albrecht J. Taurine reduces ammonia- and N-methyl-D-aspartate-induced accumulation of cyclic GMP and hydroxyl radicals in microdialysates of the rat striatum. *Eur J Pharmacol* 2003; **468**: 21-25 [PMID: 12729839 DOI: 10.1016/S0014-2999(03)01667-4]
57 **Norenberg MD**, Jayakumar AR, Rama Rao KV. Oxidative stress in the pathogenesis of hepatic encephalopathy. *Metab Brain Dis* 2004; **19**: 313-329 [PMID: 15554424]
58 **Albrecht J**, Norenberg MD. Glutamine: a Trojan horse in ammonia neurotoxicity. *Hepatology* 2006; **44**: 788-794 [PMID: 17006913 DOI: 10.1002/hep.21357]
59 **Görg B**, Qvartskhava N, Bidmon HJ, Palomero-Gallagher N, Kircheis G, Zilles K, Häussinger D. Oxidative stress markers in the brain of patients with cirrhosis and hepatic encephalopathy. *Hepatology* 2010; **52**: 256-265 [PMID: 20583283 DOI: 10.1002/hep.23656]
60 **Häussinger D**, Görg B, Reinehr R, Schliess F. Protein tyrosine nitration in hyperammonemia and hepatic encephalopathy. *Metab Brain Dis* 2005; **20**: 285-294 [PMID: 16382339 DOI: 10.1007/s11011-005-7908-2]
61 **Häussinger D**, Schliess F. Astrocyte swelling and protein tyrosine nitration in hepatic encephalopathy. *Neurochem Int* 2005; **47**: 64-70 [PMID: 15908046 DOI: 10.1016/j.neuint.2005.04.008]
62 **Persidsky Y**, Ramirez SH, Haorah J, Kanmogne GD. Blood-brain barrier: structural components and function under physiologic and pathologic conditions. *J Neuroimmune Pharmacol* 2006; **1**: 223-236 [PMID: 18040800 DOI: 10.1007/s11481-006-9025-3]
63 **Nout YS.** Gait deficits in liver disease: hepatic encephalopathy and hepatic myelopathy. *Equine Vet Educ* 2011; **23:** 11-13 [DOI: 10.1111/j.2042-3292.2010.00177.x]
64 **Amiry-Moghaddam M**, Ottersen OP. The molecular basis of water transport in the brain. *Nat Rev Neurosci* 2003; **4**: 991-1001 [PMID: 14682361 DOI: 10.1038/nrn1252]
65 **Badaut J**, Regli L. Distribution and possible roles of aquaporin 9 in the brain. *Neuroscience* 2004; **129**: 971-981 [PMID: 15561412 DOI: 10.1016/j.neuroscience.2004.06.035]
66 **Geissler A**, Lock G, Fründ R, Held P, Hollerbach S, Andus T, Schölmerich J, Feuerbach S, Holstege A. Cerebral abnormalities in patients with cirrhosis detected by proton magnetic resonance spectroscopy and magnetic resonance imaging. *Hepatology* 1997; **25**: 48-54 [PMID: 8985263 DOI: 10.1002/hep.510250109]
67 **Bustamante J**, Lores-Arnaiz S, Tallis S, Roselló DM, Lago N, Lemberg A, Boveris A, Perazzo JC. Mitochondrial dysfunction as a mediator of hippocampal apoptosis in a model of hepatic encephalopathy. *Mol Cell Biochem* 2011; **354**: 231-240 [PMID: 21505893 DOI: 10.1007/s11010-011-0822-5]
68 **Todde V**, Veenhuis M, van der Klei IJ. Autophagy: principles and significance in health and disease. *Biochim Biophys Acta* 2009; **1792**: 3-13 [PMID: 19022377]
69 **Braissant O**. Ammonia toxicity to the brain: effects on creatine metabolism and transport and protective roles of creatine. *Mol Genet Metab* 2010; **100** Suppl 1: S53-S58 [PMID: 20227315]
70 **Nicotera P**, Bellomo G, Orrenius S. Calcium-mediated mechanisms in chemically induced cell death. *Annu Rev Pharmacol Toxicol* 1992; **32**: 449-470 [PMID: 1605574 DOI: 10.1146/annurev.pa.32.040192.002313]
71 **Hermenegildo C**, Monfort P, Felipo V. Activation of N-methyl-D-aspartate receptors in rat brain in vivo following acute ammonia intoxication: characterization by in vivo brain microdialysis. *Hepatology* 2000; **31**: 709-715 [PMID: 10706562 DOI: 10.1002/hep.510310322]
72 **Nicholls DG**, Budd SL. Mitochondria and neuronal survival. *Physiol Rev* 2000; **80**: 315-360 [PMID: 10617771]
73 **Friberg H**, Wieloch T. Mitochondrial permeability transition in acute neurodegeneration. *Biochimie* 2007; **84**: 241-250 [PMID: 12022955 DOI: 10.1016/S0300-9084(02)01381-0]
74 **Romero-Gómez M**, Jover M, Del Campo JA, Royo JL, Hoyas E, Galán JJ, Montoliu C, Baccaro E, Guevara M, Córdoba J, Soriano G, Navarro JM, Martínez-Sierra C, Grande L, Galindo A, Mira E, Mañes S, Ruiz A. Variations in the promoter region of the glutaminase gene and the development of hepatic encephalopathy in patients with cirrhosis: a cohort study. *Ann Intern Med* 2010; **153**: 281-288 [PMID: 20820037]
75 **Krieger D**, Krieger S, Jansen O, Gass P, Theilmann L, Lichtnecker H. Manganese and chronic hepatic encephalopathy. *Lancet* 1995; **346**: 270-274 [PMID: 7630246 DOI: 10.1016/S0140-6736(95)92164-8]
76 **Spahr L**, Butterworth RF, Fontaine S, Bui L, Therrien G, Milette PC, Lebrun LH, Zayed J, Leblanc A, Pomier-Layrargues G. Increased blood manganese in cirrhotic patients: relationship to pallidal magnetic resonance signal hyperintensity and neurological symptoms. *Hepatology* 1996; **24**: 1116-1120 [PMID: 8903385]
77 **Takeda A**. Manganese action in brain function. *Brain Res Brain Res Rev* 2003; **41**: 79-87 [PMID: 12505649 DOI: 10.1016/S0165-0173(02)00234-5]
78 **Takeda A**, Sotogaku N, Oku N. Manganese influences the levels of neurotransmitters in synapses in rat brain. *Neuroscience* 2002; **114**: 669-674 [PMID: 12220568 DOI: 10.1016/S0306-4522(02)00353-6]
79 **Hirata Y**, Adachi K, Kiuchi K. Activation of JNK pathway and induction of apoptosis by manganese in PC12 cells. *J Neurochem* 1998; **71**: 1607-1615 [PMID: 9751194 DOI: 10.1046/j.1471-4159.1998.71041607.x]
80 **Guevara M**, Baccaro ME, Torre A, Gómez-Ansón B, Ríos J, Torres F, Rami L, Monté-Rubio GC, Martín-Llahí M, Arroyo V, Ginès P. Hyponatremia is a risk factor of hepatic encephalopathy in patients with cirrhosis: a prospective study

with time-dependent analysis. *Am J Gastroenterol* 2009; **104**: 1382-1389 [PMID: 19455124 DOI: 10.1038/ajg.2009.293]
81 **Häberle J**. Clinical practice: the management of hyperammonemia. *Eur J Pediatr* 2011; **170**: 21-34 [PMID: 21165747]
82 **Kwan L**, Wang C, Levitt L. Hyperammonemic encephalopathy in multiple myeloma. *N Engl J Med* 2002; **346**: 1674-1675 [PMID: 12024007 DOI: 10.1056/NEJM200205233462119]
83 **Jaing TH**, Lin JL, Lin YP, Yang SH, Lin JJ, Hsia SH. Hyperammonemic encephalopathy after induction chemotherapy for acute lymphoblastic leukemia. *J Pediatr Hematol Oncol* 2009; **31**: 955-956 [PMID: 19887962 DOI: 10.1097/MPH.0b013e3181b8beb1]
84 **Laish I**, Ben Ari Z. Noncirrhotic hyperammonaemic encephalopathy. *Liver Int* 2011; **31**: 1259-1270 [PMID: 21745294 DOI: 10.1111/j.1478-3231.2011.02550.x]
85 **Wadzinski J**, Franks R, Roane D, Bayard M. Valproate-associated hyperammonemic encephalopathy. *J Am Board Fam Med* 2007; **20**: 499-502 [PMID: 17823470 DOI: 10.3122/jabfm.2007.05.070062]
86 **Lokrantz CM**, Eriksson B, Rosén I, Asztely F. Hyperammonemic encephalopathy induced by a combination of valproate and pivmecillinam. *Acta Neurol Scand* 2004; **109**: 297-301 [PMID: 15016014]
87 **Tsai MF**, Chen CY. Valproate-induced hyperammonemic encephalopathy treated by hemodialysis. *Ren Fail* 2008; **30**: 822-824 [PMID: 18791959 DOI: 10.1080/08860220802272613]

P- Reviewers Takuma Y, Weigand K
S- Editor Gou SX **L- Editor** A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i1.35

World J Gastroenterol 2013 January 7; 19(1): 35-41
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

REVIEW

Retroperitoneal fibrosis associated with immunoglobulin G4-related disease

Nao Fujimori, Tetsuhide Ito, Hisato Igarashi, Takamasa Oono, Taichi Nakamura, Yusuke Niina, Masayuki Hijioka, Lingaku Lee, Masahiko Uchida, Ryoichi Takayanagi

Nao Fujimori, Tetsuhide Ito, Hisato Igarashi, Takamasa Oono, Taichi Nakamura, Yusuke Niina, Masayuki Hijioka, Lingaku Lee, Masahiko Uchida, Ryoichi Takayanagi, Department of Medicine and Bioregulatory Science, Graduate School of Medical Sciences, Kyushu University, Fukuoka 812-8582, Japan

Author contributions: Fujimori N, Igarashi H, Ito T, Oono T and Takayanagi R designed research; Fujimori N, Lee L, Nakamura T, Niina Y, Hijioka M and Uchida M performed research and reviewed the literature; Fujimori N, Igarashi H and Ito T analyzed data and wrote the paper.

Supported by The Research Program of Intractable Disease and the Research Committee of Intractable Pancreatic Diseases of the Ministry of Health, Labor and Welfare of Japan

Correspondence to: Tetsuhide Ito, MD, PhD, Department of Medicine and Bioregulatory Science, Graduate School of Medical Sciences, Kyushu University, 3-1-1 Maidashi, Higashi-ku, Fukuoka 812-8582, Japan. itopapa@intmed3.med.kyushu-u.ac.jp
Telephone: +81-92-6425285 Fax: +81-92-6425287

Received: April 30, 2012 Revised: September 18, 2012
Accepted: September 22, 2012
Published online: January 7, 2013

Abstract

Retroperitoneal fibrosis is a rare disease characterized by the development of inflammation and fibrosis in the soft tissues of the retroperitoneum and other abdominal organs. Retroperitoneal fibrosis can be of 2 types: idiopathic and secondary. The recently advocated concept and diagnostic criteria of immunoglobulin G4 (IgG4)-related disease, derived from research on autoimmune pancreatitis (AIP), has led to widespread recognition of retroperitoneal fibrosis as a condition caused by IgG4-related disease. We now know that previously diagnosed idiopathic retroperitoneal fibrosis includes IgG4-related disease; however, the actual prevalence is unclear. Conversely, some reports on AIP suggest that retroperitoneal fibrosis is concurrently found in about 10% of IgG4-related disease. Because retroperitoneal fibrosis has no specific symptoms, diagnosis is primarily based on diagnostic imaging (computed tomography and magnetic resonance imaging), which is also useful in evaluating the effect of therapy. Idiopathic retroperitoneal fibrosis can occur at different times with other lesions of IgG4-related disease including AIP. Thus, the IgG4 assay is recommended to diagnose idiopathic retroperitoneal fibrosis. High serum IgG4 levels should be treated and monitored as a symptom of IgG4-related disease. The first line of treatment for retroperitoneal fibrosis is steroid therapy regardless of its cause. For patients with concurrent AIP, i.e., IgG4-related retroperitoneal fibrosis, the starting dose of steroid is usually 30-40 mg/d. The response to steroid therapy is generally favorable. In most cases, the pancreatic lesion and retroperitoneal fibrosis improve after the initial treatment. However, the epidemiology, treatment for recurring retroperitoneal fibrosis, and long-term prognosis are still largely unknown. Further analysis of such cases and research are necessary.

© 2013 Baishideng. All rights reserved.

Key words: Autoimmune pancreatitis; Extrapancreatic lesion; Immunoglobulin G4; Immunoglobulin G4-related disease; Retroperitoneal fibrosis

Fujimori N, Ito T, Igarashi H, Oono T, Nakamura T, Niina Y, Hijioka M, Lee L, Uchida M, Takayanagi R. Retroperitoneal fibrosis associated with immunoglobulin G4-related disease. *World J Gastroenterol* 2013; 19(1): 35-41 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i1/35.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i1.35

INTRODUCTION

Retroperitoneal fibrosis is a rare disease characterized by

development of inflammation and fibrosis in the soft tissue of the retroperitoneum and other abdominal organs. The fibro-inflammatory tissue is most frequently found peripheral to the abdominal aorta, and the next most frequent location is peripheral to the iliac arteries, urinary duct, and renal arteries. In some cases, fibro-inflammatory tissue is most frequently found surrounding the pancreas. In a recently advocated concept, retroperitoneal fibrosis is regarded as part of chronic periaortitis[1]. Retroperitoneal fibrosis is generally divided into 2 types: idiopathic retroperitoneal fibrosis for which no clear cause is found, and secondary retroperitoneal fibrosis which occurs secondary to, for example, drug therapy and malignant tumors[1]. Idiopathic retroperitoneal fibrosis was first reported as retroperitoneal fibrosis causing ureteral obstruction by Albarran, a French urologist, in 1905. In 1948, 2 similar cases were reported by Ormond. Since then, idiopathic retroperitoneal fibrosis is also known as Ormond's disease[1,2]. About 30% of retroperitoneal fibrosis occurs secondary to drug therapy and malignant tumors[1,3].

The recently advocated concept of immunoglobulin G4 (IgG4)-related disease has led to widespread recognition of retroperitoneal fibrosis as one of the conditions caused by IgG4-related disease. The aforementioned concept of IgG4-related disease was derived from clinical experience and research on autoimmune pancreatitis (AIP). AIP was first reported as pancreatitis caused by autoimmunity in Japan[4,5]; subsequently, AIP was found to be often associated with increased serum IgG4 levels[6]. Hamano *et al*[7] reported in 2002 that abundant infiltration of IgG4-positive plasma cells was found in both pancreatic and retroperitoneal lesions from patients with AIP having concurrent retroperitoneal fibrosis. AIP is concurrently found not only with retroperitoneal fibrosis, but also with sclerosing cholangitis, sclerosing sialadenitis, and other various extrapancreatic lesions. Histopathologically, these lesions are characterized by the infiltration of IgG4-positive plasma cells as seen in pancreatic lesions. Based on these observations, the concept of IgG4-related sclerosing disease was proposed by Kamisawa *et al*[8,9]. Since the same disease condition can be found all over the body, the concept and diagnostic criteria for IgG4-related disease have been published not only in association with AIP, but also with rheumatological and nephrological diseases, and have attracted considerable attention in recent times[10,11]. In a consensus meeting, Japanese investigators recommended the adoption of the term "IgG4-related disease" from among many suggested names, and proposed comprehensive diagnostic criteria for this condition[12,13]. The concept of IgG4-related disease has been more or less established, and AIP is now regarded as a pancreatic lesion of IgG4-related disease. The number of reports on retroperitoneal fibrosis as an extrapancreatic lesion of AIP has increased, and retroperitoneal fibrosis is now regarded as a typical lesion of IgG4-related disease[12].

Because retroperitoneal fibrosis is essentially a rare disease, and the concept of IgG4-related disease is relatively new, not many studies have been published on IgG4-related retroperitoneal fibrosis. Moreover, the percentage of retroperitoneal fibrosis that is IgG4-related and the clinical differences, if any, between IgG4- and non-IgG4-related retroperitoneal fibrosis are unknown. In this report, based on our observation of patients with AIP, we describe the relationship between retroperitoneal fibrosis and IgG4-related disease, their clinical features, and the effect of therapies.

EPIDEMIOLOGY

Idiopathic retroperitoneal fibrosis is a rare disease, and epidemiological data are seldom found. However, epidemiological data from Finland indicate that idiopathic retroperitoneal fibrosis affects 0.1 in every 100 000 people, and the prevalence is 1.38 in every 100 000 people[14]. Onset of idiopathic retroperitoneal fibrosis most frequently occurs at and above the age of 50 years. Idiopathic retroperitoneal fibrosis is 2-3 times more common in men than in women[1,14]. About 30% of retroperitoneal fibrosis occurs secondary to a particular cause such as drug therapy, malignant tumors, infection, radiation therapy, or surgery[1,3] (Table 1).

Owing to the establishment of the concepts of AIP and IgG4-related disease, we now know that many previously diagnosed cases of idiopathic retroperitoneal fibrosis are IgG4-related (extrapancreatic lesions of AIP)[3,15]. However, the concept of IgG4-related disease is relatively new, and no large-scale epidemiological data are available to estimate what percentage of retroperitoneal fibrosis is actually IgG4-related. Thus, the exact frequency of IgG4-related retroperitoneal fibrosis is unknown. Corradi *et al*[3] reported that IgG4-positive cells were found in most cases of idiopathic retroperitoneal fibrosis. Neild *et al*[16] studied 12 patients with idiopathic retroperitoneal fibrosis and reported that the number of IgG4-positive plasma cells was > 20 per high power field in 7 of 9 patients whose retroperitoneal tissues were examined. Zen *et al*[15] reported that among 17 patients who had a histological diagnosis of retroperitoneal fibrosis, 10 patients (59%) had increased serum IgG4 levels and extensive IgG4-positive plasma cell infiltration, all of whom were men 50 years or older. Yamashita *et al*[17] reported that among 29 patients studied, the ratio of IgG4-positive plasma cells to IgG-positive plasma cells was > 20% in 9 patients (31%), and these 9 patients were considered to have IgG4-related retroperitoneal fibrosis. As described above, the proportion of IgG4-related retroperitoneal fibrosis among all cases of retroperitoneal fibrosis varies between the studies (30%-60%), and the scale of the studies is invariably small. Further studies at a larger scale need to be conducted.

On the other hand, most of the studies to assess the frequency of concurrent retroperitoneal fibrosis in IgG4-related disease were conducted in patients with AIP[18-27]. In many of the studies, concurrent retroperi-

Table 1 Etiological factors of retroperitoneal fibrosis

Etiological factors	
Idiopathic RF	
IgG4-related lesions	Frequency unknown
Secondary RF	
Drugs	Analgesics, β-blockers, *etc.*
Malignant diseases	Malignant lymphoma, *etc.*
Infections	Tuberculosis, *etc.*
Radiotherapy	Colon cancer, pancreatic cancer, *etc.*
Surgery	Lymphadenectomy, colectomy, *etc.*
Others	Trauma, *etc.*

RF: Retroperitoneal fibrosis.

Table 2 Retroperitoneal fibrosis as extrapancreatic lesions of autoimmune pancreatitis

Cases studied: 51 patients with AIP (2002–2011)	
Concurrent RF	8 patients (15.7)
Synchronized onset	5 patients
Heterochronic onset	3 patients
Preceding the diagnosis of AIP	2 patients
Following the diagnosis of AIP	1 patient
Treatment: Steroid therapy at a dose of 30–40 mg per day	
Improved	7 patients (87.5)
Unchanged	1 patient (12.5)

RF: Retroperitoneal fibrosis; AIP: Autoimmune pancreatitis.

Table 3 Frequency of concurrent retroperitoneal fibrosis in immunoglobulin G4-related disease

Ref.	Disease	Total number of patients	Patients with RF *n*	Frequency of concurrent RF
Takuma *et al*[18]	AIP	56	4	7.1
Kamisawa *et al*[19]	AIP	459	50	10.9
Hirano *et al*[20]	AIP	42	8	19.0
Ohara *et al*[21]	AIP	132	8	6.1
Hamano *et al*[22]	AIP	64	8	12.5
Ryu *et al*[23]	AIP	67	2	3.0
Song *et al*[24]	AIP	25	1	4.0
Chari *et al*[25]	AIP	29	3	10.3
Raina *et al*[26]	AIP	26	2	7.7
Maire *et al*[27]	AIP	28	1	3.6
Ohta *et al*[28]	IgG4-related sclerosing sialadenitis	10	1	10.0
Zen *et al*[29]	IgG4-related sclerosing disease	114	13	11.4
Our cases	AIP	51	8	15.7
Total	IgG4-related disease	1103	109	9.9

RF: Retroperitoneal fibrosis; AIP: Autoimmune pancreatitis; IgG4: Immunoglobulin G4.

toneal fibrosis, represented by an extrapancreatic lesion, was reported in about 10% of patients with AIP. Takuma *et al*[18] reported that concurrent retroperitoneal fibrosis was found in 4 out of 56 patients with AIP (7%); of these, the onset of retroperitoneal fibrosis coincided with the onset of AIP in 2 patients, preceded the onset of AIP in 1 patient, and occurred 1 year after bypass surgery performed for AIP in 1 patient. Hamano *et al*[22] reported that concurrent retroperitoneal fibrosis was found in 8 out of 64 patients with AIP (12.5%). The onset of retroperitoneal fibrosis was heterochronic to the onset of AIP in 6 patients; the onset of retroperitoneal fibrosis preceded the onset of AIP in 3 patients and followed the onset of AIP in the other 3 patients[22]. Hirano *et al*[20] reported that concurrent retroperitoneal fibrosis was found in 8 out of 42 patients with AIP (19%). Ohara *et al*[21] reported that retroperitoneal fibrosis, as represented by an extrapancreatic lesion, was found in 8 out of 132 Japanese patients with AIP (6.1%). Moreover, the results of a Japanese multicenter study indicate that concurrent retroperitoneal fibrosis was found in 50 out of 459 patients with AIP who received steroid therapy (11%)[19]. The frequencies of concurrent retroperitoneal fibrosis reported in other countries are as follows: about 3%-4% in Asian countries including South Korea and China[23,24], about 8%-10% in the United States[25,26], and 3.6% in Europe[27]. In our experience, retroperitoneal fibrosis, as represented by an extrapancreatic lesion, was found in 8 of 51 patients (15.7%) diagnosed with AIP and treated at Kyushu University Hospital (Table 2). Of these, the onset of retroperitoneal fibrosis was synchronized with the onset of AIP in 5 patients (case presented in Figure 1), and was heterochronic to the onset of AIP in 3 patients. Regarding heterochronic onset of retroperitoneal fibrosis, the onset of retroperitoneal fibrosis preceded the onset of AIP in 2 patients (cases presented in Figure 2) and occurred 6.5 years after pancreatectomy in 1 patient with suspected pancreatic cancer.

There are reports on IgG4-related retroperitoneal fibrosis in diseases other than AIP. Ohta *et al*[28] reported concurrent retroperitoneal fibrosis in 1 out of 10 patients (10%) with IgG4-related sclerosing sialadenitis. Zen *et al*[29] reported concurrent retroperitoneal fibrosis in 13 out of 114 patients (11.4%) with histologically diagnosed IgG4-related sclerosing disease. Although further accumulation of case data is necessary to determine the accurate frequency of concurrent retroperitoneal fibrosis, the aforementioned studies suggest that concurrent retroperitoneal fibrosis occurs in about 10% of IgG4-related disease (Table 3). The concept of IgG4-related disease encompasses various disease conditions occurring in various organs; however, the primary cause of IgG4-related disease has not been elucidated. Moreover, it is unclear what clinical features determine the involvement of various organs, including the retroperitoneum. This needs to be elucidated in the future.

DIAGNOSIS

Retroperitoneal fibrosis has no specific symptoms. However, dull abdominal (flank) pain, back pain, general malaise, fever, and weight loss have been known to occur.

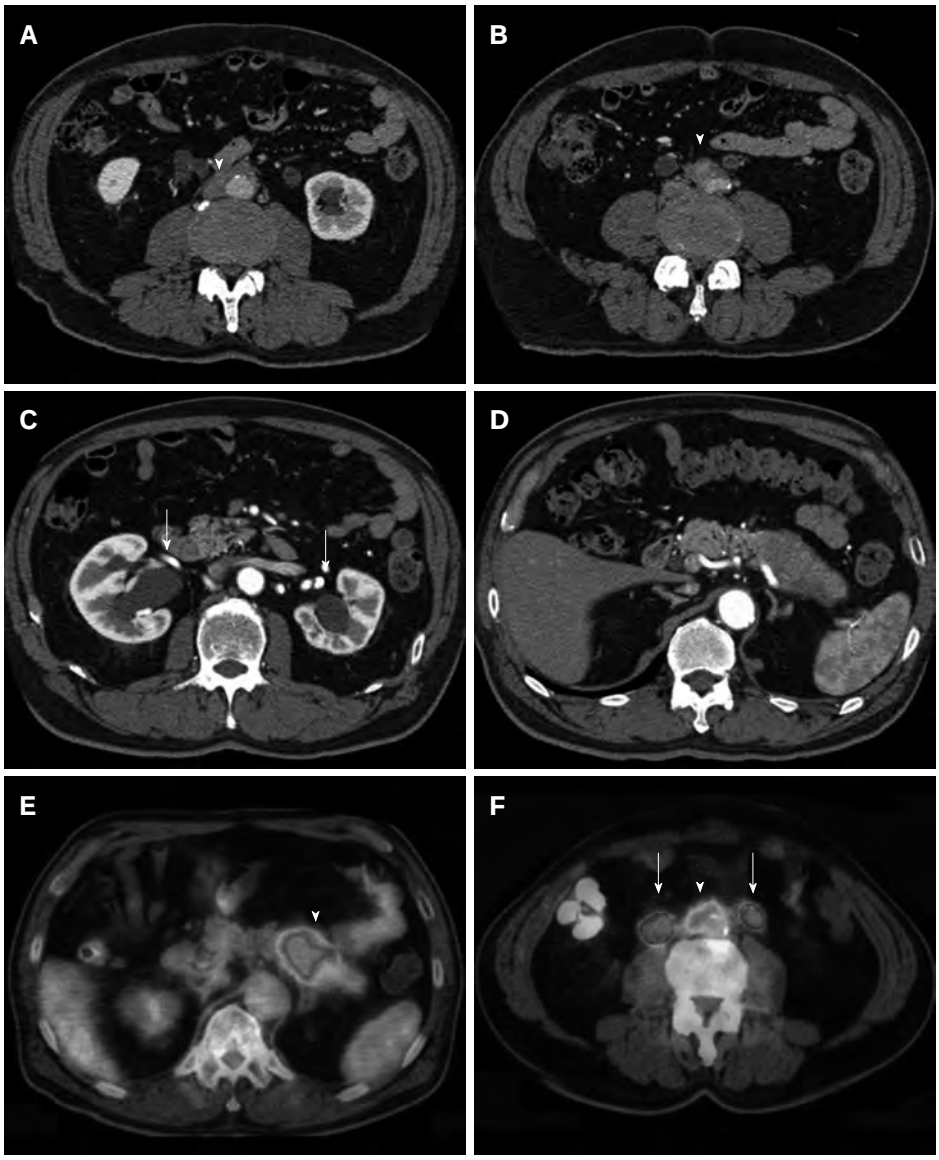


Figure 1 Typical retroperitoneal fibrosis associated with autoimmune pancreatitis. A, B: Soft tissue (arrow) surrounds the abdominal aorta and common iliac artery; C: Bilateral hydronephrosis (arrows); D: Swelling and capsule-like rim are seen in the body and tail of the pancreas. This case was diagnosed as autoimmune pancreatitis; E, F: Fluorodeoxyglucose positron emission tomography images from the same patient; high fluorodeoxyglucose uptake is seen in the body and tail of the pancreas (arrowhead) and in the retroperitoneal fibrosis lesions (arrowhead) surrounding the common iliac artery (arrows indicate dilated urinary ducts).

Retroperitoneal fibrosis often surrounds the urinary duct or inferior vena cava, and is often found in association with hydronephrosis or swelling of the lower extremities[1]. In recent times, retroperitoneal fibrosis is often detected during diagnostic imaging, such as a computed tomography (CT) scan, in asymptomatic patients undergoing a work-up for AIP (Figure 1). However, it should be noted that the onset of retroperitoneal fibrosis may or may not be synchronized with the onset of AIP. Furthermore, the onset of retroperitoneal fibrosis may either follow or precede the onset of AIP as described earlier. The histological features of retroperitoneal fibrosis associated with AIP (i.e., IgG4-related retroperitoneal fibrosis) include diffused fibrosis, infiltration of IgG4-positive plasma cells, obliterative phlebitis, eosinophilic infiltration, and the formation of lymphoid follicles; these are similar to other lesions of IgG4-related disease[15,16]. Zen *et al*[15] reported that obliterative phlebitis is significantly more frequent in IgG4-related retroperitoneal fibrosis than in non-IgG4-related retroperitoneal fibrosis. However, because surgery for retroperitoneal fibrosis is rarely performed and because a biopsy of the retroperitoneum cannot be easily carried out, it is often difficult to obtain the tissue specimens needed for diagnosis. Clinically, retroperitoneal fibrosis is diagnosed based on the results of diagnostic imaging such as CT scan and magnetic resonance imaging (MRI)[30]. Involvement of IgG4 is usually determined based on serum IgG4 levels and the presence/absence of other lesions such as AIP. Moreover, the effect of therapies is usually assessed using diagnostic imaging techniques.

There is no hematological parameter specifically associated with retroperitoneal fibrosis; however, an increased white blood cell count and increased C-reactive

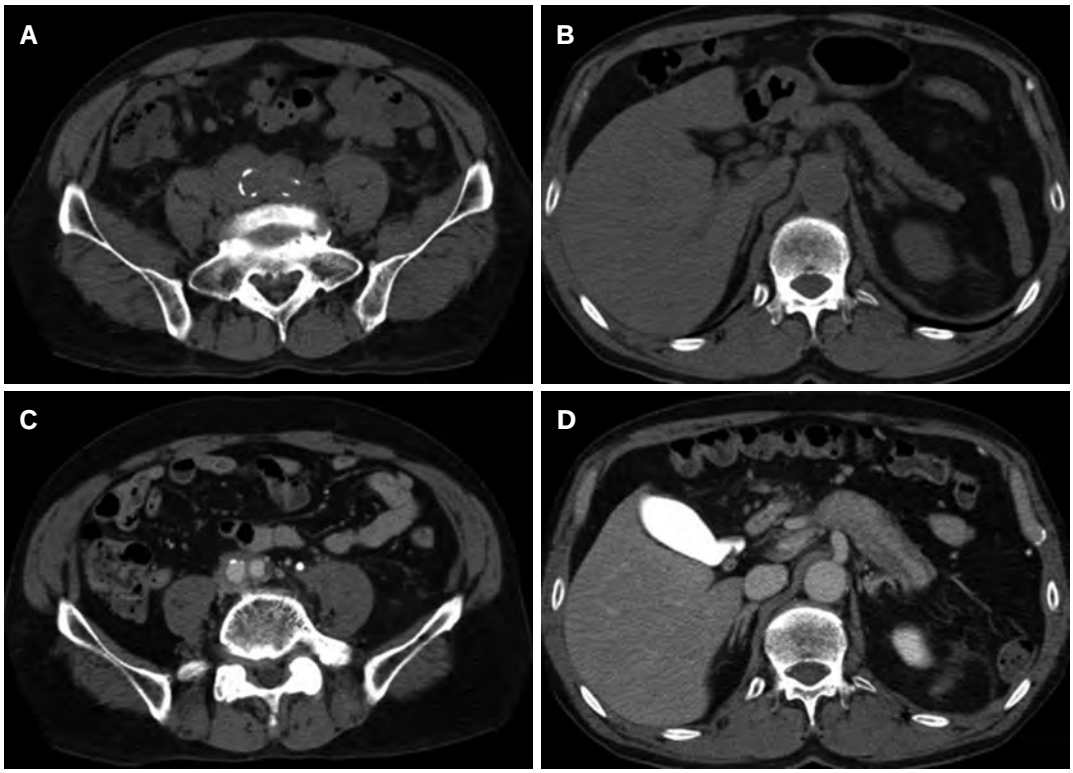


Figure 2 Retroperitoneal fibrosis occurring before the onset of autoimmune pancreatitis. A 65-year-old man developed hydronephrosis and renal failure. A, B: Soft tissue was observed surrounding the abdominal aorta and common iliac artery. No abnormality was found in the pancreas. The patient was diagnosed with idiopathic retroperitoneal fibrosis, and started receiving prednisolone (PSL) at the dose of 40 mg/d. The dose was gradually reduced over approximately 6 mo and discontinued. The hydronephrosis improved, and the patient continued to be followed up; C, D: Swelling of the pancreas observed on computed tomography images taken about 1 yr and 6 mo after the discontinuation of PSL. Compared with the initial observation, retroperitoneal fibrosis improved, but persisted. The serum immunoglobulin G4 level was 366 mg/dL. At that point, the patient was diagnosed with autoimmune pancreatitis, and started receiving PSL again at the dose of 40 mg/d. The swelling of the pancreas and retroperitoneal fibrosis were improved.

protein levels can be seen as a reflection of acute inflammation. Moreover, renal dysfunction may be observed due to ureteral obstruction. Diagnostic imaging techniques, such as ultrasound (US), CT, and MRI, are used to diagnose retroperitoneal fibrosis. US is usually performed first, because it is a simple and minimally invasive technique. Typically, retroperitoneal fibrosis is depicted as a hypo- to iso-echoic mass encasing an aorta; however, the sensitivity is low, and US is rather important in determining the presence/absence of concurrent hydronephrosis. The most useful diagnostic imaging techniques are CT and MRI. On CT images, retroperitoneal fibrosis is depicted as a soft tissue mass encasing an aorta, which often spreads laterally to involve the inferior vena cava and urinary duct[31] (Figure 1A-C). On MRI images, retroperitoneal fibrosis shows low signal intensity on T1-weighted images, and high signal intensity on T2-weighted images[1,31]. Moreover, although CT and MRI excel in their specificity, fluorodeoxyglucose positron emission tomography is also a useful tool for determination of the presence/absence of retroperitoneal fibrosis as well as other lesions of IgG4-related disease[1,32,33] (Figure 1D-F).

The retroperitoneum is also a site where tumor lesions, such as malignant lymphoma, desmoid fibromatosis, and liposarcoma, often develop. Thus, these diseases need to be differentiated from retroperitoneal fibrosis[1,3]. Although the onset of retroperitoneal fibrosis surrounding the pancreas is rare, this condition is sometimes difficult to distinguish from the invasion of pancreatic cancer to the area surrounding the superior mesenteric artery. In patients with previously diagnosed AIP in whom the onset of retroperitoneal fibrosis has occurred simultaneously with the onset (Figure 1) or relapse of AIP, retroperitoneal fibrosis can be relatively easily diagnosed based on the aforementioned characteristic findings on US/CT/MRI images and the presence of increased serum IgG4 levels. On the other hand, in some patients, the onset of retroperitoneal fibrosis precedes the onset of AIP (Figure 2); thus, the measurement of serum IgG4 levels is recommended for idiopathic retroperitoneal fibrosis[34]. When the IgG4 level is high, it should be treated and monitored as an IgG4-related disease.

TREATMENT AND PROGNOSIS

The first line of treatment for retroperitoneal fibrosis is steroid therapy, regardless of its cause. Although a few asymptomatic patients might need only monitoring, steroid therapy is usually necessary for patients with clinical symptoms (e.g., fatigue, weight loss, and abdominal or back pain) or those with hydronephrosis and acute renal

failure caused by ureteral obstruction[35]. The efficacy of immunosuppressants (e.g., azathioprine) and tamoxifen has been reported mainly in Europe and the United States[1,36,37]. However, a randomized controlled trial recently conducted in patients with idiopathic retroperitoneal fibrosis demonstrated that the efficacy of steroids in preventing the relapse of retroperitoneal fibrosis was significantly better than that of tamoxifen[35]. Moreover, Zen *et al*[15] reported that steroid therapy was effective regardless of the presence/absence of IgG4-related disease or serum IgG4 levels[15].

Steroid therapy is also recognized as a standard therapy for AIP[38]. The indications of steroid are symptoms such as obstructive jaundice due to sclerosing cholangitis, abdominal pain, and hydronephrosis due to associated retroperitoneal fibrosis[19]. Therefore, steroid therapy is strongly recommended for patients with concurrent AIP, that is, IgG4-related retroperitoneal fibrosis. The starting dose of steroid is usually 30-40 mg/d (0.6 mg/kg per day)[38]. The response to steroid therapy is generally favorable. In most cases, the pancreatic lesion and retroperitoneal fibrosis improve after the initial treatment. Among the 8 patients we studied, clinical improvement was achieved in 7 patients. In 1 patient, the disease condition did not change (Table 2). As mentioned earlier, the response to steroid therapy is generally good; however, some patients are resistant to steroid. Further, the mechanism of steroid resistance is unclear, and it is difficult to predict the efficacy of steroid therapy before treatment. In our patient who was refractory to steroid, soft tissue mass surrounding the abdominal aorta and hydronephrosis did not improve; however, his pancreatic lesion was improved and renal function was stabilized. Therefore, we continued with the maintenance steroid treatment. Zen *et al*[15] reported that 8 of 10 patients with IgG4-related retroperitoneal fibrosis received steroid therapy. One of these steroid-treated patients experienced relapse twice; however, remission of retroperitoneal fibrosis was achieved by re-administration of steroid. In some cases, relapse occurs after withdrawal from steroid therapy. In other cases, the ureteral stent cannot be removed because of inadequate response to steroid therapy. Thus, further study should be conducted to assess the long-term prognoses of retroperitoneal fibrosis. Although the use of immunosuppressants has been reported in patients with IgG4-related disease that is recurrent or non-responsive to steroid therapy, no consensus has been reached yet[26,27,39].

CONCLUSION

Owing to the newly emerged recognition of retroperitoneal fibrosis as an extrapancreatic lesion of AIP and the establishment of the concept of IgG4-related disease, diagnostic methods and therapies for retroperitoneal fibrosis have made great progress. Retroperitoneal fibrosis, as well as other lesions of IgG4-related disease, responds to steroid therapy in most cases. However, data from more cases need to be collected in order to assess the long-term prognoses of retroperitoneal fibrosis.

REFERENCES

1 **Vaglio A**, Salvarani C, Buzio C. Retroperitoneal fibrosis. *Lancet* 2006; **367**: 241-251 [PMID: 16427494 DOI: 10.1016/S0140-6736(06)68035-5]

2 **Ormond JK**. Bilateral ureteral obstruction due to envelopment and compression by an inflammatory retroperitoneal process. *J Urol* 1948; **59**: 1072-1079 [PMID: 18858051]

3 **Corradi D**, Maestri R, Palmisano A, Bosio S, Greco P, Manenti L, Ferretti S, Cobelli R, Moroni G, Dei Tos AP, Buzio C, Vaglio A. Idiopathic retroperitoneal fibrosis: clinicopathologic features and differential diagnosis. *Kidney Int* 2007; **72**: 742-753 [PMID: 17622270 DOI: 10.1038/sj.ki.5002427]

4 **Yoshida K**, Toki F, Takeuchi T, Watanabe S, Shiratori K, Hayashi N. Chronic pancreatitis caused by an autoimmune abnormality. Proposal of the concept of autoimmune pancreatitis. *Dig Dis Sci* 1995; **40**: 1561-1568 [PMID: 7628283 DOI: 10.1007/BF02285209]

5 **Ito T**, Nakano I, Koyanagi S, Miyahara T, Migita Y, Ogoshi K, Sakai H, Matsunaga S, Yasuda O, Sumii T, Nawata H. Autoimmune pancreatitis as a new clinical entity. Three cases of autoimmune pancreatitis with effective steroid therapy. *Dig Dis Sci* 1997; **42**: 1458-1468 [PMID: 9246047]

6 **Hamano H**, Kawa S, Horiuchi A, Unno H, Furuya N, Akamatsu T, Fukushima M, Nikaido T, Nakayama K, Usuda N, Kiyosawa K. High serum IgG4 concentrations in patients with sclerosing pancreatitis. *N Engl J Med* 2001; **344**: 732-738 [PMID: 11236777 DOI: 10.1056/NEJM200103083441005]

7 **Hamano H**, Kawa S, Ochi Y, Unno H, Shiba N, Wajiki M, Nakazawa K, Shimojo H, Kiyosawa K. Hydronephrosis associated with retroperitoneal fibrosis and sclerosing pancreatitis. *Lancet* 2002; **359**: 1403-1404 [PMID: 11978339 DOI: 10.1016/S0140-6736(02)08359-9]

8 **Kamisawa T**, Funata N, Hayashi Y, Eishi Y, Koike M, Tsuruta K, Okamoto A, Egawa N, Nakajima H. A new clinicopathological entity of IgG4-related autoimmune disease. *J Gastroenterol* 2003; **38**: 982-984 [PMID: 14614606 DOI: 10.1007/s00535-003-1175-y]

9 **Kamisawa T**, Okamoto A. Autoimmune pancreatitis: proposal of IgG4-related sclerosing disease. *J Gastroenterol* 2006; **41**: 613-625 [PMID: 16932997 DOI: 10.1007/s00535-006-1862-6]

10 **Masaki Y**, Dong L, Kurose N, Kitagawa K, Morikawa Y, Yamamoto M, Takahashi H, Shinomura Y, Imai K, Saeki T, Azumi A, Nakada S, Sugiyama E, Matsui S, Origuchi T, Nishiyama S, Nishimori I, Nojima T, Yamada K, Kawano M, Zen Y, Kaneko M, Miyazaki K, Tsubota K, Eguchi K, Tomoda K, Sawaki T, Kawanami T, Tanaka M, Fukushima T, Sugai S, Umehara H. Proposal for a new clinical entity, IgG4-positive multiorgan lymphoproliferative syndrome: analysis of 64 cases of IgG4-related disorders. *Ann Rheum Dis* 2009; **68**: 1310-1315 [PMID: 18701557 DOI: 10.1136/ard.2008.089169]

11 **Kawano M**, Saeki T, Nakashima H, Nishi S, Yamaguchi Y, Hisano S, Yamanaka N, Inoue D, Yamamoto M, Takahashi H, Nomura H, Taguchi T, Umehara H, Makino H, Saito T. Proposal for diagnostic criteria for IgG4-related kidney disease. *Clin Exp Nephrol* 2011; **15**: 615-626 [PMID: 21898030 DOI: 10.1007/s10157-011-0521-2]

12 **Stone JH**, Zen Y, Deshpande V. IgG4-related disease. *N Engl J Med* 2012; **366**: 539-551 [PMID: 22316447 DOI: 10.1056/NEJMra1104650]

13 **Umehara H**, Okazaki K, Masaki Y, Kawano M, Yamamoto M, Saeki T, Matsui S, Yoshino T, Nakamura S, Kawa S, Hamano H, Kamisawa T, Shimosegawa T, Shimatsu A, Nakamura S, Ito T, Notohara K, Sumida T, Tanaka Y, Mimori

T, Chiba T, Mishima M, Hibi T, Tsubouchi H, Inui K, Ohara H. Comprehensive diagnostic criteria for IgG4-related disease (IgG4-RD), 2011. *Mod Rheumatol* 2012; **22**: 21-30 [PMID: 22218969 DOI: 10.1007/s10165-011-0571-z]

14 **Uibu T**, Oksa P, Auvinen A, Honkanen E, Metsärinne K, Saha H, Uitti J, Roto P. Asbestos exposure as a risk factor for retroperitoneal fibrosis. *Lancet* 2004; **363**: 1422-1426 [PMID: 15121404 DOI: 10.1016/S0140-6736(04)16100-X]

15 **Zen Y**, Onodera M, Inoue D, Kitao A, Matsui O, Nohara T, Namiki M, Kasashima S, Kawashima A, Matsumoto Y, Katayanagi K, Murata T, Ishizawa S, Hosaka N, Kuriki K, Nakanuma Y. Retroperitoneal fibrosis: a clinicopathologic study with respect to immunoglobulin G4. *Am J Surg Pathol* 2009; **33**: 1833-1839 [PMID: 19950407 DOI: 10.1097/PAS.0b013e3181b72882]

16 **Neild GH**, Rodriguez-Justo M, Wall C, Connolly JO. Hyper-IgG4 disease: report and characterisation of a new disease. *BMC Med* 2006; **4**: 23 [PMID: 17026742 DOI: 10.1186/1741-7015-4-23]

17 **Yamashita K**, Haga H, Mikami Y, Kanematsu A, Nakashima Y, Kotani H, Ogawa O, Manabe T. Degree of IgG4+ plasma cell infiltration in retroperitoneal fibrosis with or without multifocal fibrosclerosis. *Histopathology* 2008; **52**: 404-409 [PMID: 18269592 DOI: 10.1111/j.1365-2559.2007.02959.x]

18 **Takuma K**, Kamisawa T, Anjiki H, Egawa N, Igarashi Y. Metachronous extrapancreatic lesions in autoimmune pancreatitis. *Intern Med* 2010; **49**: 529-533 [PMID: 20228586 DOI: 10.2169/internalmedicine.49.3038]

19 **Kamisawa T**, Shimosegawa T, Okazaki K, Nishino T, Watanabe H, Kanno A, Okumura F, Nishikawa T, Kobayashi K, Ichiya T, Takatori H, Yamakita K, Kubota K, Hamano H, Okamura K, Hirano K, Ito T, Ko SB, Omata M. Standard steroid treatment for autoimmune pancreatitis. *Gut* 2009; **58**: 1504-1507 [PMID: 19398440 DOI: 10.1136/gut.2008.172908]

20 **Hirano K**, Tada M, Isayama H, Yagioka H, Sasaki T, Kogure H, Nakai Y, Sasahira N, Tsujino T, Yoshida H, Kawabe T, Omata M. Long-term prognosis of autoimmune pancreatitis with and without corticosteroid treatment. *Gut* 2007; **56**: 1719-1724 [PMID: 17525092 DOI: 10.1136/gut.2006.115246]

21 **Ohara H**, Nakazawa T, Ando T, Joh T. Systemic extrapancreatic lesions associated with autoimmune pancreatitis. *J Gastroenterol* 2007; **42** Suppl 18: 15-21 [PMID: 17520218 DOI: 10.1007/s00535-007-2045-9]

22 **Hamano H**, Arakura N, Muraki T, Ozaki Y, Kiyosawa K, Kawa S. Prevalence and distribution of extrapancreatic lesions complicating autoimmune pancreatitis. *J Gastroenterol* 2006; **41**: 1197-1205 [PMID: 17287899 DOI: 10.1007/s00535-006-1908-9]

23 **Ryu JK**, Chung JB, Park SW, Lee JK, Lee KT, Lee WJ, Moon JH, Cho KB, Kang DW, Hwang JH, Yoo KS, Yoo BM, Lee DH, Kim HK, Moon YS, Lee J, Lee HS, Choi HS, Lee SK, Kim YT, Kim CD, Kim SJ, Hahm JS, Yoon YB. Review of 67 patients with autoimmune pancreatitis in Korea: a multicenter nationwide study. *Pancreas* 2008; **37**: 377-385 [PMID: 18953249 DOI: 10.1097/MPA.0b013e31817a0914]

24 **Song Y**, Liu QD, Zhou NX, Zhang WZ, Wang DJ. Diagnosis and management of autoimmune pancreatitis: experience from China. *World J Gastroenterol* 2008; **14**: 601-606 [PMID: 18203294 DOI: 10.3748/wjg.14.601]

25 **Chari ST**, Smyrk TC, Levy MJ, Topazian MD, Takahashi N, Zhang L, Clain JE, Pearson RK, Petersen BT, Vege SS, Farnell MB. Diagnosis of autoimmune pancreatitis: the Mayo Clinic experience. *Clin Gastroenterol Hepatol* 2006; **4**: 1010-1016; quiz 934 [PMID: 16843735 DOI: 10.1016/j.cgh.2006.05.017]

26 **Raina A**, Yadav D, Krasinskas AM, McGrath KM, Khalid A, Sanders M, Whitcomb DC, Slivka A. Evaluation and management of autoimmune pancreatitis: experience at a large US center. *Am J Gastroenterol* 2009; **104**: 2295-2306 [PMID: 19532132 DOI: 10.1038/ajg.2009.325]

27 **Maire F**, Le Baleur Y, Rebours V, Vullierme MP, Couvelard A, Voitot H, Sauvanet A, Hentic O, Lévy P, Ruszniewski P, Hammel P. Outcome of patients with type 1 or 2 autoimmune pancreatitis. *Am J Gastroenterol* 2011; **106**: 151-156 [PMID: 20736934 DOI: 10.1038/ajg.2010.314]

28 **Ohta N**, Kurakami K, Ishida A, Furukawa T, Saito F, Kakehata S, Izuhara K. Clinical and pathological characteristics of IgG4-related sclerosing sialadenitis. *Laryngoscope* 2012; **122**: 572-577 [PMID: 22241660 DOI: 10.1002/lary.22449]

29 **Zen Y**, Nakanuma Y. IgG4-related disease: a cross-sectional study of 114 cases. *Am J Surg Pathol* 2010; **34**: 1812-1819 [PMID: 21107087 DOI: 10.1097/PAS.0b013e3181f7266b]

30 **Kermani TA**, Crowson CS, Achenbach SJ, Luthra HS. Idiopathic retroperitoneal fibrosis: a retrospective review of clinical presentation, treatment, and outcomes. *Mayo Clin Proc* 2011; **86**: 297-303 [PMID: 21454732 DOI: 10.4065/mcp.2010.0663]

31 **Vivas I**, Nicolás AI, Velázquez P, Elduayen B, Fernández-Villa T, Martínez-Cuesta A. Retroperitoneal fibrosis: typical and atypical manifestations. *Br J Radiol* 2000; **73**: 214-222 [PMID: 10884739]

32 **Ozaki Y**, Oguchi K, Hamano H, Arakura N, Muraki T, Kiyosawa K, Momose M, Kadoya M, Miyata K, Aizawa T, Kawa S. Differentiation of autoimmune pancreatitis from suspected pancreatic cancer by fluorine-18 fluorodeoxyglucose positron emission tomography. *J Gastroenterol* 2008; **43**: 144-151 [PMID: 18306988 DOI: 10.1007/s00535-007-2132-y]

33 **Shimosegawa T**, Kanno A. Autoimmune pancreatitis in Japan: overview and perspective. *J Gastroenterol* 2009; **44**: 503-517 [PMID: 19377842 DOI: 10.1007/s00535-009-0054-6]

34 **Miura H**, Miyachi Y. IgG4-related retroperitoneal fibrosis and sclerosing cholangitis independent of autoimmune pancreatitis. A recurrent case after a 5-year history of spontaneous remission. *JOP* 2009; **10**: 432-437 [PMID: 19581751]

35 **Vaglio A**, Palmisano A, Alberici F, Maggiore U, Ferretti S, Cobelli R, Ferrozzi F, Corradi D, Salvarani C, Buzio C. Prednisone versus tamoxifen in patients with idiopathic retroperitoneal fibrosis: an open-label randomised controlled trial. *Lancet* 2011; **378**: 338-346 [PMID: 21733570 DOI: 10.1016/S0140-6736(11)60934-3]

36 **Marcolongo R**, Tavolini IM, Laveder F, Busa M, Noventa F, Bassi P, Semenzato G. Immunosuppressive therapy for idiopathic retroperitoneal fibrosis: a retrospective analysis of 26 cases. *Am J Med* 2004; **116**: 194-197 [PMID: 14749165 DOI: 10.1016/j.amjmed.2003.08.033]

37 **van Bommel EF**, Hendriksz TR, Huiskes AW, Zeegers AG. Brief communication: tamoxifen therapy for nonmalignant retroperitoneal fibrosis. *Ann Intern Med* 2006; **144**: 101-106 [PMID: 16418409]

38 **Ito T**, Nishimori I, Inoue N, Kawabe K, Gibo J, Arita Y, Okazaki K, Takayanagi R, Otsuki M. Treatment for autoimmune pancreatitis: consensus on the treatment for patients with autoimmune pancreatitis in Japan. *J Gastroenterol* 2007; **42** Suppl 18: 50-58 [PMID: 17520224 DOI: 10.1007/s00535-007-2051-y]

39 **Ghazale A**, Chari ST, Zhang L, Smyrk TC, Takahashi N, Levy MJ, Topazian MD, Clain JE, Pearson RK, Petersen BT, Vege SS, Lindor K, Farnell MB. Immunoglobulin G4-associated cholangitis: clinical profile and response to therapy. *Gastroenterology* 2008; **134**: 706-715 [PMID: 18222442 DOI: 10.1053/j.gastro.2007.12.009]

P- Reviewer Masamun A **S- Editor** Gou SX
L- Editor A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i1.42

World J Gastroenterol 2013 January 7; 19(1): 42-48
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

REVIEW

Nestin: A novel angiogenesis marker and possible target for tumor angiogenesis

Yoko Matsuda, Masahito Hagio, Toshiyuki Ishiwata

Yoko Matsuda, Masahito Hagio, Toshiyuki Ishiwata, Departments of Pathology and Integrative Oncological Pathology, Nippon Medical School, Tokyo 113-8602, Japan
Author contributions: Matsuda Y and Ishiwata T conceived the topic, reviewed the literature, and prepared the initial manuscript; Hagio M produced the final manuscript.
Supported by Grants-in-Aid for Young Scientists, No. 22689038 (to Matsuda Y); Grants-in-Aid for Challenging Exploratory Research, No. 23650604 (to Matsuda Y); Grants-in-Aid for Scientific Research, No. 22591531 (to Ishiwata T) from the Japan Society for the Promotion of Science; and the Pancreas Research Foundation of Japan (to Hagio M)
Correspondence to: Toshiyuki Ishiwata, MD, PhD, Departments of Pathology and Integrative Oncological Pathology, Nippon Medical School, 1-1-5 Sendagi, Bunkyo-ku, Tokyo 113-8602, Japan. ishiwata@nms.ac.jp
Telephone: +81-3-38222131 Fax: +81-3-58146274
Received: June 5, 2012 Revised: July 31, 2012
Accepted: August 3, 2012
Published online: January 7, 2013

Abstract

Abnormal vasculature, termed tumor vessels, is a hallmark of solid tumors. The degree of angiogenesis is associated with tumor aggressiveness and clinical outcome. Therefore, exact quantification of tumor vessels is useful to evaluate prognosis. Furthermore, selective detection of newly formed tumor vessels within cancer tissues using specific markers raises the possibility of molecular targeted therapy *via* the inhibition of tumor angiogenesis. Nestin, an intermediate filament protein, is reportedly expressed in repair processes, various neoplasms, and proliferating vascular endothelial cells. Nestin expression is detected in endothelial cells of embryonic capillaries, capillaries of the corpus luteum, which replenishes itself by angiogenesis, and proliferating endothelial progenitor cells, but not in mature endothelial cells. Therefore, expression of nestin is relatively limited to proliferating vascular endothelial cells and endothelial progenitor cells. Nestin expression is also reported in blood vessels within glioblastoma, prostate cancer, colorectal cancer, and pancreatic cancer, and its expression is more specific for newly formed blood vessels than other endothelial cell markers. Nestin-positive blood vessels form smaller vessels with high proliferation activity in tumors. Knockdown of nestin in vascular endothelial cells suppresses endothelial cell growth and tumor formation ability of pancreatic cancers *in vivo*. Using nestin to more accurately evaluate microvessel density in cancer specimens may be a novel prognostic indicator. Furthermore, nestin-targeted therapy may suppress tumor proliferation *via* inhibition of angiogenesis in numerous malignancies, including pancreatic cancer. In this review article, we focus on nestin as a novel angiogenesis marker and possible therapeutic target *via* inhibition of tumor angiogenesis.

© 2013 Baishideng. All rights reserved.

Key words: Nestin; Angiogenesis; Molecular targeting therapy; Gastrointestinal cancer; Pancreatic cancer; Microvessel density; CD34; CD31; Factor Ⅷ

Matsuda Y, Hagio M, Ishiwata T. Nestin: A novel angiogenesis marker and possible target for tumor angiogenesis. *World J Gastroenterol* 2013; 19(1): 42-48 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i1/42.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i1.42

INTRODUCTION

A hallmark of solid tumors is abnormal vasculature, termed tumor vessels, which is adjacent to the tumor itself. Greater number of tumor vessels increases the risk that tumor cells will enter systemic circulation. The basement membranes of newly formed tumor vessels are highly permeable, enabling tumor cells to penetrate them more easily than mature vessels[1]. Furthermore, tumor angiogenesis enhances the supply of oxygen and nutrients to solid tumor cells. As a result, the tumor grows more rapidly and easily when tumor vessels are formed

in close proximity. After a tumor has attained a size of 1-2 mm, the induction of new blood vessel formation is required for further growth and expansion[2]. In fact, the degree of tumor angiogenesis reportedly correlates with clinical outcome, and angiogenic properties correlate with tumor aggressiveness in gastrointestinal (GI) cancers including colorectal cancers[3], gastric cancers[4], esophageal cancers[5], and pancreatic cancers[6,7]. These findings suggest that exact quantification of tumor vessels is useful to evaluate prognosis. Furthermore, selective detection of newly formed tumor vessels within cancer tissues using specific markers raises the possibility of molecular targeted therapy *via* the inhibition of tumor angiogenesis. Microvessel density (MVD), measured immunohistochemically in tumors, is often reported to correlate with prognosis in various malignancies[8,9], including GI cancers[10-14]. However, the clinical significance of MVD is controversial; for example, pancreatic cancer is characterized by hypovascularization[15,16]. For MVD analysis, CD34, CD31, and factor Ⅷ are commonly used as endothelial cell markers in tumor vessels (Table 1). CD34 and CD31 are cell surface markers, and factor Ⅷ is an essential blood clotting protein in vascular endothelial cells of normal tissues. However, these markers do not specifically identify newly formed tumor vessels, but also detect pre-existing large sized blood vessels[17]; therefore, they are not considered suitable for the sensitive analysis of tumor angiogenesis. Inclusion of pre-existing mature vessels in MVD of various tumors may cause controversial results when determining the relationship between tumor angiogenesis, clinicopathologic features, and prognosis.

Nestin, an intermediate filament (IF) protein, has recently been recognized as a more specific marker for newly formed blood vessels and as a therapeutic target *via* inhibition of angiogenesis. In this review, we summarize and emphasize recent research evidences concerning nestin in tumor angiogenesis in GI cancers, including pancreatic cancer.

BASIC CHARACTERIZATION OF NESTIN

Nestin is a class Ⅵ IF protein originally described as a neuronal stem cell marker. After cellular differentiation, nestin expression is downregulated and replaced by neurofilament, a tissue specific IF[18]. Nestin is also expressed in non-neuronal immature or progenitor cells in some normal tissues[19]. It is a large protein (> 1600 amino acids) containing a short N terminus and an unusually long C terminus. The C terminus interacts with other IFs including vimentin, desmin, or internexin, subsequently forming heterodimers and mixed polymers[19-21]. The long C-terminal portion of nestin protrudes from the filamentous body and may function as a link or cross-bridge between IFs and microtubules. Nestin is known to contribute to the disassembly of vimentin during mitosis, inactivation of cyclin-dependent kinase 5[22], and modulation of mitosis-associated cytoplasmic reorganization during mitosis *via* phosphorylation at Thr^{316} by cdc2 kinase[23]. Neural cell-specific expression is usually regulated by the second intron, whereas nestin expression in tumor endothelium is enhanced by the first intron[24]. Although nestin is expressed at the early stages of normal development, under pathological conditions, it is re-expressed in repair processes, various neoplasms, and proliferating vascular endothelial cells[25].

NESTIN IN ANGIOGENESIS DURING DEVELOPMENT

Much evidence has shown that nestin expression in vascular endothelial cells is associated with proliferation and angiogenesis[24,26-29]. Nestin expression was detected in endothelial cells of embryonic capillaries[28], capillaries of the corpus luteum, which replenishes itself by angiogenesis[30], and proliferating endothelial progenitor cells, but not in mature endothelial cells[24].

At the early stage of development, high levels of nestin are identified in endothelial cells lining all blood vessels of E14-15 rat fetuses and extraembryonic (chorion, placenta, umbilical cord) and intraembryonic blood vessels. Expression of nestin by vascular endothelial cells is greatly reduced in adult tissues[28].

These findings suggest that nestin plays important roles in vasculogenesis during development.

NESTIN IN ANGIOGENESIS IN NON-CANCEROUS DISEASES

Angiogenesis is a process of new blood vessel formation from pre-existing vessels; it is the mechanism mediating the growth and modification of a capillary network[31]. In non-cancerous diseases, nestin plays important roles in angiogenesis of wound healing in various tissues. In an adult rat necrotizing pancreatitis model, nestin is expressed in reactive stellate cells, or submesothelial cells, and endothelial cells during active angiogenesis[32], and nestin-positive cells may participate in tissue repair of the pancreas.

Nestin-expressing interfollicular blood vessel networks contribute to skin transplant survival and wound healing[33]. Scar formation following an ischemic insult to the heart is referred to as reparative fibrosis and represents an essential physiological response to damaged myocardium. Several studies reported that scar formation was associated with the recruitment of neural crest-derived cardiac resident nestin-positive cells that display characteristics consistent with a neural progenitor/stem cell phenotype[34,35]. During the reparative fibrotic response, these nestin-positive cells participate in neural remodeling and represent a novel cellular substrate of angiogenesis.

In the rat ovary, the endogenous luteinizing hormone surge induces nestin expression in capillary endothelial cells of the theca interna *via* the vascular endothelial growth factor (VEGF) signaling pathway[36]. Angiogenic induction of chorion-derived stem cells results in higher mRNA levels of pro-angiogenic protein, fibroblast growth factor-4, and stem cell markers ABCG-2, Sox-2, FZD9, BST-1, nestin, and Oct-4[37]. Endothelial precursors express nestin, and it participates in the formation of the cytoskeleton of newly formed endothelial cells.

Table 1 Angiogenesis markers for analysis of microvessel density

	Factor Ⅷ	CD 31	CD 34	Nestin
Roles	Glycoprotein Blood coagulation	Glycoprotein Endothelial cell intercellular junctions[57], migration	Glycoprotein Cell-cell adhesion factor, facilitate opening of vascular lumens[58]	Cytoskeletal protein Cell cycle[22,59], migration
Character of positive vessels	Large size Mature vessels	Small to large size Immature to mature vessels	Small to large size Immature to mature vessels	Small size[27] Immature vessels[26,30] High-proliferating activity[26,27]
Clinical outcome				
Esophageal cancer	Poor prognosis[60]	Poor prognosis[61,62]	Poor prognosis[63,64]	-
Gastric cancer	Advanced stage[65]	No relation[66]	No relation[45]	No relation[44]
Colorectal cancer	No relation[68] Liver metastasis[69]	Poor prognosis[68]	Poor prognosis[67] Poor prognosis[68] Liver metastasis[69]	Poor prognosis[42]
Liver cancer	No relation[70]			
Pancreatic cancer	Poor prognosis[16]	No relation[71] Lymph node meta[76]	Poor prognosis[70,72-75] No relation[28] Poor prognosis[77,78]	No relation[27]
Prostate cancer	Poor prognosis[79-81]	No relation[82]	Poor prognosis[83]	Poor prognosis[26]
Glioblastoma	Tumor grade[84]	No relation[85]	No relation[86]	Tumor grade[45]

VEGF is essential for the differentiation of the primitive embryonic vascular system and has been implicated in the vascularization of organs. VEGF is considered a modulator involved in neurogenesis as well as angiogenesis[38,39]. Human VEGF overexpressed transgenic mice showed an increased number of nestin positive cells after temporal ischemia of the central nervous system, as compared with wild type mice[40]. Therefore, VEGF is involved in nestin expression. Furthermore, pre-eclamptic human placenta showed higher nestin expression levels[41], thus hypoxia may be involved in expression of nestin.

NESTIN IN TUMOR ANGIOGENESIS

Expression of nestin by newly formed blood vessels in various tumor tissues, including pancreatic cancers, has been reported[26,27,30,42,43]. Its expression is not reported in mature vessels within cancer tissues. This suggests that nestin may be an angiogenesis-specific marker in malignant tumors[27,44]. Strongly nestin-positive MVD colorectal carcinomas[42] and prostate cancers[26] show a worse prognosis. In colorectal cancer, nestin is strongly localized to vascular endothelial cells, but not to cancer cells. Nestin-expressing vessels in colorectal cancer tissues are of smaller size and show greater positivity for proliferating cell nuclear antigen than CD34-expressing cells[42]. In glioblastomas, expression of nestin in both tumor cells and endothelial cells increased with tumor grade[45]. Similarly, nestin expression correlates with more advanced stage in melanoma[46]. In gastric cancers, there is no correlation between nestin-positive MVD and patient's clinical outcome, while in patients with larger carcinomas, MVD determined by nestin correlates better with longer survival than MVD determined by CD34[44]. We previously reported that nestin-positive blood vessels in pancreatic cancer exhibit high proliferating ability, but does not correlate with prognosis[27]. Tumor angiogenesis is essential for tumor progression, but increased angiogenesis may be influenced by variations in the existing vasculature of the host organ. Compared to MVD studies using other endothelial markers, only a limited number of investigations have used nestin. Large scale studies of various different malignancies using nestin as a MVD marker are needed to determine the efficacy of nestin as a predictive and prognostic marker.

The origin of nestin-positive vascular endothelial cells in tumor tissue is still controversial. Migration of mesenchymal stem cells from the bone marrow has been proposed[47]. Dong *et al*[48] showed that human glioma stem/progenitor cells transdifferentiate into vascular endothelial cells *in vitro* and *in vivo*. In skin angiogenesis, nestin-positive hair follicle cells form neovasculature[49]; therefore tissue stem cells are one possible origin of nestin-positive vessels.

Recent studies showed that nestin is an effective marker for *in vivo* imaging of tumor angiogenesis[50,51] and vascular formation in metastatic tumors[52].

NESTIN IN PANCREATIC CANCER

Pancreatic cancer is an aggressive malignancy with an overall five-year survival rate of less than 5%[53]. Since the majority of patients have locally advanced or metastatic disease at the time of diagnosis, there has been little progress in extending survival[54]. For over ten years, chemotherapy with gemcitabine, whose strategy is to inhibit tumor growth, has been the standard treatment for patients with advanced pancreatic cancer, prolonging survival by only five to six months.

To improve upon this modest benefit, several investigations explored other strategies aimed at inhibiting pancreatic cancer growth, including the use of anti-angiogenic agents. Tumor angiogenesis is an important factor in the proliferation, invasion, metastasis, and drug sensitivity of pancreatic cancers[6]. For a tumor to develop a highly malignant and deadly phenotype, it must first recruit and sustain its own blood supply. The process of angiogenesis is also sustained by secretion of specific angiogenic factors by cancer and non-cancer cells. Angiogenic factors can initiate a multi-step process that begins with vasodila-

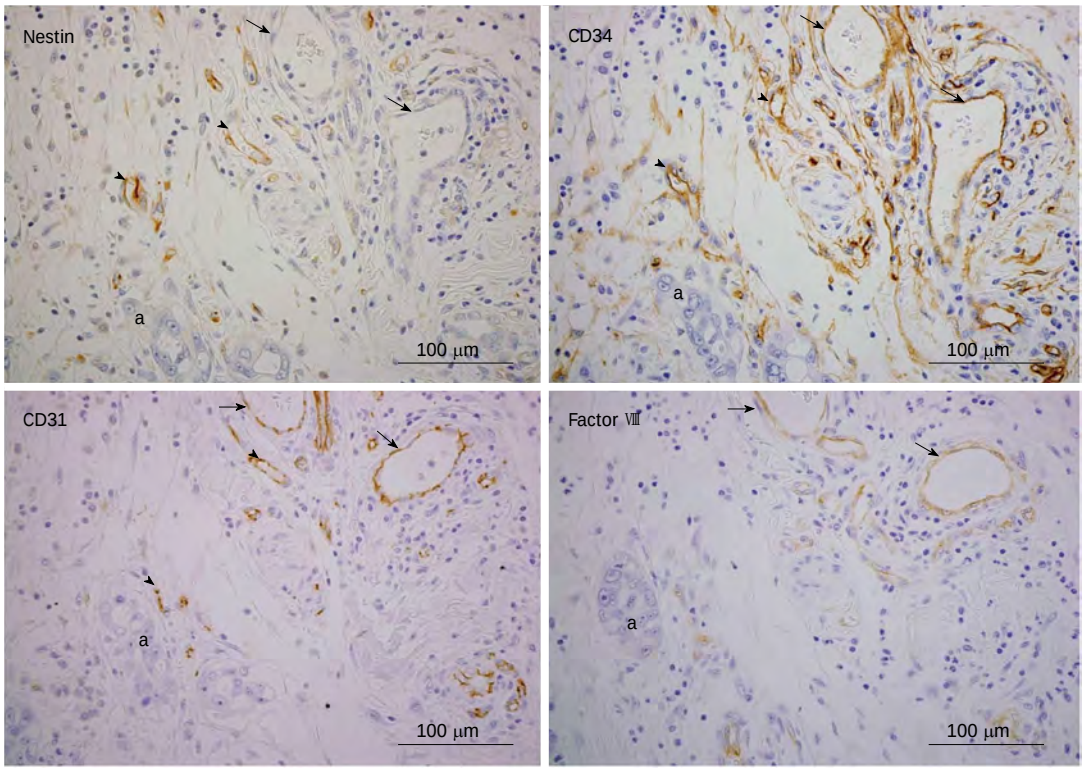


Figure 1 Immunohistochemical images of blood vessels in pancreatic cancer. Nestin was expressed in small vessels (arrowheads) and in the surrounding cancer cells (a), but not in large vessels (arrows). CD34 and CD31 were expressed in both small and large vessels (arrowheads and arrows), whereas factor Ⅷ was expressed only in large vessels (arrows).

tation, followed by the enhancement of vessel permeability and stroma degradation. Angiogenic factors can also promote endothelial cell migration and proliferation.

Several clinical agents have been developed to inhibit the angiogenesis process, including the VEGF pathway[55], and a number of phase Ⅱ and phase Ⅲ trials combined gemcitabine with novel anti-angiogenic agents with the hope of improving patient survival. Bevacizumab, which inhibits only the VEGF receptor, and sorafenib, which inhibits raf-1 kinase and the platelet-derived growth factor receptor tyrosine kinase in addition to the VEGF receptor, were studied in combination with gemcitabine. However, this approach has largely been unsuccessful and additional novel strategies are clearly needed.

In pancreatic cancers, we found that CD34, CD31, and factor Ⅷ, which are commonly used for MVD quantification by immunohistochemical analysis, were expressed in both newly formed small sized blood vessels and pre-existing vessels (Figure 1, arrows and arrowheads), whereas nestin was expressed only in newly formed blood vessels (arrowheads). Therefore, nestin is a potentially useful marker to distinguish newly formed blood vessels from pre-existing vessels in tumor tissues[27].

More importantly, we observed that knockdown of nestin in vascular endothelial cells suppresses cell growth and tumor formation of pancreatic cancers *in vivo*[27]. Therefore, nestin is a potential molecular target *via* inhibition of angiogenesis in pancreatic cancer.

Nestin is expressed in both pancreatic cancer cells and tumor vessels. Inhibition of nestin in cancer cells reduces tumor cell migration and metastasis[56] and down-regulation of nestin in tumor vessels also inhibits tumor formation[27]. These findings may indicate that nestin is a target not only for inhibition of tumor angiogenesis, but also within some cancers themselves that expression nestin, such as glioblastomas, melanomas, and pancreatic cancer (Figure 2).

CONCLUSION

More accurate estimation of MVD using nestin in GI cancers may reveal a strong relationship between tumor vessels and clinical outcome. Future studies on MVD with a large patient population are needed to clarify the efficacy of nestin as a predictive and prognostic marker. Nestin-targeting small interfering RNA (siRNA) has shown a tumor inhibitory effect *in vivo via* inhibition of tumor angiogenesis[27], thus nestin may be a novel therapeutic target for tumor angiogenesis. Treatment resistance often occurs with the isolated use of anti-VEGF therapy. Therefore, combination therapy that also includes nestin-targeting agents is a potentially effective therapeutic strategy. Nestin is expressed in some cancer cells, thus targeted therapy may exert inhibitory effects both on cancer cells and vascular endothelium in certain tumor types, including pancreatic cancers. In addition to small molecule compounds, emerging biotechnology including RNA aptamer and siRNA targeting nestin will possibly be adopted as new therapeutics for GI cancers, especially for pancreatic cancers.

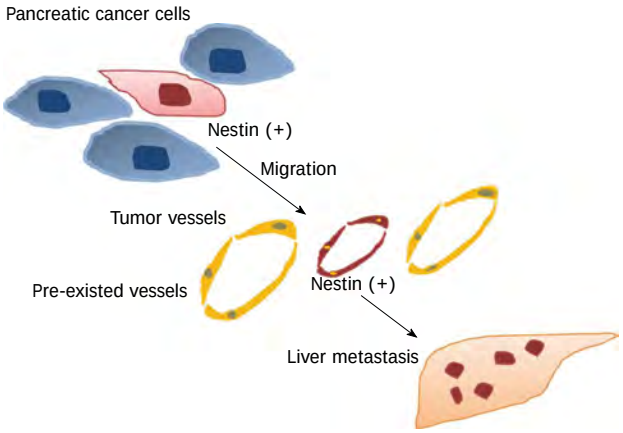


Figure 2 In pancreatic cancer, nestin is highly expressed in some pancreatic cancer cells and small proliferating tumor vessels.

ACKNOWLEDGMENTS

The authors thank Dr. Kosuke Narita from Departments of Pathology and Integrative Oncological Pathology for helpful discussion.

REFERENCES

1 **Liotta LA**, Saidel MG, Kleinerman J. The significance of hematogenous tumor cell clumps in the metastatic process. *Cancer Res* 1976; **36**: 889-894 [PMID: 1253177]
2 **Folkman J**. What is the evidence that tumors are angiogenesis dependent? *J Natl Cancer Inst* 1990; **82**: 4-6 [PMID: 1688381]
3 **Papamichael D**. Prognostic role of angiogenesis in colorectal cancer. *Anticancer Res* 2001; **21**: 4349-4353 [PMID: 11908690]
4 **Saito H**, Tsujitani S. Angiogenesis, angiogenic factor expression and prognosis of gastric carcinoma. *Anticancer Res* 2001; **21**: 4365-4372 [PMID: 11908692]
5 **Nakagawa S**, Nishimaki T, Suzuki T, Kanda T, Kuwabara S, Hatakeyama K. Tumor angiogenesis as an independent prognostic factor after extended radical esophagectomy for invasive squamous cell carcinoma of the esophagus. *Surgery* 2001; **129**: 302-308 [PMID: 11231458 DOI: 10.1067/msy.2001.111122]
6 **Ikeda N**, Adachi M, Taki T, Huang C, Hashida H, Takabayashi A, Sho M, Nakajima Y, Kanehiro H, Hisanaga M, Nakano H, Miyake M. Prognostic significance of angiogenesis in human pancreatic cancer. *Br J Cancer* 1999; **79**: 1553-1563 [PMID: 10188906 DOI: 10.1038/sj.bjc.6690248]
7 **Kuwahara K**, Sasaki T, Kuwada Y, Murakami M, Yamasaki S, Chayama K. Expressions of angiogenic factors in pancreatic ductal carcinoma: a correlative study with clinicopathologic parameters and patient survival. *Pancreas* 2003; **26**: 344-349 [PMID: 12717266]
8 **Macchiarini P**, Fontanini G, Hardin MJ, Squartini F, Angeletti CA. Relation of neovascularisation to metastasis of non-small-cell lung cancer. *Lancet* 1992; **340**: 145-146 [PMID: 1378165 DOI: 0140-6736(92)93217-B]
9 **Hansen S**, Grabau DA, Rose C, Bak M, Sørensen FB. Angiogenesis in breast cancer: a comparative study of the observer variability of methods for determining microvessel density. *Lab Invest* 1998; **78**: 1563-1573 [PMID: 9881956]
10 **Schoell WM**, Pieber D, Reich O, Lahousen M, Janicek M, Guecer F, Winter R. Tumor angiogenesis as a prognostic factor in ovarian carcinoma: quantification of endothelial immunoreactivity by image analysis. *Cancer* 1997; **80**: 2257-2262 [PMID: 9404702 DOI: 10.1002/(SICI)1097-0142(19971215)80]
11 **Tanigawa N**, Matsumura M, Amaya H, Kitaoka A, Shimomatsuya T, Lu C, Muraoka R, Tanaka T. Tumor vascularity correlates with the prognosis of patients with esophageal squamous cell carcinoma. *Cancer* 1997; **79**: 220-225 [PMID: 9010094 DOI: 10.1002/(SICI)1097-0142(19970115)79]
12 **Tanigawa N**, Amaya H, Matsumura M, Shimomatsuya T. Association of tumour vasculature with tumour progression and overall survival of patients with non-early gastric carcinomas. *Br J Cancer* 1997; **75**: 566-571 [PMID: 9052412]
13 **Takahashi Y**, Tucker SL, Kitadai Y, Koura AN, Bucana CD, Cleary KR, Ellis LM. Vessel counts and expression of vascular endothelial growth factor as prognostic factors in node-negative colon cancer. *Arch Surg* 1997; **132**: 541-546 [PMID: 9161399]
14 **Wakui S**, Furusato M, Itoh T, Sasaki H, Akiyama A, Kinoshita I, Asano K, Tokuda T, Aizawa S, Ushigome S. Tumour angiogenesis in prostatic carcinoma with and without bone marrow metastasis: a morphometric study. *J Pathol* 1992; **168**: 257-262 [PMID: 1281874 DOI: 10.1002/path.1711680303]
15 **Takagi K**, Takada T, Amano H, Yoshida M, Miura H, Toyota N, Wada K, Takahashi I. Analysis of microvessels in pancreatic cancer: by light microscopy, confocal laser scan microscopy, and electron microscopy. *J Hepatobiliary Pancreat Surg* 2008; **15**: 384-390 [PMID: 18670839 DOI: 10.1007/s00534-007-1241-6]
16 **Takagi K**, Takada T, Amano H. A high peripheral microvessel density count correlates with a poor prognosis in pancreatic cancer. *J Gastroenterol* 2005; **40**: 402-408 [PMID: 15870976 DOI: 10.1007/s00535-004-1556-x]
17 **Meert AP**, Paesmans M, Martin B, Delmotte P, Berghmans T, Verdebout JM, Lafitte JJ, Mascaux C, Sculier JP. The role of microvessel density on the survival of patients with lung cancer: a systematic review of the literature with meta-analysis. *Br J Cancer* 2002; **87**: 694-701 [PMID: 12232748 DOI: 10.1038/sj.bjc.6600551]
18 **Lendahl U**, Zimmerman LB, McKay RD. CNS stem cells express a new class of intermediate filament protein. *Cell* 1990; **60**: 585-595 [PMID: 1689217 DOI: 0092-8674(90)90662-X]
19 **Steinert PM**, Chou YH, Prahlad V, Parry DA, Marekov LN, Wu KC, Jang SI, Goldman RD. A high molecular weight intermediate filament-associated protein in BHK-21 cells is nestin, a type VI intermediate filament protein. Limited co-assembly in vitro to form heteropolymers with type III vimentin and type IV alpha-internexin. *J Biol Chem* 1999; **274**: 9881-9890 [PMID: 10092680]
20 **Marvin MJ**, Dahlstrand J, Lendahl U, McKay RD. A rod end deletion in the intermediate filament protein nestin alters its subcellular localization in neuroepithelial cells of transgenic mice. *J Cell Sci* 1998; **111** (Pt 14): 1951-1961 [PMID: 9645943]
21 **Sjöberg G**, Edström L, Lendahl U, Sejersen T. Myofibers from Duchenne/Becker muscular dystrophy and myositis express the intermediate filament nestin. *J Neuropathol Exp Neurol* 1994; **53**: 416-423 [PMID: 8021716]
22 **Sahlgren CM**, Mikhailov A, Vaittinen S, Pallari HM, Kalimo H, Pant HC, Eriksson JE. Cdk5 regulates the organization of Nestin and its association with p35. *Mol Cell Biol* 2003; **23**: 5090-5106 [PMID: 12832492]
23 **Sahlgren CM**, Mikhailov A, Hellman J, Chou YH, Lendahl U, Goldman RD, Eriksson JE. Mitotic reorganization of the intermediate filament protein nestin involves phosphorylation by cdc2 kinase. *J Biol Chem* 2001; **276**: 16456-16463 [PMID: 11278541 DOI: 10.1074/jbc.M009669200]
24 **Suzuki S**, Namiki J, Shibata S, Mastuzaki Y, Okano H. The neural stem/progenitor cell marker nestin is expressed in proliferative endothelial cells, but not in mature vasculature. *J Histochem Cytochem* 2010; **58**: 721-730 [PMID: 20421592 DOI: 10.1369/jhc.2010.955609]
25 **Ishiwata T**, Matsuda Y, Naito Z. Nestin in gastrointestinal and other cancers: effects on cells and tumor angiogenesis. *World J Gastroenterol* 2011; **17**: 409-418 [PMID: 21274370 DOI: 10.3748/wjg.v17.i4.409]
26 **Gravdal K**, Halvorsen OJ, Haukaas SA, Akslen LA. Proliferation of immature tumor vessels is a novel marker of clinical progression in prostate cancer. *Cancer Res* 2009; **69**: 4708-4715 [PMID: 19487287 DOI: 10.1158/0008-5472.CAN-08-4417]
27 **Yamahatsu K**, Matsuda Y, Ishiwata T, Uchida E, Naito Z.

Nestin as a novel therapeutic target for pancreatic cancer via tumor angiogenesis. *Int J Oncol* 2012; **40**: 1345-1357 [PMID: 22246533 DOI: 10.3892/ijo.2012.1333]
28 **Mokrý J**, Nemecek S. Angiogenesis of extra- and intraembryonic blood vessels is associated with expression of nestin in endothelial cells. *Folia Biol* (Praha) 1998; **44**: 155-161 [PMID: 10732706]
29 **Mokrý J**, Nemecek S. Cerebral angiogenesis shows nestin expression in endothelial cells. *Gen Physiol Biophys* 1999; **18** Suppl 1: 25-29 [PMID: 10707828]
30 **Mokrý J**, Cízková D, Filip S, Ehrmann J, Osterreicher J, Kolár Z, English D. Nestin expression by newly formed human blood vessels. *Stem Cells Dev* 2004; **13**: 658-664 [PMID: 15684833 DOI: 10.1089/scd.2004.13.658]
31 **Hanahan D**, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 1996; **86**: 353-364 [PMID: 8756718 DOI: S0092-8674(00)80108-7]
32 **Ishiwata T**, Kudo M, Onda M, Fujii T, Teduka K, Suzuki T, Korc M, Naito Z. Defined localization of nestin-expressing cells in L-arginine-induced acute pancreatitis. *Pancreas* 2006; **32**: 360-368 [PMID: 16670618 DOI: 10.1097/01.mpa.0000220860.01120.21]
33 **Aki R**, Amoh Y, Li L, Katsuoka K, Hoffman RM. Nestin-expressing interfollicular blood vessel network contributes to skin transplant survival and wound healing. *J Cell Biochem* 2010; **110**: 80-86 [PMID: 20225276 DOI: 10.1002/jcb.22512]
34 **El-Helou V**, Beguin PC, Assimakopoulos J, Clement R, Gosselin H, Brugada R, Aumont A, Biernaskie J, Villeneuve L, Leung TK, Fernandes KJ, Calderone A. The rat heart contains a neural stem cell population; role in sympathetic sprouting and angiogenesis. *J Mol Cell Cardiol* 2008; **45**: 694-702 [PMID: 18718475 DOI: 10.1016/j.yjmcc.2008.07.013]
35 **Calderone A**. Nestin+ cells and healing the infarcted heart. *Am J Physiol Heart Circ Physiol* 2012; **302**: H1-H9 [PMID: 22003051 DOI: 10.1152/ajpheart.00716.2011]
36 **Takahashi N**, Itoh MT, Ishizuka B. Human chorionic gonadotropin induces nestin expression in endothelial cells of the ovary via vascular endothelial growth factor signaling. *Endocrinology* 2008; **149**: 253-260 [PMID: 17916630 DOI: 10.1210/en.2007-0774]
37 **Abdul Rahman H**, Manzor NF, Tan GC, Tan AE, Chua KH. Upregulation of SOX-2, FZD9, Nestin, OCT-4 and FGF-4 expression in human chorion derived-stem cells after angiogenic induction. *Med J Malaysia* 2008; **63** Suppl A: 57-58 [PMID: 19024982]
38 **Kim BK**, Kim SE, Shim JH, Woo DH, Gil JE, Kim SK, Kim JH. Neurogenic effect of vascular endothelial growth factor during germ layer formation of human embryonic stem cells. *FEBS Lett* 2006; **580**: 5869-5874 [PMID: 17027979 DOI: 10.1016/j.febslet.2006.09.053]
39 **Raab S**, Beck H, Gaumann A, Yüce A, Gerber HP, Plate K, Hammes HP, Ferrara N, Breier G. Impaired brain angiogenesis and neuronal apoptosis induced by conditional homozygous inactivation of vascular endothelial growth factor. *Thromb Haemost* 2004; **91**: 595-605 [PMID: 14983237 DOI: 10.1160/TH03-09-058]
40 **Plaschke K**, Staub J, Ernst E, Marti HH. VEGF overexpression improves mice cognitive abilities after unilateral common carotid artery occlusion. *Exp Neurol* 2008; **214**: 285-292 [PMID: 18822285 DOI: 10.1016/j.expneurol.2008.08.014]
41 **Hwang HS**, Cho NH, Maeng YS, Kang MH, Park YW, Kim YH. Differential expression of nestin in normal and pre-eclamptic human placentas. *Acta Obstet Gynecol Scand* 2007; **86**: 909-914 [PMID: 17653873 DOI: 10.1080/00016340701417018]
42 **Teranishi N**, Naito Z, Ishiwata T, Tanaka N, Furukawa K, Seya T, Shinji S, Tajiri T. Identification of neovasculature using nestin in colorectal cancer. *Int J Oncol* 2007; **30**: 593-603 [PMID: 17273760]
43 **Salehi F**, Kovacs K, Cusimano MD, Horvath E, Bell CD, Rotondo F, Scheithauer BW. Immunohistochemical expression of nestin in adenohypophysial vessels during development of pituitary infarction. *J Neurosurg* 2008; **108**: 118-123 [PMID: 18173320 DOI: 10.3171/JNS/2008/108/01/0118]
44 **Kim HS**, Kang HS, Messam CA, Min KW, Park CS. Comparative evaluation of angiogenesis in gastric adenocarcinoma by nestin and CD34. *Appl Immunohistochem Mol Morphol* 2002; **10**: 121-127 [PMID: 12051629]
45 **Hlobilkova A**, Ehrmann J, Knizetova P, Krejci V, Kalita O, Kolar Z. Analysis of VEGF, Flt-1, Flk-1, nestin and MMP-9 in relation to astrocytoma pathogenesis and progression. *Neoplasma* 2009; **56**: 284-290 [PMID: 19473053]
46 **Brychtova S**, Fiuraskova M, Hlobilková A, Brychta T, Hinak J. Nestin expression in cutaneous melanomas and melanocytic nevi. *J Cutan Pathol* 2007; **34**: 370-375 [PMID: 17448190 DOI: 10.1111/j.1600-0560.2006.00627.x]
47 **Schiffer D**, Annovazzi L, Caldera V, Mellai M. On the origin and growth of gliomas. *Anticancer Res* 2010; **30**: 1977-1998 [PMID: 20651342]
48 **Dong J**, Zhao Y, Huang Q, Fei X, Diao Y, Shen Y, Xiao H, Zhang T, Lan Q, Gu X. Glioma stem/progenitor cells contribute to neovascularization via transdifferentiation. *Stem Cell Rev* 2011; **7**: 141-152 [PMID: 20697979 DOI: 10.1007/s12015-010-9169-7]
49 **Amoh Y**, Li L, Yang M, Moossa AR, Katsuoka K, Penman S, Hoffman RM. Nascent blood vessels in the skin arise from nestin-expressing hair-follicle cells. *Proc Natl Acad Sci USA* 2004; **101**: 13291-13295 [PMID: 15331785 DOI: 10.1073/pnas.0405250101]
50 **Hoffman RM**. Nestin-driven green fluorescent protein as an imaging marker for nascent blood vessels in mouse models of cancer. *Methods Mol Biol* 2011; **689**: 183-204 [PMID: 21153793 DOI: 10.1007/978-1-60761-950-5_11]
51 **Amoh Y**, Yang M, Li L, Reynoso J, Bouvet M, Moossa AR, Katsuoka K, Hoffman RM. Nestin-linked green fluorescent protein transgenic nude mouse for imaging human tumor angiogenesis. *Cancer Res* 2005; **65**: 5352-5357 [PMID: 15958583 DOI: 10.1158/0008-5472.CAN-05-0821]
52 **Amoh Y**, Bouvet M, Li L, Tsuji K, Moossa AR, Katsuoka K, Hoffman RM. Visualization of nascent tumor angiogenesis in lung and liver metastasis by differential dual-color fluorescence imaging in nestin-linked-GFP mice. *Clin Exp Metastasis* 2006; **23**: 315-322 [PMID: 17136576 DOI: 10.1007/s10585-006-9018-x]
53 **Jemal A**, Siegel R, Ward E, Hao Y, Xu J, Murray T, Thun MJ. Cancer statistics, 2008. *CA Cancer J Clin* 2008; **58**: 71-96 [PMID: 18287387 DOI: 10.3322/CA.2007.0010]
54 **Warshaw AL**, Fernández-del Castillo C. Pancreatic carcinoma. *N Engl J Med* 1992; **326**: 455-465 [PMID: 1732772 DOI: 10.1056/NEJM199202133260706]
55 **Assifi MM**, Hines OJ. Anti-angiogenic agents in pancreatic cancer: a review. *Anticancer Agents Med Chem* 2011; **11**: 464-469 [PMID: 21521158 DOI: 10.2174/187152011179567746]
56 **Matsuda Y**, Naito Z, Kawahara K, Nakazawa N, Korc M, Ishiwata T. Nestin is a novel target for suppressing pancreatic cancer cell migration, invasion and metastasis. *Cancer Biol Ther* 2011; **11**: 512-523 [PMID: 21258211 DOI: 10.4161/cbt.11.5.14673]
57 **Newman PJ**, Berndt MC, Gorski J, White GC, Lyman S, Paddock C, Muller WA. PECAM-1 (CD31) cloning and relation to adhesion molecules of the immunoglobulin gene superfamily. *Science* 1990; **247**: 1219-1222 [PMID: 1690453 DOI: 10.1126/science.1690453]
58 **Strilić B**, Kucera T, Eglinger J, Hughes MR, McNagny KM, Tsukita S, Dejana E, Ferrara N, Lammert E. The molecular basis of vascular lumen formation in the developing mouse aorta. *Dev Cell* 2009; **17**: 505-515 [PMID: 19853564 DOI: 10.1016/j.devcel.2009.08.011]
59 **Pallari HM**, Lindqvist J, Torvaldson E, Ferraris SE, He T, Sahlgren C, Eriksson JE. Nestin as a regulator of Cdk5 in differentiating myoblasts. *Mol Biol Cell* 2011; **22**: 1539-1549 [PMID: 21346193 DOI: 10.1091/mbc.E10-07-0568]
60 **Takebayashi Y**, Natugoe S, Baba M, Fukumoto T, Takao S, Akiba S, Akiyama S, Aikou T. Angiogenesis in esophageal squamous cell carcinoma. *Oncol Rep* 1998; **5**: 401-404 [PMID:

9468567]
61 **Saad RS**, Lindner JL, Liu Y, Silverman JF. Lymphatic vessel density as prognostic marker in esophageal adenocarcinoma. *Am J Clin Pathol* 2009; **131**: 92-98 [PMID: 19095571 DOI: 10.1309/AJCPKWUQSIPVG90H]
62 **Saad RS**, El-Gohary Y, Memari E, Liu YL, Silverman JF. Endoglin (CD105) and vascular endothelial growth factor as prognostic markers in esophageal adenocarcinoma. *Hum Pathol* 2005; **36**: 955-961 [PMID: 16153457 DOI: 10.1016/j.humpath.2005.06.019]
63 **Elpek GO**, Gelen T, Aksoy NH, Erdoğan A, Dertsiz L, Demircan A, Keleş N. The prognostic relevance of angiogenesis and mast cells in squamous cell carcinoma of the oesophagus. *J Clin Pathol* 2001; **54**: 940-944 [PMID: 11729214 DOI: 10.1136/jcp.54.12.940]
64 **Igarashi M**, Dhar DK, Kubota H, Yamamoto A, El-Assal O, Nagasue N. The prognostic significance of microvessel density and thymidine phosphorylase expression in squamous cell carcinoma of the esophagus. *Cancer* 1998; **82**: 1225-1232 [PMID: 9529012]
65 **Du JR**, Jiang Y, Zhang YM, Fu H. Vascular endothelial growth factor and microvascular density in esophageal and gastric carcinomas. *World J Gastroenterol* 2003; **9**: 1604-1606 [PMID: 12854174]
66 **Coşkun U**, Akyürek N, Dursun A, Yamaç D. Peritumoral lymphatic microvessel density associated with tumor progression and poor prognosis in gastric carcinoma. *J Surg Res* 2010; **164**: 110-115 [PMID: 19628230]
67 **Wei YZ**, Li CF, Xue YW. [Expression of transcription factor SP1, vascular endothelial growth factor and CD34 in serosa-infiltrating gastric cancer and their relationship with biological behavior and prognosis]. *Zhonghua Weichang Waike Zazhi* 2009; **12**: 145-149 [PMID: 19296249]
68 **Des Guetz G**, Uzzan B, Nicolas P, Cucherat M, Morere JF, Benamouzig R, Breau JL, Perret GY. Microvessel density and VEGF expression are prognostic factors in colorectal cancer. Meta-analysis of the literature. *Br J Cancer* 2006; **94**: 1823-1832 [PMID: 16773076 DOI: 10.1038/sj.bjc.6603176]
69 **Tomisaki S**, Ohno S, Ichiyoshi Y, Kuwano H, Maehara Y, Sugimachi K. Microvessel quantification and its possible relation with liver metastasis in colorectal cancer. *Cancer* 1996; **77**: 1722-1728 [PMID: 8608569]
70 **Poon RT**, Ng IO, Lau C, Yu WC, Yang ZF, Fan ST, Wong J. Tumor microvessel density as a predictor of recurrence after resection of hepatocellular carcinoma: a prospective study. *J Clin Oncol* 2002; **20**: 1775-1785 [PMID: 11919234 DOI: 10.1200/JCO.2002.07.089]
71 **Wang SN**, Yeh YT, Yang SF, Chai CY, Lee KT. Potential role of leptin expression in hepatocellular carcinoma. *J Clin Pathol* 2006; **59**: 930-934 [PMID: 16565221 DOI: 10.1136/jcp.2005.035477]
72 **Yang XR**, Xu Y, Yu B, Zhou J, Qiu SJ, Shi GM, Zhang BH, Wu WZ, Shi YH, Wu B, Yang GH, Ji Y, Fan J. High expression levels of putative hepatic stem/progenitor cell biomarkers related to tumour angiogenesis and poor prognosis of hepatocellular carcinoma. *Gut* 2010; **59**: 953-962 [PMID: 20442200 DOI: 10.1136/gut.2008.176271]
73 **Yang P**, Yuan W, He J, Wang J, Yu L, Jin X, Hu Y, Liao M, Chen Z, Zhang Y. Overexpression of EphA2, MMP-9, and MVD-CD34 in hepatocellular carcinoma: Implications for tumor progression and prognosis. *Hepatol Res* 2009; **39**: 1169-1177 [PMID: 19788698 DOI: 10.1111/j.1872-034X.2009.00563.x]
74 **Tseng PL**, Tai MH, Huang CC, Wang CC, Lin JW, Hung CH, Chen CH, Wang JH, Lu SN, Lee CM, Changchien CS, Hu TH. Overexpression of VEGF is associated with positive p53 immunostaining in hepatocellular carcinoma (HCC) and adverse outcome of HCC patients. *J Surg Oncol* 2008; **98**: 349-357 [PMID: 18646041 DOI: 10.1002/jso.21109]
75 **Sun HC**, Tang ZY, Li XM, Zhou YN, Sun BR, Ma ZC. Microvessel density of hepatocellular carcinoma: its relationship with prognosis. *J Cancer Res Clin Oncol* 1999; **125**: 419-426 [PMID: 10394963 DOI: 10.1007/s004320050296]
76 **van der Zee JA**, van Eijck CH, Hop WC, van Dekken H, Dicheva BM, Seynhaeve AL, Koning GA, Eggermont AM, ten Hagen TL. Angiogenesis: a prognostic determinant in pancreatic cancer? *Eur J Cancer* 2011; **47**: 2576-2584 [PMID: 21958461]
77 **Niedergethmann M**, Hildenbrand R, Wostbrock B, Hartel M, Sturm JW, Richter A, Post S. High expression of vascular endothelial growth factor predicts early recurrence and poor prognosis after curative resection for ductal adenocarcinoma of the pancreas. *Pancreas* 2002; **25**: 122-129 [PMID: 12142733 DOI: 10.1097/00006676-200208000-00002]
78 **Niedergethmann M**, Hildenbrand R, Wolf G, Verbeke CS, Richter A, Post S. Angiogenesis and cathepsin expression are prognostic factors in pancreatic adenocarcinoma after curative resection. *Int J Pancreatol* 2000; **28**: 31-39 [PMID: 11185708]
79 **Offersen BV**, Borre M, Overgaard J. Immunohistochemical determination of tumor angiogenesis measured by the maximal microvessel density in human prostate cancer. *APMIS* 1998; **106**: 463-469 [PMID: 9637268 DOI: 10.1111/j.1699-0463.1998.tb01372.x]
80 **Borre M**, Offersen BV, Nerstrøm B, Overgaard J. Microvessel density predicts survival in prostate cancer patients subjected to watchful waiting. *Br J Cancer* 1998; **78**: 940-944 [PMID: 9764587 DOI: 10.1038/bjc.1998.605]
81 **Borre M**, Offersen IB, Nerstrøm B, Overgaard J. [Angiogenesis: prognostic marker in prostatic cancer]. *Ugeskr Laeger* 1999; **161**: 3832-3836 [PMID: 10412301]
82 **Rubin MA**, Buyyounouski M, Bagiella E, Sharir S, Neugut A, Benson M, de la Taille A, Katz AE, Olsson CA, Ennis RD. Microvessel density in prostate cancer: lack of correlation with tumor grade, pathologic stage, and clinical outcome. *Urology* 1999; **53**: 542-547 [PMID: 10096381 DOI: 10.1016/S0090-4295(98)00561-5]
83 **de la Taille A**, Katz AE, Bagiella E, Buttyan R, Sharir S, Olsson CA, Burchardt T, Ennis RD, Rubin MA. Microvessel density as a predictor of PSA recurrence after radical prostatectomy. A comparison of CD34 and CD31. *Am J Clin Pathol* 2000; **113**: 555-562 [PMID: 10761458 DOI: 10.1309/02W2-KE50-PKEF-G2G4]
84 **Mahzouni P**, Mohammadizadeh F, Mougouei K, Moghaddam NA, Chehrei A, Mesbah A. Determining the relationship between "microvessel density" and different grades of astrocytoma based on immunohistochemistry for "factor VIII-related antigen" (von Willebrand factor) expression in tumor microvessels. *Indian J Pathol Microbiol* 2010; **53**: 605-610 [PMID: 21045378]
85 **Yao Y**, Kubota T, Takeuchi H, Sato K. Prognostic significance of microvessel density determined by an anti-CD105/endoglin monoclonal antibody in astrocytic tumors: comparison with an anti-CD31 monoclonal antibody. *Neuropathology* 2005; **25**: 201-206 [PMID: 16193836 DOI: 10.1111/j.1440-1789.2005.00632.x]
86 **Preusser M**, Heinzl H, Gelpi E, Schonegger K, Haberler C, Birner P, Marosi C, Hegi M, Gorlia T, Hainfellner JA. Histopathologic assessment of hot-spot microvessel density and vascular patterns in glioblastoma: Poor observer agreement limits clinical utility as prognostic factors: a translational research project of the European Organization for Research and Treatment of Cancer Brain Tumor Group. *Cancer* 2006; **107**: 162-170 [PMID: 16721804]

P- Reviewer Vitiani LR **S- Editor** Gou SX
L- Editor A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i1.49

World J Gastroenterol 2013 January 7; 19(1): 49-56
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Performance of liver stiffness measurements by transient elastography in chronic hepatitis

Giovanna Ferraioli, Carmine Tinelli, Barbara Dal Bello, Mabel Zicchetti, Raffaella Lissandrin, Gaetano Filice, Carlo Filice, Elisabetta Above, Giorgio Barbarini, Enrico Brunetti, Willy Calderon, Marta Di Gregorio, Roberto Gulminetti, Paolo Lanzarini, Serena Ludovisi, Laura Maiocchi, Antonello Malfitano, Giuseppe Michelone, Lorenzo Minoli, Mario Mondelli, Stefano Novati, Savino FA Patruno, Alessandro Perretti, Gianluigi Poma, Paolo Sacchi, Domenico Zanaboni, Marco Zaramella

Giovanna Ferraioli, Mabel Zicchetti, Raffaella Lissandrin, Carlo Filice, Elisabetta Above, Gianluigi Poma, Marta Di Gregorio, Ultrasound Unit, Department of Infectious Diseases, Fondazione IRCCS Policlinico San Matteo, Medical School University of Pavia, 27100 Pavia, Italy
Carmine Tinelli, Clinical Epidemiology and Biometric Unit, Fondazione IRCCS Policlinico San Matteo, 27100 Pavia, Italy
Barbara Dal Bello, Department of Pathology, Fondazione IRCCS Policlinico San Matteo, 27100 Pavia, Italy
Gaetano Filice, Enrico Brunetti, Serena Ludovisi, Lorenzo Minoli, Mario Mondelli, Alessandro Perretti, Marco Zaramella, Department of Infectious Diseases, Fondazione IRCCS Policlinico San Matteo, Medical School University of Pavia, 27100 Pavia, Italy
Giorgio Barbarini, Willy Calderon, Roberto Gulminetti, Paolo Lanzarini, Laura Maiocchi, Antonello Malfitano, Giuseppe Michelone, Stefano Novati, Savino FA Patruno, Paolo Sacchi, Domenico Zanaboni, Department of Infectious Diseases, Fondazione IRCCS Policlinico San Matteo, 27100 Pavia, Italy
Author contributions: Ferraioli G and Tinelli C designed the study; Ferraioli G, Zicchetti M, Lissandrin R, Dal Bello B performed the research; Tinelli C performed the statistical analysis; Ferraioli G, Zicchetti M, Lissandrin R wrote the manuscript, Tinelli C, Dal Bello B, Filice G and Filice C reviewed the manuscript; Ferraioli G, Tinelli C, Dal Bello B, Zicchetti M, Lissandrin R, Filice G and Filice C approved the final version; all authors of the Liver Fibrosis Study Group (Above E, Barbarini G, Brunetti E, Calderon W, Di Gregorio M, Gulminetti R, Lanzarini P, Ludovisi S, Maiocchi L, Malfitano A, Michelone G, Minoli L, Mondelli M, Novati S, Patruno SFA, Perretti A, Poma G, Sacchi P, Zanaboni D, Zaramella M) performed the research, reviewed the manuscript and approved the final version.
Correspondence to: Giovanna Ferraioli, MD, Ultrasound Unit, Department of Infectious Diseases, Fondazione IRCCS Policlinico San Matteo, Medical School University of Pavia, Via Taramelli 5, 27100 Pavia, Italy. giovanna.ferraioli@unipv.it
Telephone: +39-382-502887 Fax: +39-382-501505
Received: June 12, 2012 Revised: October 24, 2012
Accepted: October 30, 2012
Published online: January 7, 2013

Abstract

AIM: To compare results of liver stiffness measurements by transient elastography (TE) obtained in our patients population with that used in a recently published meta-analysis.

METHODS: This was a single center cross-sectional study. Consecutive patients with chronic viral hepatitis scheduled for liver biopsy at the outpatient ward of our Infectious Diseases Department were enrolled. TE was carried out by using FibroScan™ (Echosens, Paris, France). Liver biopsy was performed on the same day as TE, as day case procedure. Fibrosis was staged according to the Metavir scoring system. The diagnostic performance of TE was assessed by using receiver operating characteristic (ROC) curves and the area under the ROC curve analysis.

RESULTS: Two hundred and fifty-two patients met the inclusion criteria. Six (2%) patients were excluded due to unreliable TE measurements. Thus, 246 (171 men and 75 women) patients were analyzed. One hundred and ninety-five (79.3%) patients had chronic hepatitis C, 41 (16.7%) had chronic hepatitis B, and 10 (4.0%) were coinfected with human immunodeficiency virus. ROC curve analysis identified optimal cut-off value of TE as high as 6.9 kPa for $F \geqslant 2$; 7.9 kPa for $F \geqslant 3$; 9.6 kPa for $F = 4$ in all patients ($n = 246$), and as high as 6.9 kPa for $F \geqslant 2$; 7.3 kPa for $F \geqslant 3$; 9.3 kPa for $F = 4$ in patients with hepatitis C ($n = 195$). Cut-off values

of TE obtained by maximizing only the specificity were as high as 6.9 kPa for $F \geqslant 2$; 9.6 kPa for $F \geqslant 3$; 12.2 kPa for $F = 4$ in all patients ($n = 246$), and as high as 7.0 kPa for $F \geqslant 2$; 9.3 kPa for $F \geqslant 3$; 12.3 kPa for $F = 4$ in patients with hepatitis C ($n = 195$).

CONCLUSION: The cut-off values of TE obtained in this single center study are comparable to that obtained in a recently published meta-analysis that included up to 40 studies.

© 2013 Baishideng. All rights reserved.

Key words: Chronic viral hepatitis; Hepatitis C; Liver; Liver biopsy; Liver fibrosis; Elastography; Transient elastography; FibroScan; Ultrasound

Ferraioli G, Tinelli C, Dal Bello B, Zicchetti M, Lissandrin R, Filice G, Filice C, Above E, Barbarini G, Brunetti E, Calderon W, Di Gregorio M, Gulminetti R, Lanzarini P, Ludovisi S, Maiocchi L, Malfitano A, Michelone G, Minoli L, Mondelli M, Novati S, Patruno SFA, Perretti A, Poma G, Sacchi P, Zanaboni D, Zaramella M. Performance of liver stiffness measurements by transient elastography in chronic hepatitis. *World J Gastroenterol* 2013; 19(1): 49-56 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i1/49.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i1.49

INTRODUCTION

In chronic viral hepatitis, the degree of liver fibrosis affects both prognosis and treatment. Biopsy is still considered the reference standard for the assessment of liver fibrosis, even though it evaluates 1/50 000 only of the liver parenchyma and is subject to intra and inter-observer variability[1,2]. Moreover, it is an invasive procedure not well accepted by patients that is burdened by complications even though at a low rate[3,4]. For these reasons, in recent years imaging[5-8] or biochemical methods[9-13] for a noninvasive assessment of liver fibrosis have been tested. The imaging methods estimate elasticity of liver parenchyma, which is directly related to the amount of fibrosis. The method most widely used and validated is transient elastography (TE) (FibroScan Echosens, Paris)[9,14-17], which allows to evaluate the elasticity of a portion of liver parenchyma that is around 100 times greater than that examined by biopsy[18].

Several studies have shown significant positive correlation between TE and the stage of liver fibrosis[14-20]. The performance of TE is optimal for advanced stages of fibrosis[15,21-27].

A limitation of all the studies performed so far is that reference cut-off values of TE for each fibrosis stage were calculated from their own cohort of patients after data collection, and they are different across studies[8]. Few metanalysis have been carried out that have analysed the heterogeneity between studies and the overall performance of TE[5-8]. In the most recent one the cut-off values of 7, 9.5, and 12 kPa in stages F2, F3, and F4, respectively, were used[8].

The aim of this study was to compare the cut-offs of TE obtained by receiver operating characteristic (ROC) curve analysis in our series of consecutive patients, with chronic viral hepatitis referring to our center for liver biopsy, to those used in the recently published meta-analysis[8].

MATERIALS AND METHODS

Patients

This was a single center cross-sectional study. From June, 2009 through February, 2012, all consecutive patients with chronic viral hepatitis scheduled for liver biopsy at the outpatient ward of our Infectious Diseases Department were enrolled into the study.

Diagnosis of chronic hepatitis was based on the presence, at the time of the first observation, of active viral replication or detectable hepatitis C virus (HCV)-RNA in hepatitis B surface antigen or anti-HCV positive carriers, respectively, and, at least transiently, elevated serum alanine aminotransferase level. Patients characteristics, epidemiological data, and biochemical tests were recorded. Liver biopsy was performed on the same day as TE, as day case procedure.

The study protocol was approved by the institutional Ethics Committee. Participants gave their informed written consent.

Transient elastography

TE was carried out by using FibroScan™ (Echosens, Paris, France), a dedicated medical device which provides a quantifiable estimate of liver stiffness in kilopascal (kPa). Two physicians (Ferraioli G and Zicchetti M), with experience of at least 50 TE procedures as previously recommended[9], performed all the examinations. Measurements of liver stiffness were performed on the right lobe through intercostal spaces on patients lying in the dorsal decubitus position with the right arm in maximal abduction, following the examination procedure previously described[14]. Only patients with 10 validated measurements, a success rate of at least 60%, and interquartile range (IQR) of less than 30% of the median liver stiffness value were included. Values were expressed in kPa.

Liver biopsy and histology

Ultrasound-assisted percutaneous liver biopsy (LB) was performed by three experienced physicians (Filice C, Brunetti E, Michelone G) by using an intercostal approach. A disposable 1.4 mm-diameter modified Menghini needle (Hepafix; Braun, Melsungen, Germany) was used. All biopsy specimens were fixed in formalin and embedded in paraffin. The length of each LB specimen (in centimeters) was recorded.

Table 1 Patients demographics, and their biochemical and histological data at liver biopsy examination (%)

Characteristics	All patients (n = 246)	HCV patients (n = 195)	HBV patients (n = 41)
Sex (men)	171 (69.5)	139 (71.3)	30 (73.2)
Age (yr)	44.7 (11.8)[1]	45.3 (11.5)[1]	41.2 (13.7)[1]
BMI (kg/m^2)	25.2 (3.9)[1]	25.3 (3.6)[1]	25.2 (5.3)[1]
AST (IU/L)	39 (24-78)[2]	40 (26-80)[2]	28 (21-48)[2]
ALT (IU/L)	65 (32-116)[2]	70 (35-120)[2]	50 (23-109)[2]
Platelets count (10^3/mm^3)	209 (171-262)[2]	212 (176-270)[2]	203 (172-253)[2]
Fibrosis score (Metavir)			
F0-F1	129 (52.3)	98 (50.3)	30 (73.2)
F2	54 (22.0)	46 (23.6)	6 (14.6)
F3	24 (9.8)	19 (9.7)	3 (7.3)
F4	39 (15.9)	32 (16.4)	2 (4.9)
Steatosis			
S0 (0%)	157 (63.8)	126 (64.6)	26 (63.4)
S1 (< 33%)	56 (22.8)	44 (22.6)	10 (24.4)
S2 (33%-66%)	19 (7.7)	15 (7.7)	4 (9.8)
S3 (> 66%)	14 (5.7)	10 (5.1)	1 (2.4)
HAI			
Mild	109 (44.3)	80 (41.0)	28 (68.3)
Moderate	102 (41.5)	89 (45.7)	7 (17.1)
Severe	35 (14.2)	26 (13.3)	6 (14.6)

[1]mean ± SD [2]Median value and interquartile range (IQR). BMI: Body mass index; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; HAI: Histological activity index; HCV: Hepatitis C virus; HBV: Hepatitis B virus.

The specimens were read on site by a single expert liver pathologist (Dal Bello B), blind to the TE results, but not to the patient's clinical and biochemical data. Fibrosis was evaluated semiquantitatively and staged according to the Metavir scoring system (F0, absent; F1, enlarged fibrotic portal tract; F2, periportal or initial portal-portal septa but intact architecture; F3, architectural distortion but no obvious cirrhosis; and F4, cirrhosis)[28]. For the purpose of this study, F0 and F1 scores were grouped in the subsequent statistical analysis. Necroinflammatory activity was evaluated using the histologic activity index (HAI) described by Knodell *et al* and classified as follows for statistical analysis: HAI score, 1-5 = mild; HAI score, 6-9 = moderate; HAI score, ⩾ 10 = severe[29]. Steatosis was expressed as a percentage of fat in the hepatocytes and classified as absent (S0), < 33% (S1), between 33% and 66% (S2), and > 66% (S3)[30].

Statistical analysis

Descriptive statistics were produced for demographic, clinical and laboratory characteristics for this study sample of patients. The Shapiro-Wilk test was used to test the normal distribution of quantitative variables. When quantitative variables were normally distributed, the results were expressed as mean values and SD, otherwise median and IQR (25th-75th percentile) were reported; qualitative variables were summarized as counts and percentages. Spearman rank coefficient was used to test correlation between two study variables. Quantile regression was used for multivariate model to assess the association between TE and fibrosis, HAI, steatosis, and biochemical tests. A frequency distribution was obtained for choosing optimal cut-off values of TE to maximize the sum of sensitivity and specificity for different fibrosis thresholds: F0-F1 *vs* F2- F4 ($F \geqslant 2$), F0-F2 *vs* F3-F4 ($F \geqslant 3$), F0-F3 *vs* F4 ($F = 4$). The diagnostic performance of TE was assessed by using ROC curves and the area under the ROC (AUC) curve analysis.

Data analysis was performed with STATA statistical package (release 11, 1, 2010, Stata Corporation, College Station, Texas, United States) and Medcalc (Version 11.2, 2011 MedCalc Software bvba , Be).

RESULTS

Patients

Two hundred and fifty-two patients met the inclusion criteria. Six (2%) patients were excluded due to unreliable TE measurements (no successful acquisitions in four patients; IQR > 30% in two patients). Thus, 246 patients were analyzed. Their characteristics are summarized in Table 1. There were 171 men and 75 women. One hundred and ninety-five (79.3%) patients had chronic hepatitis C, 41 (16.7%) had chronic hepatitis B, and 10 (4.0%) were coinfected with HIV. The mean length of the LB samples was 2.7 cm (± 0.8 cm). The fibrosis grade distribution was as follows: F0-F1, 129 patients; F2, 54 patients; F3, 24 patients; F4, 39 patients.

Liver stiffness measurements

All patients (n = 246): Medians and IQR of TE values for each fibrosis stage were 5.2 kPa (4.4-6.4) for F0-F1, 6.4 kPa (5.6-8.6) for F2, 9.0 kPa (7.8-14.8) for F3, and 17.8 kPa (12.9-23.7) for F4. Multiple comparison between groups provided an adjusted P value for significance of 0.004 and showed that there was a statistical significant difference for F0-F1 *vs* F2 (P = 0.000 026), and F2 *vs* F3 (P = 0.001), but not for F3 *vs* F4 (P = 0.02).

In univariate analysis, TE showed a good correlation with the degree of fibrosis (r = 0.72, P < 0.00 001), a moderate correlation with the degree of histological activity index (r = 0.49, P < 0.00 001) and the aspartate aminotransferase (AST) value (r = 0.40, P < 0.00 001), and a poor correlation with the degree of steatosis despite being statistically significant (r = 0.19, P = 0.002). TE also showed a moderate negative correlation with platelet counts (r = -0.41, P < 0.00 001). Multivariate regression analysis including fibrosis stage, histological activity index, steatosis, AST, platelet counts confirmed the correlation with fibrosis stage (P = 0.0001) but not with all other variables.

ROC curve analysis identified optimal cut-off values of liver stiffness measurements as high as 6.9 kPa for $F \geqslant 2$; 7.9 kPa for $F \geqslant 3$; 9.6 kPa for $F = 4$ (Figure 1). Corresponding values of sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (LR), negative LR and AUCs are shown in Table 2.

Table 2 Cut-off values of transient elastography obtained by receiver operating characteristic curve analysis for each Metavir fibrosis stage

	Sensitivity%	Specificity%	PPV%	NPV%	LR+	LR-	AUC
$F \geqslant 2$[1]							
7.0 kPa	70 (66-75)	81 (77-85)					
$F \geqslant 2$[2]							
6.9 kPa	70.9 (61.8-79.0)	86.1 (78.8-91.5)	82.2 (73.3-89.1)	76.6 (68.8-83.2)	5.1 (4.4-5.8)	0.34 (0.2-0.6)	0.86 (0.81-0.90)
$F \geqslant 2$[3]							
6.9 kPa	69.1 (58.9-78.1)	89.8 (82.0-95.0)	87.0 (77.4-93.6)	74.6 (65.7-82.1)	6.8 (5.8-7.9)	0.34 (0.2-0.7)	0.86 (0.80-0.90)
$F \geqslant 3$[1]							
9.5 kPa	80 (72-85)	85 (79-90)					
$F \geqslant 3$[2]							
7.9 kPa	88.9 (78.4-95.4)	85.8 (79.9-90.5)	68.3 (57.1-78.1)	95.7 (91.4-98.3)	6.3 (5.6-7.0)	0.13 (0.06-0.3)	0.94 (0.91-0.97)
9.6 kPa[4]	76.3 (73.8-86.9)	94.5 (90.2-97.3)	82.8 (70.6-91.4)	92.0 (87.2-95.5)	13.9 (12.1-16.1)	0.25 (0.1-0.5)	
$F \geqslant 3$[3]							
7.3 kPa	94.1 (83.8-98.8)	85.4 (70.6-90.7)	69.6 (57.3-80.1)	97.6 (93.2-99.4)	6.5 (5.9-7.1)	0.07 (0.02-0.2)	0.95 (0.91-0.98)
9.3 kPa[4]	74.5 (60.4-85.7)	96.5 (92.1-98.9)	88.4 (74.9-96.1)	91.4 (85.8-95.4)	21.5 (18.2-25.3)	0.3 (0.1-0.7)	
$F = 4$[1]							
12 kPa	86 (80-91)	88 (82-91)					
$F = 4$[2]							
9.6 kPa	95 (83-99)	90 (85-94)	64 (50-76)	99 (96-100)	9.4 (8.6-10.2)	0.06 (0.01-0.2)	0.96 (0.93-0.98)
12.2 kPa[4]	84.6 (69.5-94.1)	94.7 (90.7-97.3)	75.0 (59.7-86.8)	97.0 (93.6-98.9)	15.9 (13.9-18.3)	0.16 (0.06-0.4)	
$F = 4$[3]							
9.3 kPa	93.8 (79.2-99.2)	92.0 (86.7-95.7)	69.0 (53.9-82.8)	98.7 (93.3-99.8)	11.8 (10.6-13.0)	0.07 (0.02-0.3)	0.97 (0.93-0.99)
12.3 kPa[4]	81.3 (63.6-92.8)	95.7 (91.4-98.3)	78.8 (61.1-91.0)	96.3 (92.1-98.6)	18.9 (16.0-22.4)	0.2 (0.07-0.5)	

[1]Cut-off values of transient elastography from the paper of Tsochatzis *et al*[8]; [2]Cut-off values of transient elastography in all patients (n = 246); [3]Cut-off values of transient elastography in patients with chronic hepatitis C (n = 195); [4]Cut-off values obtained by maximizing specificity. PPV: Positive predictive value; NPV: Negative predictive value; LR+: Positive likelihood ratio; LR-: Negative likelihood ratio; AUC: The area under the receiver operating characteristic.

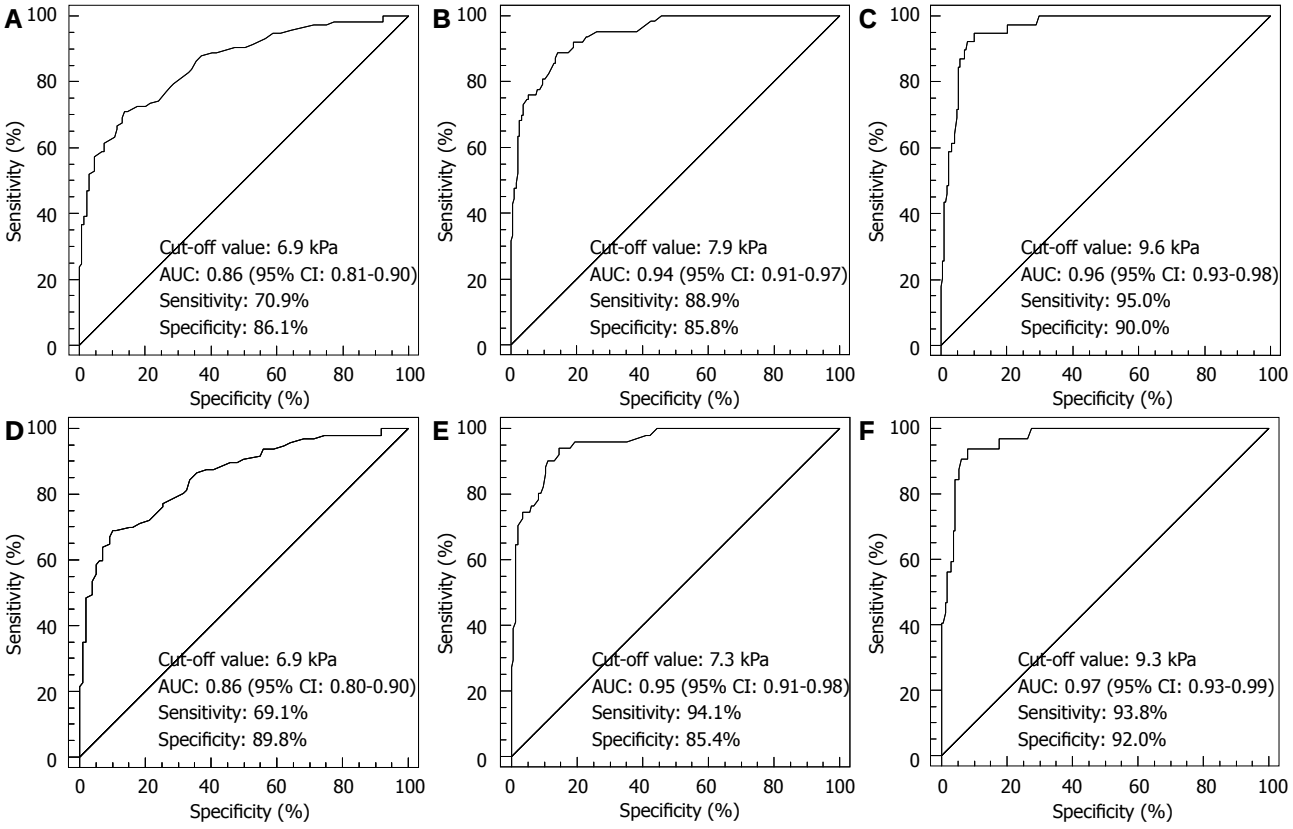


Figure 1 Receiver operating characteristic curve. A: F0-F1 *vs* F2- F4 (F > 2) in all patients (n = 246); B: F0-F2 *vs* F3- F4 (F > 3) in all patients (n = 246); C: F0-F3 *vs* F4 (F = 4) in all patients (n = 246); D: F0-F1 *vs* F2- F4 (F > 2) in patients with chronic hepatitis C (n = 195); E: F0-F2 *vs* F3- F4 (F > 3) in patients with chronic hepatitis C (n = 195); F: F0-F3 *vs* F4 (F = 4) in patients with chronic hepatitis C (n = 195).

Cut-off values of liver stiffness measurements obtained maximizing only the specificity were as high as 6.9 kPa for $F \geqslant 2$; 9.6 kPa for $F \geqslant 3$; 12.2 kPa for $F = 4$.

Patients with hepatitis C (n = 195): Medians and IQR of TE values for each fibrosis stage were 5.1 kPa (4.2-6.3) for F0-F1, 6.4 kPa (5.4-7.9) for F2, 8.9 kPa (8.0-13.3) for F3, and 17.4 kPa (12.8-22.2) for F4.

Multiple comparison between groups gave an adjusted P-value for significance of 0.004 and showed that there was a statistical significant difference for F0-F1 *vs* F2 (P = 0.0002), and F2 *vs* F3 (P = 0.001), but not for F3 *vs* F4 (P = 0.04).

In univariate analysis, TE showed a good correlation with the degree of fibrosis (r = 0.72, P < 0.00 001), a moderate correlation with the degree of histological activity index (r = 0.43, P < 0.00 001) and the AST value (r = 0.36, P < 0.00 001), and a poor correlation with the degree of steatosis despite being statistically significant (r = 0.19, P = 0.002). TE also showed a moderate negative correlation with platelet counts (r = -0.39, P = 0.002). Multivariate regression analysis including fibrosis stage, histological activity index, steatosis, AST, platelet counts confirmed the correlation with fibrosis stage (P < 0.0001) but not with all other variables.

ROC curve analysis identified optimal cut-off values of liver stiffness measurements as high as 6.9 kPa for $F \geqslant 2$; 7.3 kPa for $F \geqslant 3$; 9.3 kPa for $F = 4$ (Figure 1). Corresponding values of sensitivity, specificity, PPV, NPV, positive LR, negative LR and AUCs are shown in Table 2.

Cut-off values of liver stiffness measurements obtained maximizing only the specificity were as high as 7.0 kPa for $F \geqslant 2$; 9.3 kPa for $F \geqslant 3$; 12.3 kPa for $F = 4$.

Patients with hepatitis B (n = 41): Due to the small number of patients, only patients with F0-F1 stages *vs* F2-F4 stages were considered for statistical analysis. There was a statistical significant difference for F0-F1 *vs* F2-F4 (P = 0.0006).

In univariate analysis, TE showed a good correlation with the degree of fibrosis (r = 0.60, P < 0.00 001), a high correlation with the degree of histological activity index (r = 0.70, P < 0.00 001) and the AST value (r = 0.76, P < 0.00 001); no correlation was found with steatosis. Due to the small number of patients, multivariate regression analysis was not performed.

DISCUSSION

The results of this study show that the cut-off values of TE obtained for each fibrosis stage in a population of consecutive patients with chronic viral hepatitis from a single center are comparable to that obtained in the recent meta-analysis by Tsochatzis *et al*[8] that included up to 40 studies. In their study, the meta-analysis was conducted for cut-offs 7.0, 9.5 and 12 kPa in stages F2, F3, and F4, respectively.

In our series, when maximizing the sum of sensitivity and specificity for different fibrosis thresholds, the optimal cut-offs of TE were similar to those of the meta-analysis for the diagnosis of significant fibrosis ($F \geqslant 2$), and were lower for the diagnosis of advanced fibrosis ($F \geqslant 3$) and cirrhosis ($F = 4$). However, when maximizing specificity, the cut-offs were similar to those used by Tsochatzis *et al*[8], and showed specificity even higher with respect to those of the meta-analysis for all fibrosis stages.

In our series the results obtained in all patients with chronic viral hepatitis were almost similar to that obtained excluding patients with chronic hepatitis B. On the other hand, it should be underlined that our series included only 41/246 (16.7%) patients with chronic hepatitis B. Due to the small number, the statistical analysis of the data obtained in this group of patients was not performed.

An uneven distribution of patients over fibrosis stage, as observed in our study, is commonly found when consecutive patients undergoing liver biopsy are enrolled. Indeed, in our series the distribution of patients over fibrosis stages is almost similar to that observed in other studies[17,20,31,32], or when large series - as in the study of Degos *et al*[17] were enrolled.

Unlike to that reported by other authors[33,34], who found that the prevalence of liver steatosis in patients with hepatitis C was more than 50%, in our series the majority of patients had no to mild steatosis. On the other hand, it should be noted that, in our study, the mean value of BMI was within the normal range. This patient's characteristic could also explain the lower rate of TE unreliable results with respect to that reported in the literature[35,36].

In the seminal work of Sandrin *et al*[14] 93% of patients with TE values ⩽ 5.1 kPa were F0-F1, whereas 94% of patients with TE values ⩾ 7.6 kPa were F2 or more. On the other hand, patients with cirrhosis presented a very large range (from 14 kPa to 69 kPa). A limitation of that study is that liver biopsy and TE measurements were not performed in the same day; indeed, an inclusion criterion was liver biopsy performed less than one year before TE assessment. Since then , several studies have been published[15,31,37,38]. A wide range of TE cut-off value for F4 stage - from 11.8 kPa to 26.5 kPa - is found when comparing the results of several studies[5-9,15,17,31,35,37-39]. The different optimal TE cut-offs for F4 stage found in these studies by ROC curve analysis depend also on the fact that the characteristics of the patient population are not similar. Harada *et al*[37], that found a cut-off value of 26.5 kPa, enrolled patients with HCV recurrence after liver transplantation. In the study of Masuzaki *et al*[38], in which a cut-off of 15.9 kPa was found for F4 stage, there was a prevalence of patients with cirrhosis or pre-cirrhosis. In the study of Ziol *et al*[31], in which the optimal cut-off value for F4 stage was 14.5 kPa, liver biopsy was performed by the tran-

sjugular route in 63/251 (25.1%) of patients. It could be inferred that these patients presented already with clinical signs of cirrhosis, thus higher values of TE in the F4 stage - respect to patients without a clinical diagnosis of liver cirrhosis - could be expected[15,40]. Indeed, it has been demonstrated that there is an increase of TE values with liver disease progression beyond the F4 stage[15,19], and a highly positive correlation between TE values and portal pressure has been found[24,26]. Results similar to that obtained in our series were found in studies in which consecutive patients without clinical signs of liver cirrhosis were enrolled[39,41]. Cirrhosis should be regarded as a dynamic process, thus it is likely that patients in the initial stage of liver cirrhosis show lower values of TE respect to patients with compensated disease. Moreover, in the study of Foucher *et al*[15] liver stiffness was significantly correlated with Child-Pugh score. In their study, which included a large patient population with liver disease of different etiologies and also patients with decompensated cirrhosis, the optimal TE cut-off for F4 stage 17.6 kPa.

In conclusion, the results of our study show that the TE values used in the metanalysis of Tsochatzis *et al*[8] - namely 7.0, 9.5, and 12.0 kPa in stages F2, F3, and F4, respectively - could be used as given reference values in future studies aimed at evaluating the performance of TE in the assessment of liver fibrosis.

ACKNOWLEDGMENTS

The authors would like to thank Ms. Livia Astroni, Ms. Natali Calabrese, Mr. Filippo Cuda, Mr. Lorenzo Guioli, Ms. Maura Marchisoni, Ms. Giampiera Nava, Ms. Loredana Pavesi, Ms. Barbara Ricci, nurses in the outpatient ward of the Infectious Diseases Department, for their valuable help in complying with the study protocol.

COMMENTS

Background

Several studies have shown significant positive correlation between transient elastography (TE) and the stage of liver fibrosis. A limitation of all the studies is that the cut-off values for each fibrosis stage were calculated from their own cohort of patients after data collection, and they are different across studies.

Research frontiers

The aim of this study was to compare the cut-offs of TE obtained by receiver operating characteristic (ROC) curve analysis in their series of consecutive patients with chronic viral hepatitis to that used in a recently published meta-analysis.

Innovations and breakthroughs

The results of this study show that the cut-off values of TE obtained for each fibrosis stage in a population of consecutive patients with chronic viral hepatitis from a single center are comparable to that obtained in a recent meta-analysis that included up to 40 studies.

Applications

The values of TE obtained in the recently published meta-analysis could be used as reference values in future studies aimed at evaluating the performance of the technique in the assessment of liver fibrosis.

Terminology

Transient elastography is a technique that noninvasively quantify liver stiffness by estimating the velocity of propagation of a shear wave along an ultrasonic A-line. The harder the tissue, the faster the shear wave travels.

Peer review

The authors conducted this study to compare the cut-offs of TE obtained by ROC curve analysis in their series of consecutive patients, with chronic viral hepatitis referred to their center for liver biopsy, to that used in a recently published meta-analysis.

REFERENCES

1 **Bravo AA**, Sheth SG, Chopra S. Liver biopsy. *N Engl J Med* 2001; **344**: 495-500 [PMID: 11172192 DOI: 10.1056/NEJM200102153440706]
2 **Regev A**, Berho M, Jeffers LJ, Milikowski C, Molina EG, Pyrsopoulos NT, Feng ZZ, Reddy KR, Schiff ER. Sampling error and intraobserver variation in liver biopsy in patients with chronic HCV infection. *Am J Gastroenterol* 2002; **97**: 2614-2618 [PMID: 12385448 DOI: 10.1111/j.1572-0241.2002.06038]
3 **Cadranel JF**, Rufat P, Degos F. Practices of liver biopsy in France: results of a prospective nationwide survey. For the Group of Epidemiology of the French Association for the Study of the Liver (AFEF). *Hepatology* 2000; **32**: 477-481 [PMID: 10960438 DOI: 10.1053/jhep.2000.16602]
4 **Castéra L**, Nègre I, Samii K, Buffet C. Pain experienced during percutaneous liver biopsy. *Hepatology* 1999; **30**: 1529-1530 [PMID: 10610352 DOI: 10.1002/hep.510300624]
5 **Shaheen AA**, Wan AF, Myers RP. FibroTest and FibroScan for the prediction of hepatitis C-related fibrosis: a systematic review of diagnostic test accuracy. *Am J Gastroenterol* 2007; **102**: 2589-2600 [PMID: 17850410 DOI: 10.1111/j.1572-0241.2007.01466.x]
6 **Friedrich-Rust M**, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, Herrmann E. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. *Gastroenterology* 2008; **134**: 960-974 [PMID: 18395077 DOI: 10.1053/j.gastro.2008.01.034]
7 **Stebbing J**, Farouk L, Panos G, Anderson M, Jiao LR, Mandalia S, Bower M, Gazzard B, Nelson M. A meta-analysis of transient elastography for the detection of hepatic fibrosis. *J Clin Gastroenterol* 2010; **44**: 214-219 [PMID: 19745758 DOI: 10.1097/MCG.0b013e3181b4af1f]
8 **Tsochatzis EA**, Gurusamy KS, Ntaoula S, Cholongitas E, Davidson BR, Burroughs AK. Elastography for the diagnosis of severity of fibrosis in chronic liver disease: a meta-analysis of diagnostic accuracy. *J Hepatol* 2011; **54**: 650-659 [PMID: 21146892 DOI: 10.1016/j.jhep.2010.07.033]
9 **Castéra L**, Vergniol J, Foucher J, Le Bail B, Chanteloup E, Haaser M, Darriet M, Couzigou P, De Lédinghen V. Prospective comparison of transient elastography, Fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005; **128**: 343-350 [PMID: 15685546 DOI: 10.1053/j.gastro.2004.11.018]
10 **Poynard T**, Morra R, Halfon P, Castera L, Ratziu V, Imbert-Bismut F, Naveau S, Thabut D, Lebrec D, Zoulim F, Bourliere M, Cacoub P, Messous D, Munteanu M, de Ledinghen V. Meta-analyses of FibroTest diagnostic value in chronic liver disease. *BMC Gastroenterol* 2007; **7**: 40 [PMID: 17937811 DOI: 10.1186/1471-230X-7-40]
11 **Gressner OA**, Weiskirchen R, Gressner AM. Biomarkers of liver fibrosis: clinical translation of molecular pathogenesis or based on liver-dependent malfunction tests. *Clin Chim Acta* 2007; **381**: 107-113 [PMID: 17399697 DOI: 10.1016/j.cca.2007.02.038]
12 **Shaheen AA**, Myers RP. Diagnostic accuracy of the aspartate aminotransferase-to-platelet ratio index for the prediction of hepatitis C-related fibrosis: a systematic review. *Hepatology* 2007; **46**: 912-921 [PMID: 17705266 DOI: 10.1002/hep.21835]
13 **Pinzani M**, Vizzutti F, Arena U, Marra F. Technology In-

sight: noninvasive assessment of liver fibrosis by biochemical scores and elastography. *Nat Clin Pract Gastroenterol Hepatol* 2008; **5**: 95-106 [PMID: 18253138 DOI: 10.1038/ncpgasthep1025]

14 **Sandrin L**, Fourquet B, Hasquenoph JM, Yon S, Fournier C, Mal F, Christidis C, Ziol M, Poulet B, Kazemi F, Beaugrand M, Palau R. Transient elastography: a new noninvasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol* 2003; **29**: 1705-1713 [PMID: 14698338 DOI: 10.1016/j.ultrasmedbio.2003.07.001]

15 **Foucher J**, Chanteloup E, Vergniol J, Castéra L, Le Bail B, Adhoute X, Bertet J, Couzigou P, de Lédinghen V. Diagnosis of cirrhosis by transient elastography (FibroScan): a prospective study. *Gut* 2006; **55**: 403-408 [PMID: 16020491 DOI: 10.1136/gut.2005.069153]

16 **Takeda T**, Yasuda T, Nakayama Y, Nakaya M, Kimura M, Yamashita M, Sawada A, Abo K, Takeda S, Sakaguchi H, Shiomi S, Asai H, Seki S. Usefulness of noninvasive transient elastography for assessment of liver fibrosis stage in chronic hepatitis C. *World J Gastroenterol* 2006; **12**: 7768-7773 [PMID: 17203518]

17 **Degos F**, Perez P, Roche B, Mahmoudi A, Asselineau J, Voitot H, Bedossa P. Diagnostic accuracy of FibroScan and comparison to liver fibrosis biomarkers in chronic viral hepatitis: a multicenter prospective study (the FIBROSTIC study). *J Hepatol* 2010; **53**: 1013-1021 [PMID: 20850886 DOI: 10.1016/j.jhep.2010.05.035]

18 **Castera L**, Forns X, Alberti A. Non-invasive evaluation of liver fibrosis using transient elastography. *J Hepatol* 2008; **48**: 835-847 [PMID: 18334275 DOI: 10.1016/j.jhep.2008.02.008]

19 **Carrión JA**, Navasa M, Bosch J, Bruguera M, Gilabert R, Forns X. Transient elastography for diagnosis of advanced fibrosis and portal hypertension in patients with hepatitis C recurrence after liver transplantation. *Liver Transpl* 2006; **12**: 1791-1798 [PMID: 16823833 DOI: 10.1002/lt.20857]

20 **Fraquelli M**, Rigamonti C, Casazza G, Donato MF, Ronchi G, Conte D, Rumi M, Lampertico P, Colombo M. Etiology-related determinants of liver stiffness values in chronic viral hepatitis B or C. *J Hepatol* 2011; **54**: 621-628 [PMID: 21146243 DOI: 10.1016/j.jhep.2010.07.017]

21 **de Lédinghen V**, Douvin C, Kettaneh A, Ziol M, Roulot D, Marcellin P, Dhumeaux D, Beaugrand M. Diagnosis of hepatic fibrosis and cirrhosis by transient elastography in HIV/hepatitis C virus-coinfected patients. *J Acquir Immune Defic Syndr* 2006; **41**: 175-179 [PMID: 16394849 DOI: 10.1097/01.qai.0000194238.15831.c7]

22 **Ganne-Carrié N**, Ziol M, de Ledinghen V, Douvin C, Marcellin P, Castera L, Dhumeaux D, Trinchet JC, Beaugrand M. Accuracy of liver stiffness measurement for the diagnosis of cirrhosis in patients with chronic liver diseases. *Hepatology* 2006; **44**: 1511-1517 [PMID: 17133503 DOI: 10.1002/hep.21420]

23 **Arena U**, Vizzutti F, Abraldes JG, Corti G, Stasi C, Moscarella S, Milani S, Lorefice E, Petrarca A, Romanelli RG, Laffi G, Bosch J, Marra F, Pinzani M. Reliability of transient elastography for the diagnosis of advanced fibrosis in chronic hepatitis C. *Gut* 2008; **57**: 1288-1293 [PMID: 18448567 DOI: 10.1136/gut.2008.149708]

24 **Vizzutti F**, Arena U, Romanelli RG, Rega L, Foschi M, Colagrande S, Petrarca A, Moscarella S, Belli G, Zignego AL, Marra F, Laffi G, Pinzani M. Liver stiffness measurement predicts severe portal hypertension in patients with HCV-related cirrhosis. *Hepatology* 2007; **45**: 1290-1297 [PMID: 17464971 DOI: 10.1002/hep.21665]

25 **Rigamonti C**, Donato MF, Fraquelli M, Agnelli F, Ronchi G, Casazza G, Rossi G, Colombo M. Transient elastography predicts fibrosis progression in patients with recurrent hepatitis C after liver transplantation. *Gut* 2008; **57**: 821-827 [PMID: 18218676 DOI: 10.1136/gut.2007.135046]

26 **Kazemi F**, Kettaneh A, N'kontchou G, Pinto E, Ganne-Carrie N, Trinchet JC, Beaugrand M. Liver stiffness measurement selects patients with cirrhosis at risk of bearing large oesophageal varices. *J Hepatol* 2006; **45**: 230-235 [PMID: 16797100 DOI: 10.1016/j.jhep.2006.04.006]

27 **Rockey DC**. Noninvasive assessment of liver fibrosis and portal hypertension with transient elastography. *Gastroenterology* 2008; **134**: 8-14 [PMID: 18166342 DOI: 10.1053/j.gastro.2007.11.053]

28 **Bedossa P**, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996; **24**: 289-293 [PMID: 8690394 DOI: 10.1002/hep.510240201]

29 **Desmet VJ**. Knodell RG, Ishak KG, Black WC, Chen TS, Craig R, Kaplowitz N, Kiernan TW, Wollman J. Formulation and application of a numerical scoring system for assessing histological activity in asymptomatic chronic active hepatitis [Hepatology 1981; 1: 431-435]. *J Hepatol* 2003; **38**: 382-386 [PMID: 12663226 DOI: 10.1002/hep.1840010511]

30 **Brunt EM**, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Nonalcoholic steatohepatitis: a proposal for grading and staging the histological lesions. *Am J Gastroenterol* 1999; **94**: 2467-2474 [PMID: 10484010]

31 **Ziol M**, Handra-Luca A, Kettaneh A, Christidis C, Mal F, Kazemi F, de Lédinghen V, Marcellin P, Dhumeaux D, Trinchet JC, Beaugrand M. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. *Hepatology* 2005; **41**: 48-54 [PMID: 15690481 DOI: 10.1002/hep.20506]

32 **Karlas T**, Pfrepper C, Wiegand J, Wittekind C, Neuschulz M, Mössner J, Berg T, Tröltzsch M, Keim V. Acoustic radiation force impulse imaging (ARFI) for non-invasive detection of liver fibrosis: examination standards and evaluation of interlobe differences in healthy subjects and chronic liver disease. *Scand J Gastroenterol* 2011; **46**: 1458-1467 [PMID: 21916815 DOI: 10.3109/00365521.2011.610004]

33 **Leandro G**, Mangia A, Hui J, Fabris P, Rubbia-Brandt L, Colloredo G, Adinolfi LE, Asselah T, Jonsson JR, Smedile A, Terrault N, Pazienza V, Giordani MT, Giostra E, Sonzogni A, Ruggiero G, Marcellin P, Powell EE, George J, Negro F. Relationship between steatosis, inflammation, and fibrosis in chronic hepatitis C: a meta-analysis of individual patient data. *Gastroenterology* 2006; **130**: 1636-1642 [PMID: 16697727 DOI: 10.1053/j.gastro.2006.03.014]

34 **Asselah T**, Rubbia-Brandt L, Marcellin P, Negro F. Steatosis in chronic hepatitis C: why does it really matter? *Gut* 2006; **55**: 123-130 [PMID: 16344578 DOI: 10.1136/gut.2005.069757]

35 **Fraquelli M**, Rigamonti C, Casazza G, Conte D, Donato MF, Ronchi G, Colombo M. Reproducibility of transient elastography in the evaluation of liver fibrosis in patients with chronic liver disease. *Gut* 2007; **56**: 968-973 [PMID: 17255218 DOI: 10.1136/gut.2006.111302]

36 **Castéra L**, Foucher J, Bernard PH, Carvalho F, Allaix D, Merrouche W, Couzigou P, de Lédinghen V. Pitfalls of liver stiffness measurement: a 5-year prospective study of 13,369 examinations. *Hepatology* 2010; **51**: 828-835 [PMID: 20063276 DOI: 10.1002/hep.23425]

37 **Harada N**, Soejima Y, Taketomi A, Yoshizumi T, Ikegami T, Yamashita Y, Itoh S, Kuroda Y, Maehara Y. Assessment of graft fibrosis by transient elastography in patients with recurrent hepatitis C after living donor liver transplantation. *Transplantation* 2008; **85**: 69-74 [PMID: 18192914 DOI: 10.1097/01.tp.0000297248.18483.16]

38 **Masuzaki R**, Tateishi R, Yoshida H, Goto E, Sato T, Ohki T, Goto T, Yoshida H, Kanai F, Sugioka Y, Ikeda H, Shiina S, Kawabe T, Omata M. Comparison of liver biopsy and transient elastography based on clinical relevance. *Can J Gastroenterol* 2008; **22**: 753-757 [PMID: 18818788]

39 **Lupşor M**, Badea R, Stefănescu H, Grigorescu M, Sparchez

Z, Serban A, Branda H, Iancu S, Maniu A. Analysis of histopathological changes that influence liver stiffness in chronic hepatitis C. Results from a cohort of 324 patients. *J Gastrointestin Liver Dis* 2008; **17**: 155-163 [PMID: 18568136]

40 **Poynard T**, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. *Lancet* 1997; **349**: 825-832 [PMID: 9121257 DOI: 10.1016/S0140-6736(96)07642-8]

41 **Rizzo L**, Calvaruso V, Cacopardo B, Alessi N, Attanasio M, Petta S, Fatuzzo F, Montineri A, Mazzola A, L'abbate L, Nunnari G, Bronte F, Di Marco V, Craxì A, Cammà C. Comparison of transient elastography and acoustic radiation force impulse for non-invasive staging of liver fibrosis in patients with chronic hepatitis C. *Am J Gastroenterol* 2011; **106**: 2112-2120 [PMID: 21971536 DOI: 10.1038/ajg.2011.341]

P- Reviewer Atta HM **S- Editor** Gou SX
L- Editor A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i1.57

World J Gastroenterol 2013 January 7; 19(1): 57-64
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Comparison of fatty liver index with noninvasive methods for steatosis detection and quantification

Shira Zelber-Sagi, Muriel Webb, Nimer Assy, Laurie Blendis, Hanny Yeshua, Moshe Leshno, Vlad Ratziu, Zamir Halpern, Ran Oren, Erwin Santo

Shira Zelber-Sagi, Muriel Webb, Laurie Blendis, Hanny Yeshua, Moshe Leshno, Zamir Halpern, Ran Oren, Erwin Santo, Department of Gastroenterology, Tel Aviv Sourasky Medical Center, 64239 Tel-Aviv, Israel
Shira Zelber-Sagi, School of Public Health, Faculty of Social Welfare and Health Sciences, University of Haifa, 31905 Haifa, Israel
Nimer Assy, Liver Unit, Ziv Medical Center, Israel and Bar Ilan University, 13100 Safed, Israel
Moshe Leshno, Zamir Halpern, Ran Oren, Erwin Santo, The Sackler Faculty of Medicine, Tel-Aviv University, 69978 Ramat Aviv, Israel
Vlad Ratziu, Université Pierre et Marie Curie, Hôpital Pitié Salpêtrière, 75013 Paris, France
Author contributions: Zelber-Sagi S conceived and designed the study, performed the data collection, analyzed the data and wrote the manuscript; Webb M developed the HRI method and performed the abdominal ultrasounds and HRI calculations; Assy N, Blendis L, Yeshua H, Ratziu V critically reviewed the manuscript and contributed to the writing of the manuscript; Leshno M contributed to the data analyzes; Halpern Z, Oren R conducted data collection and critically reviewed the manuscript; Santo E helped to design the study and was involved in the development of the HRI method; all authors read and approved the final manuscript.
Correspondence to: Dr. Shira Zelber-Sagi, Researcher, Lecturer, The Liver Unit, Department of Gastroenterology, Tel-Aviv Sourasky Medical Center, 64239 Tel-Aviv, Israel. zelbersagi@bezeqint.net
Telephone: +972-3-6973984 Fax: +972-3-6974622
Received: June 15, 2012 Revised: September 18, 2012
Accepted: September 22, 2012
Published online: January 7, 2013

Abstract

AIM: To compare noninvasive methods presently used for steatosis detection and quantification in nonalcoholic fatty liver disease (NAFLD).

METHODS: Cross-sectional study of subjects from the general population, a subgroup from the First Israeli National Health Survey, without excessive alcohol consumption or viral hepatitis. All subjects underwent anthropometric measurements and fasting blood tests. Evaluation of liver fat was performed using four noninvasive methods: the SteatoTest; the fatty liver index (FLI); regular abdominal ultrasound (AUS); and the hepatorenal ultrasound index (HRI). Two of the noninvasive methods have been validated *vs* liver biopsy and were considered as the reference methods: the HRI, the ratio between the median brightness level of the liver and right kidney cortex; and the SteatoTest, a biochemical surrogate marker of liver steatosis. The FLI is calculated by an algorithm based on triglycerides, body mass index, γ-glutamyl-transpeptidase and waist circumference, that has been validated only *vs* AUS. FLI < 30 rules out and FLI ⩾ 60 rules in fatty liver.

RESULTS: Three hundred and thirty-eight volunteers met the inclusion and exclusion criteria and had valid tests. The prevalence rate of NAFLD was 31.1% according to AUS. The FLI was very strongly correlated with SteatoTest (r = 0.91, P < 0.001) and to a lesser but significant degree with HRI (r = 0.55, P < 0.001). HRI and SteatoTest were significantly correlated (r = 0.52, P < 0.001). The κ between diagnosis of fatty liver by SteatoTest (⩾ S2) and by FLI (⩾ 60) was 0.74, which represented good agreement. The sensitivity of FLI *vs* SteatoTest was 85.5%, specificity 92.6%, positive predictive value (PPV) 74.7%, and negative predictive value (NPV) 96.1%. Most subjects (84.2%) with FLI < 60 had S0 and none had S3-S4. The κ between diagnosis of fatty liver by HRI (⩾ 1.5) and by FLI (⩾ 60) was 0.43, which represented only moderate agreement. The sensitivity of FLI *vs* HRI was 56.3%, specificity 86.5%, PPV 57.0%, and NPV 86.1%. The diagnostic accuracy of FLI for steatosis > 5%, as predicted by SteatoTest, yielded an area under the receiver operating characteristic curve (AUROC) of 0.97 (95% CI: 0.95-0.98). The diagnostic accuracy of FLI for steatosis

> 5%, as predicted by HRI, yielded an AUROC of 0.82 (95% CI: 0.77-0.87). The κ between diagnosis of fatty liver by AUS and by FLI ($\geq$ 60) was 0.48 for the entire sample. However, after exclusion of all subjects with an intermediate FLI score of 30-60, the κ between diagnosis of fatty liver by AUS and by FLI either $\geq$ 60 or < 30 was 0.65, representing good agreement. Excluding all the subjects with an intermediate FLI score, the sensitivity of FLI was 80.3% and the specificity 87.3%. Only 8.5% of those with FLI < 30 had fatty liver on AUS, but 27.8% of those with FLI $\geq$ 60 had normal liver on AUS.

CONCLUSION: FLI has striking agreement with SteatoTest and moderate agreements with AUS or HRI. However, if intermediate values are excluded FLI has high diagnostic value *vs* AUS.

© 2013 Baishideng. All rights reserved.

Key words: Steatosis; Hepatorenal ultrasound index; SteatoTest; Fatty liver index; Screening; Agreement; Sensitivity; Specificity

Zelber-Sagi S, Webb M, Assy N, Blendis L, Yeshua H, Leshno M, Ratziu V, Halpern Z, Oren R, Santo E. Comparison of fatty liver index with noninvasive methods for steatosis detection and quantification. *World J Gastroenterol* 2013; 19(1): 57-64 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i1/57.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i1.57

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is emerging as a significant health burden raising serious clinical and public health concerns. Liver steatosis may predispose the liver to inflammation, fibrosis and eventually cirrhosis and hepatocellular carcinoma[1,2]. Furthermore, it is regarded as the most prevalent chronic liver disease affecting as much as 30% of the adult western population[3-5].

Besides the hepatic damage, in recent years NAFLD has emerged as an independent risk factor for type 2 diabetes and cardiovascular disease[6,7]. Therefore, efforts to prevent NAFLD progression and extrahepatic manifestations must include screening and surveillance strategies. The occult nature of the disease has led to increased efforts in achieving simple and cost-effective diagnostic methods, preferably quantitative, that would be useful for screening, follow-up and evaluation of response to treatment in both clinical practice and research.

Liver biopsy is the gold standard for quantification of liver steatosis in NAFLD[8]. However, it is not routinely performed because it is an invasive procedure with a significant degree of sampling error[9]. In addition, due to the high prevalence of NAFLD in the general population using routine liver biopsy to diagnose NAFLD is unreasonable. Therefore, noninvasive methods including imaging techniques and blood-test-based formulas have been developed to qualify and quantify liver steatosis[10-12]. However, a widely accepted examination that is easy to perform, accurate and inexpensive has yet to be found.

The aim of this study was to compare the fatty liver index (FLI)[13], which has been validated only *vs* abdominal ultrasound (AUS) with two different reference noninvasive methods for steatosis quantification that were validated *vs* liver biopsy: the hepatorenal ultrasound index (HRI)[14], and the SteatoTest, a biochemical surrogate marker of liver steatosis[15], providing together a more complete picture of construct validity. We also aimed to compare FLI with regular AUS in a qualitative manner.

MATERIALS AND METHODS

Study population and measurements

A cross-sectional study was performed during 2003-2004, consisting of 375 participants, a subgroup from the First Israeli National Health Survey[16] as described in detail elsewhere[5,17]. Exclusion criteria were: alcohol consumption $\geq$ 30 g/d in men or 20 g/d in women, presence of hepatitis B surface antigen or anti-hepatitis C virus antibodies, fatty liver suspected to be secondary to hepatotoxic drugs, inflammatory bowel disease, prior surgery that could cause fatty liver, or celiac disease. All patients underwent measurements of weight, height, and waist circumference according to uniform protocols. Blood samples were drawn following a 12-h fast, and tested for liver enzymes, serum lipid profile, and fasting serum glucose and serum insulin levels. Frozen serum samples from all participants were stored at -80 ℃ until the analysis of SteatoTest (BioPredictive, Paris, France). Biomarkers components were analyzed according to published recommendations[18]. A face-to-face interview was carried out with a questionnaire that was assembled by the Israeli Ministry of Health[16] and included demographic data, health status and a detailed questionnaire on alcohol intake.

The study protocol was approved by the institution's human research committee and all participants gave signed informed consent.

AUS for detection of fatty liver and liver fat quantification

Fatty liver was diagnosed qualitatively by AUS using standardized criteria[19]. Ultrasound was performed in all subjects with the same equipment (EUB-8500 scanner; Hitachi Medical Corporation, Tokyo, Japan) and by the same operator (Webb M) as described previously[5,20]. The radiologist was blinded to the results of the blood tests and the clinical background of the participants, and the calculation of steatosis biomarkers was performed only after the radiological examination.

Furthermore, during AUS, the same single radiologist performed steatosis quantification using the HRI. The HRI has been validated *vs* liver biopsy and is an objective operator-independent examination[14] (available in 331 subjects). As previously described in detail[14], during ultrasonography, a graphic representation of echo

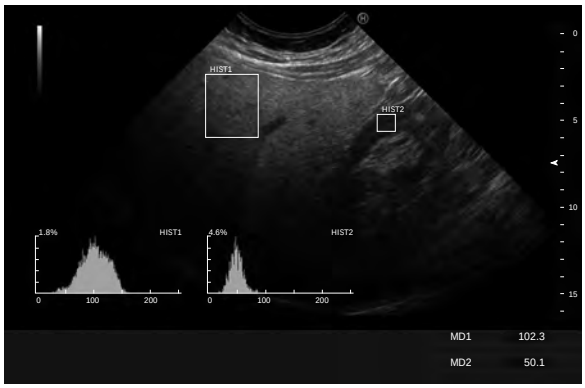


Figure 1 Ultrasound image of the liver and the right kidney cortex with graphic representation of the histogram in the region of interest rectangle. HIST1 is the histogram of the liver and HIST2 is the histogram of the right kidney cortex. The median histogram (MD) 1 of the liver is 102.3 and the MD2 of the kidney cortex is 50.1, yielding an HRI of 2.04.

intensity (histogram) within a region of interest in both the liver and the right kidney cortex was obtained on the same longitudinal sonographic plane. The ratio between the median brightness level of the liver and the right kidney cortex was calculated to determine the HRI (Figure 1). HRI $\geqslant$ 1.50 indicated fatty liver (parallel to steatosis > 5% on liver biopsy) with a sensitivity of 100% and specificity of 91% according to our validation study[14].

Surrogate measures of fatty liver

SteatoTest, the steatosis index, has been validated *vs* liver biopsy with a sensitivity of 0.90 and specificity of 0.54 at the 0.30 cut-off for the diagnosis of > 5% steatosis[15]. SteatoTest is calculated by the combination of the FibroTest with serum alanine amino transferase (ALT), body mass index (BMI), blood glucose, serum triglycerides and cholesterol adjusted for age and sex[15]. Two subjects had extremely low haptoglobin values, with suspected hemolysis and a high risk of false-positive FibroTest results, and therefore were omitted from our analysis. The definition of fatty liver was SteatoTest $\geqslant$ S2 (> 5% fat).

The FLI was calculated by an algorithm based on triglycerides, BMI, γ-glutamyl-transpeptidase and waist circumference. The FLI score range is 0-100 and estimates the percentage chance of having fatty liver. It has been validated *vs* fatty liver diagnosed qualitatively by ultrasound with a sensitivity of 0.61 and specificity of 0.86 for a cut-off of $\geqslant$ 60[13]. FLI < 30 rules out and FLI $\geqslant$ 60 rules in fatty liver. Our analyses referred to a cut-off with a value above 60 indicating NAFLD and a value below indicating no NAFLD.

Statistical analysis

Statistical analyses were performed using SPSS Version 17 (Chicago, IL, Unitd States). Continuous variables are presented as mean ± SD. The Pearson correlation coefficient was used for continuous variables. To test differences in continuous variables between two groups the independent samples *t* test was performed. For nominal variables, the Pearson χ^2 test was performed. To test the predictive value of the methods, receiver operating characteristic (ROC) curves were performed with SteatoTest ($\geqslant$ S2) or HRI ($\geqslant$ 1.5) as the reference methods, and the area under the ROC (AUROC) curve was recorded. κ was calculated for evaluation of agreement between diagnosis of fatty liver by SteatoTest ($\geqslant$ S2) or HRI ($\geqslant$ 1.5) compared to FLI ($\geqslant$ 60). κ values were interpreted by the following grades: very poor (0.00-0.20), poor (0.21-0.40), moderate (0.41-0.60), good (0.61-0.80), and excellent (0.81-1.00) agreement[21]. $P < 0.05$ was considered statistically significant.

RESULTS

Characteristics of the study population and comparison between subjects with FLI $\geqslant$ 60 and < 60

Three hundred and forty-nine volunteers met the inclusion criteria. Three hundred and forty serum samples were available for the SteatoTest (9 were either missing or hemolysed). Two subjects had a high risk of a false-positive FibroTest and thus were omitted from analysis, leaving a sample size of 338 subjects. Detailed information on the study population has been described elsewhere[5,17]. The main relevant characteristics of the study sample and comparison between the subjects with FLI $\geqslant$ 60 and and < 60 are depicted in Table 1. Subjects with FLI $\geqslant$ 60 were older, had a higher percentage of men, higher BMI, and higher serum fasting levels of liver enzymes, glucose, triglycerides, insulin and ferritin.

Distribution of steatosis as predicted by different methods in the entire sample and by FLI

Applying AUS as the reference method, most subjects with FLI $\geqslant$ 60 had fatty liver on AUS, yielding a positive predictive value (PPV) of 72.2%. Most subjects with FLI < 60 had normal liver on AUS, yielding a negative predictive value (NPV) of 81.5%. Applying HRI as the reference method, only 57.0% (PPV) of subjects with FLI $\geqslant$ 60 had HRI $\geqslant$ 1.50, but 86.1% (NPV) of the subjects with FLI < 60 also had HRI < 1.5. Applying the SteatoTest as the reference method, most subjects with FLI $\geqslant$ 60 had a SteatoTest of $\geqslant$ S2, yielding a PPV of 74.7%, and the majority of those with FLI < 60 had a SteatoTest < S2, yielding an NPV of 96.1%. Most subjects (84.2%) with FLI < 60 had S0 and none had S3-S4 (Table 1).

Correlation between FLI and SteatoTest or HRI

FLI was very strongly correlated with SteatoTest ($r = 0.91$, $P < 0.001$) and to a lesser but significant degree with HRI ($r = 0.55$, $P < 0.001$) (Figure 2). HRI and SteatoTest were also significantly correlated ($r = 0.52$, $P < 0.001$).

Furthermore, when testing the distribution of SteatoTest by FLI categories, FLI value above or below 60 discriminated the SteatoTest values with no overlap between the box plots (interquartile range). Similarly, the HRI values were discriminated by FLI categories, but to

Table 1 Characteristics of the study sample, distribution of steatosis as predicted by different methods, and comparison between subjects with fatty liver index ⩾ 60 and < 60

Characteristics/method	Range	Total (n = 338)	FLI < 60 (n = 259)	FLI ⩾ 60 (n = 79)	P value
Age (yr)		50.8 ± 10.4	50.0 ± 10.4	53.3 ± 10.0	0.01
Male %		53.0	48.3	68.4	0.002
BMI (kg/m^2)		27.2 ± 4.4	25.6 ± 3.2	32.4 ± 3.9	< 0.001
ALT (U/L)	5-39	22.1 ± 9.6	20.6 ± 7.4	26.9 ± 13.5	< 0.001
AST (U/L)	5-40	23.0 ± 5.5	22.5 ± 4.8	24.7 ± 7.0	0.008
GGT (U/L)	6-28	16.0 ± 12.1	13.9 ± 11.1	22.7 ± 12.7	< 0.001
Glucose (mg/dL)	70-110	90.8 ± 19.8	87.6 ± 16.2	101.0 ± 26.1	< 0.001
Insulin (μU/mL)	5-25	22.3 ± 11.7	19.5 ± 10.0	31.5 ± 12.3	< 0.001
Triglycerides (mg/dL)	50-175	116.8 ± 60.4	100.2 ± 44.4	170.9 ± 73.3	< 0.001
Ferritin (ng/mL)	7.1-151	72.7 ± 60.1	64.6 ± 55.3	100.1 ± 67.5	< 0.001
FL on AUS % (n = 338)		31.1	18.5	72.2	< 0.001
HRI (n = 331)		1.3 ± 0.4	1.2 ± 0.3	1.6 ± 0.4	< 0.001
HRI ⩾ 1.50 (FL) %		24.2	13.9	57.0	< 0.001
SteatoTest (n = 338)		0.3 ± 0.2	0.2 ± 0.1	0.6 ± 0.1	< 0.001
S ⩾ 2 (FL) %		20.4	3.9	74.7	< 0.001
S0 %		66.9	84.2	10.1	< 0.001
S1 %	1-5	12.7	12.0	15.2	0.45
S2 %	6-33	13.0	3.9	43.0	< 0.001
S3-S4 %	34-100	7.4	0	31.6	< 0.001
FLI (n = 338)		36.7 ± 27.7	24.2 ± 17.1	77.8 ± 11.1	< 0.001
FLI ⩾ 60 (FL) %		23.4	NA	NA	NA
FLI < 30 %		48.8	NA	NA	NA

Data are mean ± SD or proportion. BMI: Body mass index; ALT: Alanine amino transferase; AST: Abstract syntax tree; GGT: Gamma-glutamyl transpeptidase; FL: Fatty liver; FLI: Fatty liver index; AUS: Abdominal ultrasound; HRI: Hepatorenal ultrasound index; NA: Not avaliable.

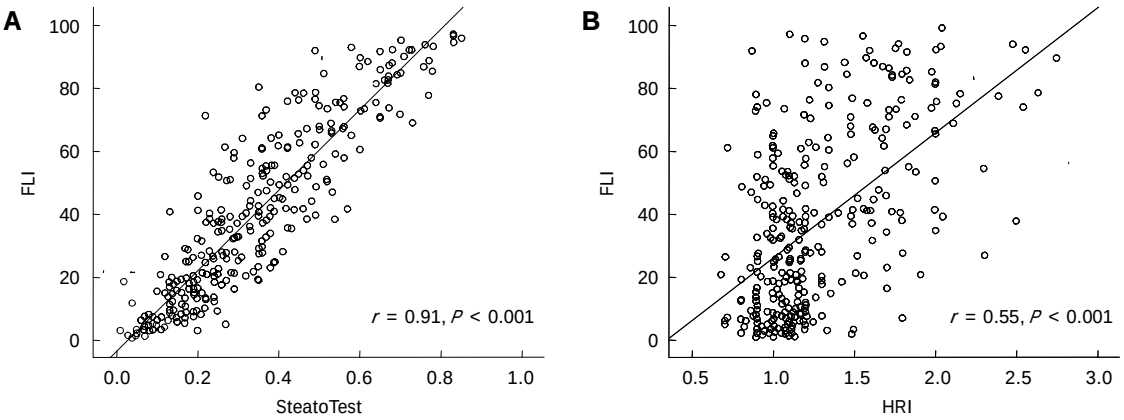


Figure 2 Correlation of fatty liver index with the reference methods. A: SteatoTest; B: Hepatorenal ultrasound index (HRI). FLI: Fatty liver index.

a lesser extent (Figure 3). The mean levels of SteatoTest and HRI were significantly different between FLI categories of above or below 60 (Table 1).

Concordance between FLI and SteatoTest or HRI in diagnosis of steatosis

The κ between diagnosis of fatty liver by SteatoTest (⩾ S2) and by FLI (⩾ 60) was 0.74, which represented good agreement (Table 2). The sensitivity of FLI *vs* SteatoTest was 85.5% (59/69) and the specificity 92.6% (249/269). The κ between diagnosis of fatty liver by HRI (⩾ 1.5) and by FLI (⩾ 60) was 0.43, which represented only moderate agreement (Table 2). The sensitivity of FLI *vs* HRI was 56.3% (45/80) and the specificity 86.5% (217/251).

Concordance between FLI and regular AUS in diagnosis of steatosis

The κ between diagnosis of fatty liver by AUS and by FLI (⩾ 60) was 0.48 (data not shown). A validation study of FLI has suggested that FLI < 30 rules out and FLI ⩾ 60 rules in fatty liver[13], therefore, further analysis was performed after exclusion of all subjects with an intermediate FLI score of 30-60 (27.8%). The κ between diagnosis of fatty liver by AUS and by FLI either ⩾ 60 or < 30 was 0.65, which represented good agreement (Table 2). The sensitivity of FLI was 80.3% (57/71) and the specificity 87.3% (151/173).

Only 8.5% of those with FLI < 30 had fatty liver on AUS, but 27.8% of those with FLI ⩾ 60 had normal liver on AUS.

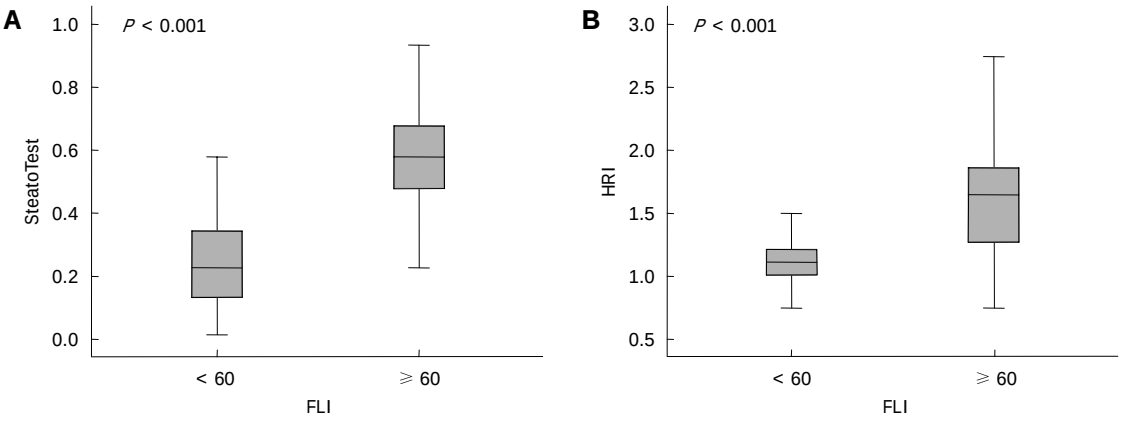


Figure 3 Distribution of SteatoTest and hepatorenal ultrasound index by fatty liver index above and below 60. A: SteatoTest; B: Hepatorenal ultrasound index (HRI). The box represents the interquartile range. The line across the box indicates the median. The "whiskers" are lines that extend from the box to the highest and lowest values, excluding outliers (defined as observations greater than 1.5 interquartile ranges). FLI: Fatty liver index.

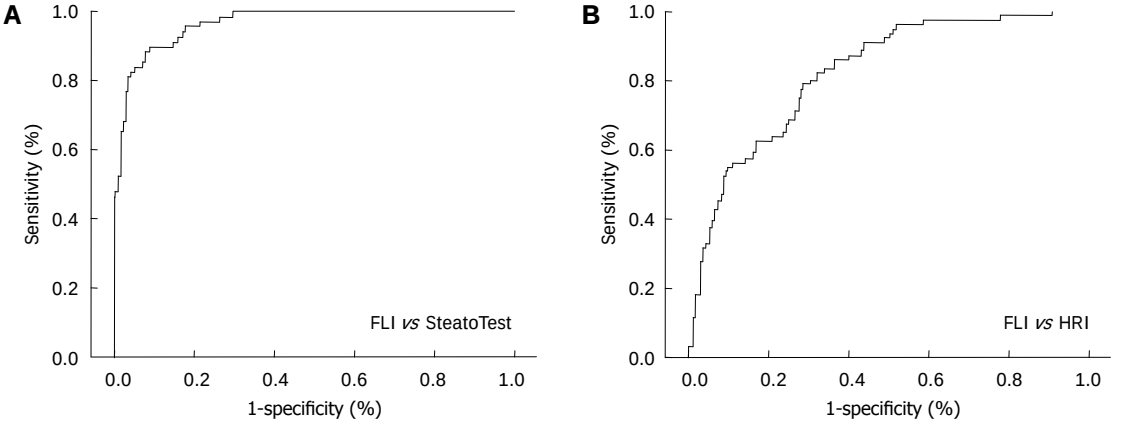


Figure 4 Receiver operating characteristic curves for diagnostic accuracy of fatty liver index *vs* SteatoTest or hepatorenal ultrasound index. A: SteatoTest; B: Hepatorenal ultrasound index (HRI). Comparing fatty liver index (FLI) to SteatoTest ⩾ S2, area under the receiver operating characteristic curve (AUROC) was 0.97 (95% CI: 0.95-0.98). Comparing FLI to HRI ⩾ 1.5, AUROC was 0.82 (95% CI: 0.77-0.87). Receiver operating characteristic curve of sensitivity (true-positive fraction) plotted against 1- specificity (false-positive fraction) of the FLI for diagnosis of steatosis.

Table 2 Diagnostic value of fatty liver index for predicting steatosis *vs* SteatoTest or hepatorenal ultrasound index or abdominal ultrasound

FLI ⩾ 60 *vs*	Sensitivity %	Specificity %	PPV %	NPV %	κ
SteatoTest ⩾ S2 (n = 338)	85.5	92.6	74.7	96.1	0.74
HRI ⩾ 1.50 (n = 331)	56.3	86.5	57	86.1	0.43
Abdominal ultrasound fatty liver (n = 244)	80.3	87.3	72.2	91.5	0.65

HRI: Hepatorenal ultrasound index; FLI: Fatty liver index; PPV: Positive predictive value; NPV: Negative predictive value.

Diagnostic accuracy of FLI for detection of steatosis > 5% in comparison to SteatoTest or HRI

The diagnostic accuracy of FLI for steatosis > 5%, as predicted by SteatoTest, yielded an AUROC of 0.97 (95% CI: 0.95-0.98). The diagnostic accuracy of FLI for steatosis > 5%, as predicted by HRI, yielded an AUROC of 0.82 (95% CI: 0.77-0.87) (Figure 4).

DISCUSSION

In view of the public health issue of the increasing prevalence of NAFLD and its hepatic and extrahepatic consequences, the development of simple cost-effective screening methods has become extremely important.

In the present study, the agreement between different potential noninvasive screening methods was evaluated. This is believed to be the first study to evaluate the agreement between FLI and steatoTest and between FLI and quantitative ultrasound methodology (HRI).

We found a striking agreement between SteatoTest and FLI, which were very highly correlated. A less impressive but still high correlation was found between FLI and HRI. The κ between diagnosis of fatty liver by SteatoTest and by FLI was 0.73, which represented good agreement. The κ between diagnosis of fatty liver by HRI and by FLI was 0.44, which represented only moderate agreement.

Although evaluation and quantification of steatosis does not provide a complete reflection of severity of

NAFLD, such evaluation is important for several reasons. As much as 23% of patients with simple steatosis may still develop nonalcoholic steatohepatitis (NASH) and fibrosis progression, as demonstrated in a recent 3-year follow-up of NAFLD patients[22]. Furthermore, recent literature indicates that NAFLD predicts the tendency to develop both diabetes mellitus[23,24] and cardiovascular disease[25-27]. Therefore, it is not surprising that patients with NAFLD have increased mortality and morbidity compared with the general population[28,29]. Moreover, NAFLD patients seem to have diminished quality of life[30], which is manifested by increased fatigue with impairment in physical function[31] and over-representation of depressive and anxiety disorders[32]. From the economic point of view, the health care costs were demonstrated to be significantly higher for individuals with NAFLD and increased serum ALT levels by 33%, controlling for BMI, lifestyle and comorbid conditions[33].

The limitation of this study was that it had no liver biopsy as a gold standard because it could not be obtained in a population-based screening study for NAFLD. Therefore, no inference can be made as for a criterion validity of the FLI. Two quantitative methods were developed and validated against liver biopsy; the HRI, a radiological method[14], and the SteatoTest[15], based on biochemical markers. Therefore, both methods were used as the best available reference for steatosis quantification, in the absence of biopsies, in the present population-based study. For that reason, the correlations presented here can only provide construct validity to the FLI. In fact, for a broader use of both SteatoTest and HRI, more validation studies including liver biopsy are warranted because only one has been performed for the HRI[14] and two for the SteatoTest in liver disease patients[15], and recently in patients with morbid obesity treated with bariatric surgery[34]. The HRI has been used in very few studies so far[35,36]; probably because it requires special ultrasonographic equipment, and a dedicated ultrasonographer. In contrast, there have been more studies using the SteatoTest[17,37-39] providing it with some construct validity.

The FLI is a continuous measure that has been validated against AUS for the qualitative detection of NAFLD and has never been validated *vs* liver biopsy. However, the presence of quantitative reference methods in the current study has enabled the testing of FLI also in a quantitative manner. The FLI has recently been used as a surrogate for NAFLD in large epidemiological studies. In a large European cross-sectional population-based study, FLI was associated with insulin resistance, higher Framingham risk score, and increased intima-media thickness[40]. More importantly, the predictive validity of FLI was demonstrated in two large cohorts. In the French general population cohort, FLI was an independent predictor for diabetes in a 9-year follow-up[41], as would be expected from ultrasound-diagnosed NAFLD[42]. In an Italian population cohort, after 15 years follow-up, FLI was independently associated with liver-related mortality[43].

The commonest noninvasive method for the evaluation of fatty liver is AUS[44,45]. AUS is the modern diagnostic test of choice for NAFLD in epidemiological surveys because it is noninvasive, safe, widely available, and with a reasonable sensitivity and specificity[4,46,47]. In a recent meta-analysis, the overall sensitivity and specificity of ultrasound for the detection of moderate-severe fatty liver compared to histology were 84.8% and 93.6%, respectively[48]. We demonstrated only moderate agreement between diagnosis of fatty liver by AUS and by FLI ($\geqslant$ 60) (κ = 0.48) , but after exclusion of all subjects with a intermediate FLI score of 30-60, κ increased to 0.65, representing good agreement, and the sensitivity and specificity of FLI were 80.3% and 87.3%, respectively. This however was at the cost of leaving almost 30% of the study population undiagnosed.

In summary, the present study provides construct validity to simple, inexpensive surrogate markers of NAFLD. FLI highly correlates and has good agreement with SteatoTest, perhaps because both are calculated measures based on overlapping parameters. FLI has moderate agreement with ultrasonographic methods; either regular AUS or HRI. These noninvasive diagnostic methods for liver steatosis should be further validated in different populations, preferably by criterion (*vs* liver histology) and predictive validity.

NAFLD has become one of the most important public health issues today. Although NASH is more relevant for the development of life-threatening liver disease, such as cirrhosis and hepatocellular carcinoma[49-51], it has now become clear from population studies that steatosis is relevant for the development of extrahepatic life-threatening diseases[52], such as diabetes[7,23,24] and cardiovascular disease[26,27]. Therefore, there is an urgent need for well-validated, quantitative, cost-effective, noninvasive methods for evaluation of steatosis in clinical practice, and epidemiological and clinical research when liver biopsy is not feasible.

COMMENTS

Background

Nonalcoholic fatty liver disease (NAFLD) is regarded as the most prevalent chronic liver disease affecting as much as 30% of the adult western population. Besides hepatic damage, in recent years, NAFLD has emerged as an independent risk factor for type 2 diabetes and cardiovascular disease.

Research frontiers

Liver biopsy is the gold standard for detection and quantification of liver steatosis in NAFLD. However, it is not routinely performed because it is an invasive procedure with a significant degree of sampling error. In addition, due to the high prevalence of NAFLD, using routine liver biopsy to diagnose or screen for NAFLD is unreasonable and also unethical in epidemiological population-based studies. Therefore, noninvasive methods including imaging techniques and blood-test-based formulas have been developed to qualify and quantify liver steatosis. However, a widely accepted examination that is easy to perform, accurate and inexpensive has yet to be found.

Innovations and breakthroughs

In view of the increasing prevalence of NAFLD and its hepatic and extrahepatic consequences, the development of simple cost-effective screening methods has become extremely important. In the present study, the agreement between different potential noninvasive screening methods was evaluated. This is be-

lieved to be the first study to evaluate the agreement between fatty liver index (FLI) and two quantitative methods, the steatoTest and the hepatorenal index (HRI), which have been validated *vs* liver biopsy. This study also adds further validation to FLI as compared to regular abdominal ultrasound (AUS), which has been demonstrated so far only in one study.

Applications

Efforts to prevent NAFLD progression and extrahepatic manifestations must include screening and surveillance strategies. The present study provides validity to FLI, a simple, inexpensive surrogate marker of NAFLD. Validated, quantitative, cost-effective methods for evaluation of steatosis can help in repeated evaluation of treatment efficacy during follow-up in clinical practice and clinical trials. Furthermore, in large epidemiological population-based studies, when liver biopsy is not feasible, noninvasive methods may serve as an alternative. Knowing the agreement and disagreement between the different noninvasive methods would help in the interpretation of results from studies using different methods. Further validation of FLI in comparison with liver biopsy is still warranted.

Terminology

Steatosis is fatty infiltration of the liver, mainly triglycerides. Fatty liver is defined as steatosis exceeding 5%-10% of its weight. NAFLD may predispose the liver to inflammation, fibrosis and eventually cirrhosis and hepatocellular carcinoma. The HRI is a quantitative ultrasound methodology. The SteatoTest and the FLI are biochemical surrogate markers of liver steatosis based on calculated algorithms. All these are noninvasive methods presently used for steatosis detection and quantification.

Peer review

The subject of the article is of interest and importance. This was a good descriptive study in which the authors compared noninvasive methods presently used for steatosis detection and quantification in NAFLD. The results are interesting and suggest that FLI has striking agreement with SteatoTest and moderate agreements with AUS or HRI.

REFERENCES

1 **Caldwell S**, Argo C. The natural history of non-alcoholic fatty liver disease. *Dig Dis* 2010; **28**: 162-168 [PMID: 20460906]
2 **Bhala N**, Angulo P, van der Poorten D, Lee E, Hui JM, Saracco G, Adams LA, Charatcharoenwitthaya P, Topping JH, Bugianesi E, Day CP, George J. The natural history of nonalcoholic fatty liver disease with advanced fibrosis or cirrhosis: an international collaborative study. *Hepatology* 2011; **54**: 1208-1216 [PMID: 21688282 DOI: 10.1002/hep.24491]
3 **Bellentani S**, Saccoccio G, Masutti F, Crocè LS, Brandi G, Sasso F, Cristanini G, Tiribelli C. Prevalence of and risk factors for hepatic steatosis in Northern Italy. *Ann Intern Med* 2000; **132**: 112-117 [PMID: 10644271]
4 **Bedogni G**, Bellentani S. Fatty liver: how frequent is it and why? *Ann Hepatol* 2004; **3**: 63-65 [PMID: 15257248]
5 **Zelber-Sagi S**, Nitzan-Kaluski D, Halpern Z, Oren R. Prevalence of primary non-alcoholic fatty liver disease in a population-based study and its association with biochemical and anthropometric measures. *Liver Int* 2006; **26**: 856-863 [PMID: 16911469]
6 **Sung KC**, Ryan MC, Wilson AM. The severity of nonalcoholic fatty liver disease is associated with increased cardiovascular risk in a large cohort of non-obese Asian subjects. *Atherosclerosis* 2009; **203**: 581-586 [PMID: 18774133]
7 **Stefan N**, Kantartzis K, Häring HU. Causes and metabolic consequences of Fatty liver. *Endocr Rev* 2008; **29**: 939-960 [PMID: 18723451]
8 **Adams LA**, Angulo P, Lindor KD. Nonalcoholic fatty liver disease. *CMAJ* 2005; **172**: 899-905 [PMID: 15795412]
9 **Ratziu V**, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, Grimaldi A, Capron F, Poynard T. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. *Gastroenterology* 2005; **128**: 1898-1906 [PMID: 15940625]
10 **Saadeh S**, Younossi ZM, Remer EM, Gramlich T, Ong JP, Hurley M, Mullen KD, Cooper JN, Sheridan MJ. The utility of radiological imaging in nonalcoholic fatty liver disease. *Gastroenterology* 2002; **123**: 745-750 [PMID: 12198701]
11 **Joy D**, Thava VR, Scott BB. Diagnosis of fatty liver disease: is biopsy necessary? *Eur J Gastroenterol Hepatol* 2003; **15**: 539-543 [PMID: 12702913 DOI: 10.1097/01.meg.0000059112.41030.2e]
12 **Siegelman ES**, Rosen MA. Imaging of hepatic steatosis. *Semin Liver Dis* 2001; **21**: 71-80 [PMID: 11296698]
13 **Bedogni G**, Bellentani S, Miglioli L, Masutti F, Passalacqua M, Castiglione A, Tiribelli C. The Fatty Liver Index: a simple and accurate predictor of hepatic steatosis in the general population. *BMC Gastroenterol* 2006; **6**: 33 [PMID: 17081293]
14 **Webb M**, Yeshua H, Zelber-Sagi S, Santo E, Brazowski E, Halpern Z, Oren R. Diagnostic value of a computerized hepatorenal index for sonographic quantification of liver steatosis. *AJR Am J Roentgenol* 2009; **192**: 909-914 [PMID: 19304694]
15 **Poynard T**, Ratziu V, Naveau S, Thabut D, Charlotte F, Messous D, Capron D, Abella A, Massard J, Ngo Y, Munteanu M, Mercadier A, Manns M, Albrecht J. The diagnostic value of biomarkers (SteatoTest) for the prediction of liver steatosis. *Comp Hepatol* 2005; **4**: 10 [PMID: 16375767]
16 **Nitzan Kaluski D,** Goldsmith R, Chinitz A, Ben Arie-Magled O, Mayer C, Green M. First National Health and Nutrition Survey, Part I 1999-2001. Jerusalem, Israel: Israel Center for Disease Control, 2003
17 **Zelber-Sagi S**, Nitzan-Kaluski D, Goldsmith R, Webb M, Zvibel I, Goldiner I, Blendis L, Halpern Z, Oren R. Role of leisure-time physical activity in nonalcoholic fatty liver disease: a population-based study. *Hepatology* 2008; **48**: 1791-1798 [PMID: 18972405 DOI: 10.1002/hep.22525]
18 **Imbert-Bismut F**, Messous D, Thibault V, Myers RB, Piton A, Thabut D, Devers L, Hainque B, Mercadier A, Poynard T. Intra-laboratory analytical variability of biochemical markers of fibrosis (Fibrotest) and activity (Actitest) and reference ranges in healthy blood donors. *Clin Chem Lab Med* 2004; **42**: 323-333 [PMID: 15080567 DOI: 10.1515/CCLM.2004.058]
19 **Gore RM.** Diffuse liver disease. In: Gore R, Levine MS, Laufer I, editors. Textbook of Gastrointestinal Radiology. Philadelphia: Saunders, 1994: 1968-2017
20 **Zelber-Sagi S**, Nitzan-Kaluski D, Halpern Z, Oren R. NAFLD and hyperinsulinemia are major determinants of serum ferritin levels. *J Hepatol* 2007; **46**: 700-707 [PMID: 17150278]
21 **Landis JR**, Koch GG. The measurement of observer agreement for categorical data. *Biometrics* 1977; **33**: 159-174 [PMID: 843571]
22 **Wong VW**, Wong GL, Choi PC, Chan AW, Li MK, Chan HY, Chim AM, Yu J, Sung JJ, Chan HL. Disease progression of non-alcoholic fatty liver disease: a prospective study with paired liver biopsies at 3 years. *Gut* 2010; **59**: 969-974 [PMID: 20581244]
23 **Sung KC**, Kim SH. Interrelationship between fatty liver and insulin resistance in the development of type 2 diabetes. *J Clin Endocrinol Metab* 2011; **96**: 1093-1097 [PMID: 21252243]
24 **Fan JG**, Li F, Cai XB, Peng YD, Ao QH, Gao Y. Effects of nonalcoholic fatty liver disease on the development of metabolic disorders. *J Gastroenterol Hepatol* 2007; **22**: 1086-1091 [PMID: 17608855]
25 **Musso G**, Gambino R, Cassader M, Pagano G. Meta-analysis: natural history of non-alcoholic fatty liver disease (NAFLD) and diagnostic accuracy of non-invasive tests for liver disease severity. *Ann Med* 2011; **43**: 617-649 [PMID: 21039302 DOI: 10.3109/07853890.2010.518623]
26 **Targher G**, Bertolini L, Poli F, Rodella S, Scala L, Tessari R, Zenari L, Falezza G. Nonalcoholic fatty liver disease and risk of future cardiovascular events among type 2 diabetic patients. *Diabetes* 2005; **54**: 3541-3546 [PMID: 16306373]
27 **Villanova N**, Moscatiello S, Ramilli S, Bugianesi E, Magalotti D, Vanni E, Zoli M, Marchesini G. Endothelial dysfunction and cardiovascular risk profile in nonalcoholic fatty liver disease. *Hepatology* 2005; **42**: 473-480 [PMID: 15981216 DOI: 10.1002/hep.20781]

28 **Adams LA**, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, Angulo P. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology* 2005; **129**: 113-121 [PMID: 16012941]

29 **Feldstein AE**, Charatcharoenwitthaya P, Treeprasertsuk S, Benson JT, Enders FB, Angulo P. The natural history of nonalcoholic fatty liver disease in children: a follow-up study for up to 20 years. *Gut* 2009; **58**: 1538-1544 [PMID: 19625277]

30 **David K**, Kowdley KV, Unalp A, Kanwal F, Brunt EM, Schwimmer JB. Quality of life in adults with nonalcoholic fatty liver disease: baseline data from the nonalcoholic steatohepatitis clinical research network. *Hepatology* 2009; **49**: 1904-1912 [PMID: 19434741 DOI: 10.1002/hep.22868]

31 **Newton JL**, Jones DE, Henderson E, Kane L, Wilton K, Burt AD, Day CP. Fatigue in non-alcoholic fatty liver disease (NAFLD) is significant and associates with inactivity and excessive daytime sleepiness but not with liver disease severity or insulin resistance. *Gut* 2008; **57**: 807-813 [PMID: 18270241]

32 **Elwing JE**, Lustman PJ, Wang HL, Clouse RE. Depression, anxiety, and nonalcoholic steatohepatitis. *Psychosom Med* 1991; **68**: 563-569 [PMID: 16868265]

33 **Baumeister SE**, Völzke H, Marschall P, John U, Schmidt CO, Flessa S, Alte D. Impact of fatty liver disease on health care utilization and costs in a general population: a 5-year observation. *Gastroenterology* 2008; **134**: 85-94 [PMID: 18005961]

34 **Lassailly G**, Caiazzo R, Hollebecque A, Buob D, Leteurtre E, Arnalsteen L, Louvet A, Pigeyre M, Raverdy V, Verkindt H, Six MF, Eberle C, Patrice A, Dharancy S, Romon M, Pattou F, Mathurin P. Validation of noninvasive biomarkers (FibroTest, SteatoTest, and NashTest) for prediction of liver injury in patients with morbid obesity. *Eur J Gastroenterol Hepatol* 2011; **23**: 499-506 [PMID: 21499110 DOI: 10.1097/MEG.0b013e3283464111]

35 **Neuman G**, Sagi R, Shalitin S, Reif S. Serum inflammatory markers in overweight children and adolescents with nonalcoholic fatty liver disease. *Isr Med Assoc J* 2010; **12**: 410-415 [PMID: 20862821]

36 **Soder RB**, Baldisserotto M, Duval da Silva V. Computer-assisted ultrasound analysis of liver echogenicity in obese and normal-weight children. *AJR Am J Roentgenol* 2009; **192**: W201-W205 [PMID: 19380523]

37 **Ratziu V**, Giral P, Munteanu M, Messous D, Mercadier A, Bernard M, Morra R, Imbert-Bismut F, Bruckert E, Poynard T. Screening for liver disease using non-invasive biomarkers (FibroTest, SteatoTest and NashTest) in patients with hyperlipidaemia. *Aliment Pharmacol Ther* 2007; **25**: 207-218 [PMID: 17229244]

38 **Rubio A**, Monpoux F, Huguon E, Truchi R, Triolo V, Rosenthal-Allieri MA, Deville A, Rosenthal E, Boutté P, Tran A. Noninvasive procedures to evaluate liver involvement in HIV-1 vertically infected children. *J Pediatr Gastroenterol Nutr* 2009; **49**: 599-606 [PMID: 19668009 DOI: 10.1097/MPG.0b013e3181a15b72]

39 **Friedrich-Rust M**, Müller C, Winckler A, Kriener S, Herrmann E, Holtmeier J, Poynard T, Vogl TJ, Zeuzem S, Hammerstingl R, Sarrazin C. Assessment of liver fibrosis and steatosis in PBC with FibroScan, MRI, MR-spectroscopy, and serum markers. *J Clin Gastroenterol* 2010; **44**: 58-65 [PMID: 19581812 DOI: 10.1097/MCG.0b013e3181a84b8d]

40 **Gastaldelli A**, Kozakova M, Højlund K, Flyvbjerg A, Favuzzi A, Mitrakou A, Balkau B. Fatty liver is associated with insulin resistance, risk of coronary heart disease, and early atherosclerosis in a large European population. *Hepatology* 2009; **49**: 1537-1544 [PMID: 19291789 DOI: 10.1002/hep.22845]

41 **Balkau B**, Lange C, Vol S, Fumeron F, Bonnet F. Nine-year incident diabetes is predicted by fatty liver indices: the French D.E.S.I.R. study. *BMC Gastroenterol* 2010; **10**: 56 [PMID: 20529259]

42 **Kim CH**, Park JY, Lee KU, Kim JH, Kim HK. Fatty liver is an independent risk factor for the development of Type 2 diabetes in Korean adults. *Diabet Med* 2008; **25**: 476-481 [PMID: 18346164]

43 **Calori G**, Lattuada G, Ragogna F, Garancini MP, Crosignani P, Villa M, Bosi E, Ruotolo G, Piemonti L, Perseghin G. Fatty liver index and mortality: the Cremona study in the 15th year of follow-up. *Hepatology* 2011; **54**: 145-152 [PMID: 21488080 DOI: 10.1002/hep.24356]

44 **Browning JD**, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, Grundy SM, Hobbs HH. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004; **40**: 1387-1395 [PMID: 15565570 DOI: 10.1002/hep.20466]

45 **Bedogni G**, Miglioli L, Masutti F, Tiribelli C, Marchesini G, Bellentani S. Prevalence of and risk factors for nonalcoholic fatty liver disease: the Dionysos nutrition and liver study. *Hepatology* 2005; **42**: 44-52 [PMID: 15895401 DOI: 10.1002/hep.20734]

46 **Fan JG**, Zhu J, Li XJ, Chen L, Li L, Dai F, Li F, Chen SY. Prevalence of and risk factors for fatty liver in a general population of Shanghai, China. *J Hepatol* 2005; **43**: 508-514 [PMID: 16006003]

47 **Sanyal AJ**. AGA technical review on nonalcoholic fatty liver disease. *Gastroenterology* 2002; **123**: 1705-1725 [PMID: 12404245]

48 **Hernaez R**, Lazo M, Bonekamp S, Kamel I, Brancati FL, Guallar E, Clark JM. Diagnostic accuracy and reliability of ultrasonography for the detection of fatty liver: a meta-analysis. *Hepatology* 2011; **54**: 1082-1090 [PMID: 21618575 DOI: 10.1002/hep.24452]

49 **Ekstedt M**, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, Kechagias S. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006; **44**: 865-873 [PMID: 17006923 DOI: 10.1002/hep.21327]

50 **Rafiq N**, Bai C, Fang Y, Srishord M, McCullough A, Gramlich T, Younossi ZM. Long-term follow-up of patients with nonalcoholic fatty liver. *Clin Gastroenterol Hepatol* 2009; **7**: 234-238 [PMID: 19049831]

51 **Sanyal AJ**, Brunt EM, Kleiner DE, Kowdley KV, Chalasani N, Lavine JE, Ratziu V, McCullough A. Endpoints and clinical trial design for nonalcoholic steatohepatitis. *Hepatology* 2011; **54**: 344-353 [PMID: 21520200 DOI: 10.1002/hep.24376]

52 **Ratziu V**, Bellentani S, Cortez-Pinto H, Day C, Marchesini G. A position statement on NAFLD/NASH based on the EASL 2009 special conference. *J Hepatol* 2010; **53**: 372-384 [PMID: 20494470]

P- Reviewer Takuma Y **S- Editor** Gou SX
L- Editor Kerr C **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i1.65

World J Gastroenterol 2013 January 7; 19(1): 65-71
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Prolonged QT dispersion in inflammatory bowel disease

Elif Yorulmaz, Aslıhan Sezgin, Hatice Yorulmaz, Gupse Adali, Hilmi Ciftci

Elif Yorulmaz, Gupse Adali, Department of Gastroenterology, Goztepe Training and Research Hospital, 34470 Istanbul, Turkey
Aslıhan Sezgin, Hilmi Ciftci, Department of III Internal Medicine, Goztepe Training and Research Hospital, 34470 Istanbul, Turkey
Hatice Yorulmaz, School of Nursing, Halic University, 34394 Istanbul, Turkey
Author contributions: Yorulmaz E and Adali G collected material and wrote the manuscript; Sezgin A and Ciftci H provided evaluating data; Yorulmaz H was involved in editing the manuscript and statistical analysis.
Correspondence to: Hatice Yorulmaz, Assistant Professor, School of Nursing, Halic University, Buyukdere Street. No.101, Mecidiyeköy, 34394 Istanbul,
Turkey. haticeyorulmaz@hotmail.com
Telephone: +90-212-2752020 Fax: +90-212-5264917
Received: July 7, 2011 Revised: April 13, 2012
Accepted: August 15, 2012
Published online: January 7, 2013

Abstract

AIM: To investigate the frequency and factors of prolonged QT dispersion that may lead to severe ventricular arrhythmias in patients with inflammatory bowel disease (IBD).

METHODS: This study included 63 ulcerative colitis (UC) and 41 Crohn's disease (CD) patients. Forty-seven healthy patients were included as the control group. Heart rate was calculated using electrocardiography, corrected QT dispersion (QTcd) and the Bazett's formula. Homeostasis model assessment (HOMA) was used to determine insulin resistance (IR). HOMA values < 1 were considered normal and values > 2.5 indicated a high probability of IR.

RESULTS: Prolonged QTcd was found in 12.2% of UC patients, and in 14.5% of CD patients compared with the control group ($P < 0.05$). A significant difference was found between the insulin values (CD: 10.95 ± 6.10 *vs* 6.44 ± 3.28, $P < 0.05$; UC: 10.88 ± 7.19 *vs* 7.20 ± 4.54, $P < 0.05$) and HOMA (CD: 2.56 ± 1.43 *vs* 1.42 ± 0.75, $P < 0.05$; UC: 2.94 ± 1.88 *vs* 1.90 ± 1.09, $P < 0.05$) in UC and CD patients with and without prolonged QTcd. Disease behavior types were determined in CD patients with prolonged QTcd. Increased systolic arterial pressure (125 ± 13.81 *vs* 114.09 ± 8.73, $P < 0.01$) and age (48.67 ± 13.93 *vs* 39.57 ± 11.58, $P < 0.05$) in UC patients were significantly associated with prolonged QTcd.

CONCLUSION: Our data show that IBD patients have prolonged QTcd in relation to controls. The routine follow-up of IBD patients should include determination of HOMA, insulin values and electrocardiogram examination.

© 2013 Baishideng. All rights reserved.

Key words: Crohn's disease; Homeostasis model assessment; Insulin; QT dispersion; Ulcerative colitis

Yorulmaz E, Sezgin A, Yorulmaz H, Adali G, Ciftci H. Prolonged QT dispersion in inflammatory bowel disease. *World J Gastroenterol* 2013; 19(1): 65-71 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i1/65.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i1.65

INTRODUCTION

The most common extra-intestinal manifestations of Crohn's disease (CD) and ulcerative colitis (UC) are iridis, uveitis, primary sclerosing cholangitis, nodal erythema and pyoderma gangrenosum. Complications within the cardiovascular system seem to be uncommon, but there are no systematic investigations concerning the epidemiology of these manifestations. There have been more than 100 cases of pericarditis and perimyocarditis reported in patients with inflammatory bowel disease (IBD). Other patients with CD or UC suffer from vasculitis, representing a further mechanism of inflammatory diseases of the cardiovascular system. Some patients developed thrombotic complications due to activation of the coagulation system, which can result in atrial thrombi, embolism of the pulmonary arteries, myocardial infarction and disseminated intravascular coagulopa-

thy. Furthermore, a few cases of atrioventricular block, amyloidosis of the heart, dilated cardiomyopathy and endomyocardial fibrosis have been reported in patients with chronic IBD[1].

The most important causes of sudden death of cardiac origin are ventricular tachycardia and ventricular fibrillation cardiac arrhythmias. The action potentials in patients with long QT electrocardiographic (ECG) changes are not homogeneous, and as they result in the development of early and late repolarization facilitate the development of ventricular fibrillation[2]. The heterogeneity of QT dispersion (QTd) in ventricular repolarization is defined by the difference between the longest and shortest QT[3]. Increased QTd in many patients, and disease group were associated with the risk of serious arrhythmia and sudden death[2,4,5]. In patients with acute myocardial infarction, measurement of QT dispersion has shown little promise as a predictor of long-term mortality risk in individuals[6]. Cardiac involvement in IBD can cause serious ventricular arrhythmias with prolonged QTd, however, very little data is available on this subject[7]. In this study, QTd was prolonged in patients with IBD, and the relationships between disease characteristics and biochemical parameters were investigated.

MATERIALS AND METHODS

Ethics aspects

This study was formally authorized by the local Ethics Committee (approval date and number: 02-06-2009, No: 57). All of the patients gave written informed consent to the study protocol.

Study design

This was an observational, case-control study. Clinical, endoscopic, histologic, and radiologic findings showed that 63 patients had UC and 41 had CD. Forty-seven healthy control subjects were also included.

Selection of controls and patients

The control group had no chronic diseases, electrolyte imbalance and did not take regular medication. In addition, the controls were selected from persons with normal QT dispersion. IBD patients with known chronic disease, electrolyte imbalance, thyroid dysfunction or treated with drugs known to affect ECG findings were excluded from the study. Smoking status, alcohol habit, family history, use of medications, and physical examination were performed in all patients. After measurement of liver enzymes, blood count and blood levels of potassium, calcium and magnesium, those with abnormal findings were excluded from the study. To evaluate the clinical activity of CD, the Crohn's disease activity index (CDAI) was used. CDAI scores less than 150 indicated quiescence, while higher scores indicated active disease[8]. In UC patients, the Truelove-Witts clinical activity index was used to evaluate disease activity[9].

Anthropometric measurements

Anthropometric measurements (height, weight, waist circumference) were carried out by same person using standard instruments in patients wearing hospital clothing, while standing. Body mass index (BMI) was calculated by dividing the patient's weight by the square of his or her height (mass/height^2, kg/m^2). Waist circumference was measured while standing at the narrowest area of the waist midway between the anterior superior iliac spine and the lower rib margin on light expiration. Average systolic and diastolic blood pressure measurements were recorded. Systolic blood pressure (SBP) of 140 mmHg and over and diastolic blood pressure of 90 mmHg and over were accepted as hypertension[10]. Patients with hypertension were excluded.

QT interval calculation

All patients and controls underwent 12-lead surface ECG to determine QT interval, and the QRS complex was defined as the distance from the beginning to the end of the T wave. Heart rate, physiological causes of shortening of the QT interval, and reduction in speed will cause QT prolongation. Various formulations of the QT interval were corrected for heart rate. Corrected QT (QTc) was calculated according to the Bazett's formula (Bazett's formula QTc = QT × $\sqrt{RR}$). QTd and minimum mean QT as the difference between the average QT were also calculated. Corrected QT dispersion (QTcd) values were calculated in the same way. Extended QTc interval was defined as a duration of > 440 ms[11].

Biochemical analysis

After 12 h of night fasting, venous blood samples were taken, centrifuged (2500 cycle/min) and the serum separated to measure biochemical indices. Glucose, total cholesterol, low density lipoprotein cholesterol, high density lipoprotein cholesterol, triglyceride and uric acid levels were determined by enzymatic methods, and insulin level was determined by electrochemiluminescence immunoassay using a Roche E170 device. Insulin sensitivity was assessed by homeostasis model assessment of insulin resistance (HOMA-IR). Accordingly, IR was calculated using the following formula: Fasting insulin level (μIU/mL) × fasting plasma glucose (mmol/L)/22.5[12].

Statistical analysis

All data are presented as mean ± SE. Data were analyzed using descriptive statistical methods (mean, standard deviation, frequency). Differences between CD, UC, and controls, demographic, anthropometric, and laboratory values and the relationship between QT duration were calculated using the Kruskal-Wallis test, Mann-Whitney *U* test, and Chi-square test. Pearson correlation analysis was used to determine the relationship between variables. All analysis were performed using SPSS version 15.0 (SPSS Inc., United States). Statistical significance was accepted at $P \leq 0.05$.

Table 1 Characteristics of patients with Crohn's disease, and the relationship between QT prolongation *n* (%)

	CD QT prolongation		Test values	
	-	+	χ^2	*P* value
Diagnosis period (yr)	26.47 ± 34.27	27.2 ± 32.88	*Z* = 0.31	0.74
Family history			0.97	0.61
None	30 (83.3)	5 (100)		
CD	3 (8.3)			
Colon cancer	3 (8.3)			
Operation			2.11	0.14
Yes	5 (13.88)	2 (40)		
No	31 (86.11)	3 (60)		
Age at diagnosis (yr)			3.01	0.22
< 17	2 (5.6)			
17-40	33 (91.70)	4 (80)		
> 40	1 (2.80)	1 (20)		
Disease behavior			23.35	0.000[b]
Non-stricturing	22 (61.1)	1 (20)		
Non penetrating				
Stricturing	14 (38.9)	1 (20)		
Penetrating	-	3 (60)		
Disease location			0.55	0.75
İleal	12 (33.3)	1 (20)		
Colonic	1 (2.80)	-		
İleocolonic	23 (63.90)	4 (80)		
CDAI value			0.013	0.91
CDAI < 150	28 (77.7)	4 (80)		
CDAI ⩾ 150	8 (22.2)	1 (20)		
Drug use			0.13	0.71
Used	26 (72.2)	4 (80)		
Not used	10 (27.8)	1 (20)		
5-ASA			0.23	0.62
Used	11 (30.6)	1 (20)		
Not used	25 (69.4)	4 (80)		
CS			0.97	0.32
Used	30 (83.3)	5 (100)		
Not used	6 (16.7)	-		
IS			0.13	0.71
Used	26 (72.2)	4 (80)		
Not used	10 (27.8)	1 (20)		
Smoking			0.66	0.72
Yes	21 (58.3)	2 (40)		
No	9 (25)	2 (40)		
Quit	6 (16.7)	1 (20)		
Alcohol			2.08	0.35
Yes	9 (25)	-		
No	25 (69.4)	5 (100)		
Quit	2 (5.6)	-		

[b]*P* < 0.01, χ^2 test and *Z*: Mann-Whitney *U* test. CD: Crohn's disease; CDAI: Crohn's disease activity index; 5-ASA: 5-aminosalicylic acid; CS: Corticosteroid; IS: immunosuppressive.

Table 2 Characteristics of patients with ulcerative colitis, and the relationship between QT prolongation *n* (%)

	UC QT prolongation		Test values	
	-	+	χ^2	*P* value
Diagnosis period (yr)	44.13 ± 42.60	45.67 ± 47.99		0.86
Family history			0.48	0.78
None	41 (77.4)	6 (66.7)		
UC	8 (15.1)	2 (22.2)		
Colon cancer	4 (7.5)	1 (11.1)		
Operation			0.17	0.67
Yes	1 (1.9)	-		
No	52 (98.1)	9 (100)		
Extent of disease			3.49	0.32
Rectosigmoid	16 (30.18)	2 (22.22)		
Left sided colon	10 (18.86)	4 (44.4)		
Extensive	6 (11.32)	-		
Pancolitis	21 (39.62)	3 (33.3)		
Clinical activity			2.19	0.33
TW1 (mild activity)	34 (64.15)	8 (88.89)		
TW2 (moderate activity)	17 (32.07)	1 (11.11)		
TW3 (severe activity)	3.77	-		
Drug use			0.09	0.75
Used	45 (84.9)	8 (88.89)		
Not used	8 (15.09)	1 (11.11)		
5-ASA			0.19	0.65
Used	9 (16.98)	1 (11.11)		
Not used	44 (83.01)	8 (88.89)		
CS			1.12	0.28
Used	47 (88.67)	9 (100)		
Not used	6 (11.32)	-		
IS			0.13	0.71
Used	49 (92.45)	8 (88.89)		
Not used	4 (7.54)	1 (11.11)		
Smoking			2.99	0.22
Yes	12 (22.64)	2 (22.2)		
No	26 (49.05)	2 (22.2)		
Quit	15 (28.30)	5 (55.56)		
Alcohol				
Yes	9 (16.98)	2 (22.2)	0.46	0.79
No	42 (79.24)	7 (77.78)		
Quit	2 (3.77)	-		

χ^2 test, Mann-Whitney *U* test. TW: Truelove-Witts clinical activity index; UC: Ulcerative colitis; 5-ASA: 5-aminosalicylic acid; CS: Corticosteroid; IS: Immunosuppressive.

RESULTS

Demographic characteristics

This study included 63 UC patients (33 female, 30 male; mean age 40.89 ± 12.25 years), 41 CD patients (16 female, 25 male; mean age 38 ± 7.79 years) and 47 healthy controls (25 female, 32 male; mean age 41.21 ± 10.55 years).

Descriptive analysis of patients

Of these patients; 12.2% UC and 14.5% CD patients had prolonged QTcd when compared with the control group ($P < 0.05$). Increased SBP ($P < 0.01$) and age ($P < 0.05$) in UC patients were significantly associated with prolonged QTcd. Disease group, disease duration, family history, smoking, alcohol use, history of surgery, disease activity, localization, and drug use were not related to prolonged QTcd ($P > 0.05$). Disease behavior type was determined to be involved in CD patients with prolonged QTcd ($P < 0.001$) (Tables 1 and 2).

Biochemical analysis and the relationship between prolonged QT intervals

Significant differences were observed in patients with UC and CD with respect to insulin values ($P < 0.05$) and HOMA ($P < 0.05$) values compared to patients without UC and CD (Tables 3 and 4). Both groups demonstrated HOMA values above 2.5 (Figure 1). Using Pearson cor-

Table 3 Crohn's disease and control group, demographic, anthropometric and laboratory values and the relationship between QT prolongation

	CD QT prolongation (-)	CD QT prolongation (+)	Control	Test values
Male, *n* (%)	23 (63.9)	2 (40)	31 (66)	$\chi^2 = 1.31$, $P = 0.51$
Female, *n* (%)	13 (36.1)	3 (60)	16 (34)	
Age (yr)	36.80 ± 7.79	41.33 ± 7.66	41.21 ± 10.55	$P = 0.53$
SBP (mmHg)	109.72 ± 13.41	105 ± 20	102.74 ± 11.93	$P = 0.071$
DBP (mmHg)	69.72 ± 9.40	70 ± 10	71.11 ± 6.55	$P = 0.62$
Heart rate (beats/min)	77.13 ± 13.4	71.4 ± 12.23	74.80 ± 13.25	$P = 0.36$
BMI (kg/m²)	23.78 ± 4.90	25.38 ± 3.99	24.77 ± 3.43	$P = 0.57$
WC (cm) male	89 ± 7.88	91 ± 6.36	90.19 ± 4.99	$P = 0.14$
WC (cm) female	87.23 ± 5.45	92.33 ± 7.08	90.68 ± 5.18	$P = 0.19$
QTcd (ms)	39.69 ± 2.32	55.4 ± 2.88	38.68 ± 2.25	CD QT prolongation (-) *vs* (+), CD QT prolongation (+) *vs* control, $^bP = 0.000$; CD QT prolongation (-) *vs* control, $^aP = 0.049$
FPG (mg/dL)	89.31 ± 6.87	93.8 ± 10.75	87.6 ± 10.13	$P = 0.078$
Total-C (mg/dL)	187.89 ± 39.46	189.8 ± 21.28	185.68 ± 37.74	$P = 0.65$
Triglyceride (mg/dL)	116.51 ± 64.85	111 ± 33.12	97.98 ± 36.74	$P = 0.50$
HDL-C (mg/dL)	55.4 ± 12.69	60 ± 19.19	58.17 ± 14.74	$P = 0.66$
LDL-C (mg/dL)	121.05 ± 32.66	118.2 ± 9.78	119.02 ± 24.48	$P = 0.77$
Insulin (μU/mL)	6.44 ± 3.28	10.95 ± 6.10	5.68 ± 3.83	CD QT prolongation (-) *vs* (+), $^aP = 0.036$; CD QT prolongation (-) *vs* control, $^aP = 0.027$; CD QT prolongation (+) *vs* control, $P = 0.16$
HOMA-IR	1.42 ± 0.75	2.56 ± 1.43	1.31 ± 0.94	CD QT prolongation (-) *vs* (+), $^aP = 0.026$; CD QT prolongation (-) *vs* control, $P = 0.12$; CD QT prolongation (+) *vs* control, $^aP = 0.023$
Uric acid (mg/dL)	4.09 ± 0.86	4.26 ± 1.52	4.01 ± 1.05	$P = 0.95$

$^aP < 0.05$, $^bP < 0.01$, χ^2 test, Mann-Whitney *U* test, Kruskall-Wallis test. CD: Crohn's disease; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; BMI: Body mass index; WC: Waist circumference; QTcd: Corrected QT dispersion; FPG: Fasting plasma glucose; C: Cholesterol; HDL: High density lipoprotein; LDL: Low density lipoprotein; HOMA-IR: Homeostasis model assessment of insulin resistance.

Table 4 Ulcerative colitis and control group, demographic, anthropometric, and laboratory values and the relationship between QT prolongation

	UC QT prolongation (-)	UC QT prolongation (+)	Control	Test values
Male, *n* (%)	25 (47.2)	5 (55.6)	31 (66)	$\chi^2 = 3.56$, $P = 0.16$
Female, *n* (%)	28 (52.8)	4 (44.4)	16 (34)	
Age (yr)	39.57 ± 11.58	48.67 ± 13.93	41.21 ± 10.55	$P = 0.14$
SBP (mmHg)	114.09 ± 8.73	125 ± 13.81	102.74 ± 9.93	UC QT prolongation (-) *vs* (+), $^aP = 0.012$; UC QT prolongation (-) *vs* control, $P = 0.51$; UC QT prolongation (+) *vs* control, $^aP = 0.037$
DBP (mmHg)	73.96 ± 6.88	74.44 ± 8.81	71.11 ± 6.55	$P = 0.66$
Heart rate (beats/min)	77.13 ± 13.4	71.4 ± 12.23	74.80 ± 13.25	$P = 0.25$
BMI (kg/m²)	25.22 ± 4.31	27.72 ± 9.11	24.77 ± 3.43	$P = 0.18$
WC (cm) male	94.92 ± 9.91	104.6 ± 22.67	93.19 ± 10.99	$P = 0.59$
WC (cm) female	92.75 ± 12.83	93.5 ± 10.96	90.68 ± 12.18	$P = 0.54$
QTcd (ms)	40.09 ± 2.34	55.1 ± 3.1	38.68 ± 2.25	UC QT prolongation (-) *vs* (+), UC QT prolongation (+) *vs* control, $^bP = 0.000$; UC QT prolongation (-) *vs* control, $^bP = 0.003$
FPG (mg/dL)	93.62 ± 9.02	95.11 ± 17.33	87.6 ± 10.13	$P = 0.53$
Total-C (mg/dL)	194.49 ± 34.31	214.33 ± 40.84	185.68 ± 37.74	$P = 0.34$
Triglyceride (mg/dL)	114.33 ± 53.89	120.11 ± 36.31	97.98 ± 36.74	$P = 0.10$
HDL-C (mg/dL)	54.62 ± 15.10	61.22 ± 14.95	58.17 ± 14.74	$P = 0.38$
LDL-C (mg/dL)	124.81 ± 24.41	137.552 ± 28.75	119.02 ± 24.48	$P = 0.49$
Insulin (μU/mL)	7.20 ± 4.54	10.88 ± 7.19	5.68 ± 3.83	UC QT prolongation (-) *vs* (+), $^aP = 0.031$; UC QT prolongation (-) *vs* control, $P = 0.43$; UC QT prolongation (+) *vs* control, $^aP = 0.047$
HOMA-IR	1.90 ± 1.09	2.94 ± 1.88	1.31 ± 0.94	UC QT prolongation (-) *vs* (+), $^aP = 0.011$; UC QT prolongation (-) *vs* control, $P = 0.51$; UC QT prolongation (+) *vs* control, $^aP = 0.027$
Uric acid (mg/dL)	4.31 ± 1.35	4.44 ± 1.29	4.01 ± 1.05	$P = 0.12$

$^aP < 0.05$, $^bP < 0.01$, χ^2 test, Mann-Whitney *U* test, Kruskall-Wallis test. UC: Ulcerative colitis; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; BMI: Body mass index; WC: Waist circumference; QTcd: Corrected QT dispersion; FPG: Fasting plasma glucose; C: Cholesterol; HDL: High density lipoprotein; LDL: Low density lipoprotein; HOMA-IR: Homeostasis model assessment of insulin resistance.

relation analysis, a moderately significant positive relationship was observed between QT time in patients with CD and UC and insulin (CD: $r = 0.542$, $P = 0.023$; UC: $r = 0.560$, $P = 0.021$) and HOMA values (CD: $r = 0.618$, $P = 0.018$; UC: $r = 0.678$, $P = 0.011$).

DISCUSSION

Homogeneous ventricular resting time is believed to be protective against arrhythmias[13]. Easy and non-invasive methods to determine the risk of ventricular arrhythmia,

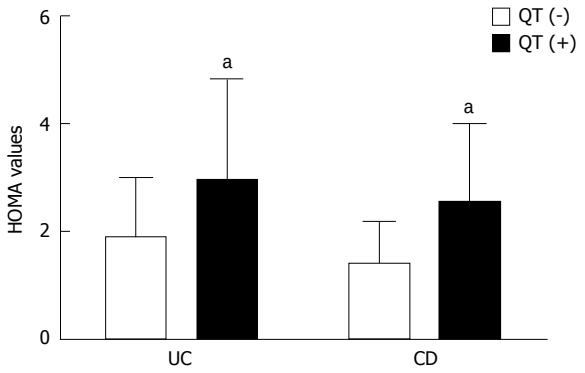


Figure 1 Homeostasis model assessment values in ulcerative colitis and Crohn's disease patient. Data are shown as mean ± SD. $^{a}P < 0.05$ *vs* QT prolongation (-) groups using Mann-Whitney *U* test. HOMA: Homeostasis model assessment; UC: Ulcerative colitis; CD: Crohn's disease.

are an advantage in patients with QTd. However, the frequency and prognostic value of IBD as an indicator of arrhythmia is not fully understood. In this study, the demographic data of patients with IBD and prolonged QT dispersion and the relationships with some cardiovascular risk parameters were investigated. Conflicting results were obtained in previous studies investigating the relationship between age and QT interval. Some studies in older healthy individuals showed a significant correlation between age and prolonged QTd[14-16]. In contrast, Merri *et al*[17] looked at descriptive ECG features of ventricular repolarization, and did not find a relationship between age and QT interval. The relationship between gender and QT interval has also been investigated. In the literature, some studies have reported long-QT dispersion in healthy women compared with men[18,19]. Mangoni and colleagues found no differences between QTd and gender[14]. In our study, UC patients showed a significant relationship between older age and prolonged QTd. Smoking may predispose to ventricular fibrillation and sudden cardiac death by altering ventricular recovery time dispersion indices. Some studies found a correlation between smoking in men and QT dispersion, this was not detected in the study by Mangoni and colleagues[14,20,21]. In our study, both UC and CD patients did not show a correlation between smoking and prolonged QTd. Drugs such as steroids and azathioprine can be cardiotoxic[22-27]. There was no correlation between QT dispersion and the use of 5-aminosalicylic acid, steroids and azathiopurine. In fact, although UC and CD are generally described as two expressions of a common pathophysiological process, they may be characterized by different autonomic alterations due to the diverse location of inflammatory lesions along the gastrointestinal tract[28]. Inflammation tends to be diffuse and uniform in UC, mainly affecting the superficial layers of the inner lining of the bowel, where autonomic neural fibers of the submucosal plexus are located[29], whereas in CD inflammation is concentrated in some areas more than in others, involving deeper layers of the bowel wall. The different location and structure of the lesions may therefore have different effects on autonomic gastrointestinal control, possibly reflected by differences in cardiovascular autonomic control[30]. In our study, prolonged QTd was significantly more frequent in patients with stricturing and penetrating CD. The relationship between physiologic BMI and QT interval confirms and extends the findings of previous reports which showed a positive relationship between these variables in human obesity[31-33]. Prolonged QTcd was observed in about 30% of subjects with impaired glucose tolerance in a report from the NHANES Ⅲ cohort, and a positive association was found between BMI and QTcd[34]. Some studies have also found a positive correlation between waist circumference and QTd[31,35,36]. In our study, prolonged QTd was found in UC and CD patients with higher BMI and waist circumference, although no statistically significant relationship was found. IR is a metabolic disorder characterized by a reduction in the use of glucose in skeletal muscle. Excess sodium retention, insulin-like cellular proliferation and matrix expansion causes vascular responses. Insulin, various growth factors, and vascular damage can accelerate atherosclerosis[37]. Nigro and colleagues found that HOMA and insulin concentrations were significantly correlated with QTd[33]. In our study, UC and CD patients with high insulin and HOMA values showed a statistically significant relationship with QT dispersion. The two groups demonstrated HOMA values above 2.5. According to JNC 7, subjects with systolic blood pressure of 120-139 mmHg are considered to have prehypertension[8]. Some studies showed a significant relationship between subjects with prehypertension and prolonged QTd[38,39]. In our study, SBP in UC patients with prolonged QTd was significantly higher than that in those without prolonged QTd, and the controls. QT dispersion seen before the development of hypertension and diabetes mellitus may be associated with IBD etiopathogenesis.

Simple, inexpensive and non-invasive methods can be used to identify QTd, ventricular arrhythmias, and the risk of cardiovascular diseases. These cardiovascular diseases may occur as a result of prolonged QTd, and IBD exacerbates ventricular arrhythmias and can cause sudden death. In IBD patients, prolonged QTd can be used to determine cardiovascular risk factors thought to be useful for the large patient groups in controlled trials.

COMMENTS

Background

Inflammatory bowel disease (IBD) involves two separate chronic idiopathic inflammatory diseases: Crohn's disease and ulcerative colitis. Increased QT dispersion (QTd) is a marker of myocardial electrical instability and predicts ventricular arrhythmias and sudden cardiac death.

Research frontiers

Easy and non-invasive methods to determine the risk of ventricular arrhythmia are an advantage in patients with QTd. However, the frequency and prognostic value of IBD as an indicator of arrhythmia is not fully understood. In this study, the demographic data of patients with IBD and prolonged QT dispersion and their relationship with some cardiovascular risk parameters were investigated.

Innovations and breakthroughs

Cardiac involvement in IBD can cause serious ventricular arrhythmias with prolonged QTd, however, very little data is available on this subject. QT disper-

sion is prolonged in patients with IBD. The relationship between QT dispersion, insulin, homeostasis model assessment (HOMA) and increased cardiovascular risk factors were investigated in this study.

Applications

Determination of the factors causing prolongation of the treatment of patients with IBD with QT dispersion and reorganization will provide additional benefit in the future prognosis of these diseases.

Peer review

The aim of the study was to investigate the whether prolonged QT dispersion as measured on the electrocardiographic, at risk of developing ventricular fibrillation, in adult patients with IBD. In addition, the authors determined the level of insulin resistance in these patients using HOMA.

REFERENCES

1 **Rellecke P**, Strauer BE. Chronic inflammatory bowel disease and cardiovascular complications. *Med Klin* (Munich) 2006; **101** Suppl 1: 56-60 [PMID: 16802521]
2 **Day CP**, McComb JM, Campbell RW. QT dispersion: an indication of arrhythmia risk in patients with long QT intervals. *Br Heart J* 1990; **63**: 342-344 [PMID: 2375895 DOI: 10.1136/hrt.63.6.342]
3 **Hnatkova K**, Malik M, Kautzner J, Gang Y, Camm AJ. Adjustment of QT dispersion assessed from 12 lead electrocardiograms for different numbers of analysed electrocardiographic leads: comparison of stability of different methods. *Br Heart J* 1994; **72**: 390-396 [PMID: 7833200 DOI: 10.1136/hrt.72.4.390]
4 **Somberg JC**, Molnar J. Usefulness of QT dispersion as an electrocardiographically derived index. *Am J Cardiol* 2002; **89**: 291-294 [PMID: 11809431 DOI: 10.1016/S0002-9149(01)02230-5]
5 **van de Loo A**, Arendts W, Hohnloser SH. Variability of QT dispersion measurements in the surface electrocardiogram in patients with acute myocardial infarction and in normal subjects. *Am J Cardiol* 1994; **74**: 1113-1118 [PMID: 7977069]
6 **Knight CJ**. New insights into the pathophysiology of acute coronary occlusion. *Eur Heart J* 1999; **1**: 36
7 **Curione M**, Aratari A, Amato S, Colotto M, Barbato M, Leone S, Tego A, Panetti D, Parlapiano C. A study on QT interval in patients affected with inflammatory bowel disease without cardiac involvement. *Intern Emerg Med* 2010; **5**: 307-310 [PMID: 20411364 DOI: 10.1007/s11739-010-0382-9]
8 **Best WR**, Becktel JM, Singleton JW, Kern F. Development of a Crohn's disease activity index. National Cooperative Crohn's Disease Study. *Gastroenterology* 1976; **70**: 439-444 [PMID: 1248701]
9 **Truelove SC**, Witts LJ. Cortisone in ulcerative colitis; final report on a therapeutic trial. *Br Med J* 1955; **2**: 1041-1048 [PMID: 13260656]
10 **Chobanian AV**, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Jones DW, Materson BJ, Oparil S, Wright JT, Roccella EJ. The Seventh Report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure: the JNC 7 report. *JAMA* 2003; **289**: 2560-2572 [PMID: 12748199]
11 **Bazett HC**. The time relations of the blood-pressure changes after excision of the adrenal glands, with some observations on blood volume changes. *J Physiol* 1920; **53**: 320-339 [PMID: 16993419]
12 **Matthews DR**, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28**: 412-419 [PMID: 3899825]
13 **Elming H**, Sonne J, Lublin HK. The importance of the QT interval: a review of the literature. *Acta Psychiatr Scand* 2003; **107**: 96-101 [PMID: 12534434 DOI: 10.1034/j.1600-0447.2003.00061.x]
14 **Mangoni AA**, Kinirons MT, Swift CG, Jackson SH. Impact of age on QT interval and QT dispersion in healthy subjects: a regression analysis. *Age Ageing* 2003; **32**: 326-331 [PMID: 12720621 DOI: 10.1093/ageing/32.3.326]
15 **Reardon M**, Malik M. QT interval change with age in an overtly healthy older population. *Clin Cardiol* 1996; **19**: 949-952 [PMID: 8957599 DOI: 10.1002/clc.4960191209]
16 **Taneja T**, Mahnert BW, Passman R, Goldberger J, Kadish A. Effects of sex and age on electrocardiographic and cardiac electrophysiological properties in adults. *Pacing Clin Electrophysiol* 2001; **24**: 16-21 [PMID: 11227963 DOI: 10.1046/j.1460-9592.2001.00016.x]
17 **Merri M**, Benhorin J, Alberti M, Locati E, Moss AJ. Electrocardiographic quantitation of ventricular repolarization. *Circulation* 1989; **80**: 1301-1308 [PMID: 2805266 DOI: 10.1161/01.CIR.80.5.1301]
18 **Molnar J**, Zhang F, Weiss J, Ehlert FA, Rosenthal JE. Diurnal pattern of QTc interval: how long is prolonged? Possible relation to circadian triggers of cardiovascular events. *J Am Coll Cardiol* 1996; **27**: 76-83 [PMID: 8522713]
19 **Sagie A**, Larson MG, Goldberg RJ, Bengtson JR, Levy D. An improved method for adjusting the QT interval for heart rate (the Framingham Heart Study) *Am J Cardiol* 1992; **70**: 797-801 [PMID: 1519533 DOI: 10.1016/0002-9149(92)90562-D]
20 **Singh K**. Effect of smoking on QT interval, QT dispersion and rate pressure product. *Indian Heart J* 2004; **56**: 140-142 [PMID: 15377136]
21 **Fauchier L**, Maison-Blanche P, Forhan A, D'Hour A, Lépinay P, Tichet J, Vol S, Coumel P, Fauchier JP, Balkau B. Association between heart rate-corrected QT interval and coronary risk factors in 2,894 healthy subjects (the DESIR Study). Data from an Epidemiological Study on the Insulin Resistance syndrome. *Am J Cardiol* 2000; **86**: 557-559, A9 [PMID: 11009279]
22 **Cassinotti A**, Massari A, Ferrara E, Greco S, Bosani M, Ardizzone S, Bianchi Porro G. New onset of atrial fibrillation after introduction of azathioprine in ulcerative colitis: case report and review of the literature. *Eur J Clin Pharmacol* 2007; **63**: 875-878 [PMID: 17598094 DOI: 10.1007/s00228-007-0328-y]
23 **Iqbal FM**, Beeharilal PS, Sadat K, Mohajer R, Lewis BE. Steroid induced atrial fibrillation. *Compr Ther* 2008; **34**: 111-114 [PMID: 18833788]
24 **Kwon HJ**, Coté TR, Cuffe MS, Kramer JM, Braun MM. Case reports of heart failure after therapy with a tumor necrosis factor antagonist. *Ann Intern Med* 2003; **138**: 807-811 [PMID: 12755552]
25 **Sofos S**, Savoye G, Ramirez S, Bauer F, Lerebours E. Transient type III atrioventricular block after infliximab infusion in a fistulizing perianal Crohn's disease patient. *Am J Gastroenterol* 2007; **102**: 217-219 [PMID: 17278277]
26 **Caviglia R**, Boskoski I, Cicala M. Long-term treatment with infliximab in inflammatory bowel disease: safety and tolerability issues. *Expert Opin Drug Saf* 2008; **7**: 617-632 [PMID: 18759714 DOI: 10.1517/14740338.7.5.617]
27 **Dubowitz M**, Gorard DA. Cardiomyopathy and pericardial tamponade in ulcerative colitis. *Eur J Gastroenterol Hepatol* 2001; **13**: 1255-1258 [PMID: 11711786 DOI: 10.1097/00042737-200110000-00022]
28 **Straub RH**, Antoniou E, Zeuner M, Gross V, Schölmerich J, Andus T. Association of autonomic nervous hyperreflexia and systemic inflammation in patients with Crohn's disease and ulcerative colitis. *J Neuroimmunol* 1997; **80**: 149-157 [PMID: 9413271 DOI: 10.1016/S0165-5728(97)00150-1]
29 **Seldenrijk CA**, Morson BC, Meuwissen SG, Schipper NW, Lindeman J, Meijer CJ. Histopathological evaluation of colonic mucosal biopsy specimens in chronic inflammatory bowel disease: diagnostic implications. *Gut* 1991; **32**: 1514-1520 [PMID: 1773958 DOI: 10.1136/gut.32.12.1514]
30 **Coruzzi P**, Castiglioni P, Parati G, Brambilla V, Brambilla L, Gualerzi M, Cademartiri F, Franzè A, De Angelis G, Di Rienzo M, Di Mario F. Autonomic cardiovascular regulation in quiescent ulcerative colitis and Crohn's disease. *Eur J Clin Invest* 2007; **37**: 964-970 [PMID: 18036030 DOI: 10.1111/

j.1365-2362.2007.01887.x]
31 **Frank S**, Colliver JA, Frank A. The electrocardiogram in obesity: statistical analysis of 1,029 patients. *J Am Coll Cardiol* 1986; **7**: 295-299 [PMID: 3944347 DOI: 10.1016/S0735-1097(86)80494-6]
32 **el-Gamal A**, Gallagher D, Nawras A, Gandhi P, Gomez J, Allison DB, Steinberg JS, Shumacher D, Blank R, Heymsfield SB. Effects of obesity on QT, RR, and QTc intervals. *Am J Cardiol* 1995; **75**: 956-959 [PMID: 7733014]
33 **Nigro G**, Russo V, Di Salvo G, De Crescenzo I, Rago A, Perrone L, Golino P, Russo MG, Calabrò R. Increased heterogenity of ventricular repolarization in obese nonhypertensive children. *Pacing Clin Electrophysiol* 2010; **33**: 1533-1539 [PMID: 20946307 DOI: 10.1111/j.1540-8159.2010.02889.x]
34 **Brown DW**, Giles WH, Greenlund KJ, Valdez R, Croft JB. Impaired fasting glucose, diabetes mellitus, and cardiovascular disease risk factors are associated with prolonged QTc duration. Results from the Third National Health and Nutrition Examination Survey. *J Cardiovasc Risk* 2001; **8**: 227-233 [PMID: 11551001 DOI: 10.1177/174182670100800407]
35 **Peiris AN**, Thakur RK, Sothmann MS, Gustafson AB, Hennes MI, Wilson CR, Kissebah AH. Relationship of regional fat distribution and obesity to electrocardiographic parameters in healthy premenopausal women. *South Med J* 1991; **84**: 961-965 [PMID: 1882272 DOI: 10.1097/00007611-199108000-00007]
36 **Mshui ME**, Saikawa T, Ito K, Hara M, Sakata T. QT interval and QT dispersion before and after diet therapy in patients with simple obesity. *Proc Soc Exp Biol Med* 1999; **220**: 133-138 [PMID: 10193440]
37 **Ferrannini E**, Natali A, Capaldo B, Lehtovirta M, Jacob S, Yki-Järvinen H. Insulin resistance, hyperinsulinemia, and blood pressure: role of age and obesity. European Group for the Study of Insulin Resistance (EGIR). *Hypertension* 1997; **30**: 1144-1149 [PMID: 9369268 DOI: 10.1291/hypres.15.67]
38 **Cagirci G**, Cay S, Karakurt O, Eryasar N, Acikel S, Dogan M, Yesilay AB, Kilic H, Akdemir R. P-wave dispersion increases in prehypertension. *Blood Press* 2009; **18**: 51-54 [PMID: 19353411 DOI: 10.1080/08037050902779441]
39 **Doğru MT**, Güneri M, Tireli E, Sahin O, Celik T, Iyisoy A. QT interval and dispersion differences between normal and prehypertensive patients: effects of autonomic and left ventricular functional and structural changes. *Anadolu Kardiyol Derg* 2009; **9**: 15-22 [PMID: 19196568]

P-Reviewer Tran CD **S- Editor** Cheng JX
L- Editor Webster JR **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i1.72

World J Gastroenterol 2013 January 7; 19(1): 72-77
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Improvement of prognosis for unresectable biliary tract cancer

Takashi Sasaki, Hiroyuki Isayama, Yousuke Nakai, Naminatsu Takahara, Naoki Sasahira, Hirofumi Kogure, Suguru Mizuno, Hiroshi Yagioka, Yukiko Ito, Natsuyo Yamamoto, Kenji Hirano, Nobuo Toda, Minoru Tada, Masao Omata, Kazuhiko Koike

Takashi Sasaki, Hiroyuki Isayama, Yousuke Nakai, Naminatsu Takahara, Naoki Sasahira, Hirofumi Kogure, Natsuyo Yamamoto, Kenji Hirano, Minoru Tada, Kazuhiko Koike, Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo, Tokyo 113-8655, Japan
Suguru Mizuno, Department of Gastroenterology, Kanto Central Hospital, Tokyo 158-8531, Japan
Hiroshi Yagioka, Department of Gastroenterology, JR Tokyo General Hospital, Tokyo 151-8528, Japan
Yukiko Ito, Department of Gastroenterology, Japanese Red Cross Medical Center, Tokyo 150-8935, Japan
Nobuo Toda, Department of Gastroenterology, Mitsui Memorial Hospital, Tokyo 101-8643, Japan
Masao Omata, Yamanashi Prefectural Hospital Organization, Yamanashi 400-8506, Japan
Author contributions: Sasaki T designed the study, analyzed the data and worked on the manuscript; Isayama H designed the study concept; Nakai Y analyzed the data and worked on the manuscript; Takahara N, Sasahira N, Kogure H, Mizuno S, Yagioka H, Ito Y and Toda N collected the clinical data; Yamamoto N, Hirano K and Tada M performed the analysis; Omata M and Koike K supervised and overviewed the study.
Correspondence to: Takashi Sasaki, MD, PhD, Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. sasakit-tky@umin.ac.jp
Telephone: +81-3-38155411 Fax: +81-3-38140021
Received: July 1, 2012 Revised: September 17, 2012
Accepted: September 22, 2012
Published online: January 7, 2013

Abstract

AIM: To evaluate the chemotherapeutic outcomes and confirm the recent improvement of prognosis for unresectable biliary tract cancer.

METHODS: A total of 186 consecutive patients with unresectable biliary tract cancer, who had been treated with chemotherapy between 2000 and 2009 at five institutions in Japan, were retrospectively analyzed. These patients were divided into three groups based on the year beginning chemotherapy: Group A (2000-2003), Group B (2004-2006), and Group C (2007-2009). The data were fixed at the end of December 2011. Overall survival and time-to-progression were analyzed and compared chronologically.

RESULTS: No patient characteristics were significantly different among the three groups. The gallbladder was involved in about half of the patients in each group, and metastatic biliary tract cancer was present in three quarters of the enrollees. In Group A, 5-fluorouracil-based chemotherapies were primarily selected as first-line chemotherapy, and only 24% were treated with second-line chemotherapy. In Group B, gemcitabine or S-1 monotherapy was mainly introduced as first-line chemotherapy, and 51% of the patients who were refractory to first-line chemotherapy were treated with second-line chemotherapy mainly with monotherapy. In Group C, the combination therapy with gemcitabine and S-1 was mainly chosen as first-line chemotherapy, and 53% of the patients refractory to first-line chemotherapy were treated with second-line chemotherapy mainly with combination therapy. The median time-to-progressions were 4.4 mo, 3.5 mo and 5.9 mo in Groups A, B and C, respectively (4.4 mo *vs* 3.5 mo *vs* 5.9 mo, $P < 0.01$). The median overall survivals were 7.1, 7.3, and 11.7 mo in Groups A, B and C (7.1 mo *vs* 7.3 mo *vs* 11.7 mo, $P = 0.03$). Induction rates of all three drugs (gemcitabine, platinum analogs, and fluoropyrimidine) in Groups A, B and C were 4%, 2% and 27% (4% *vs* 2% *vs* 27%, $P < 0.01$).

CONCLUSION: The prognosis of unresectable biliary tract cancer has improved recently. Using three effective drugs (gemcitabine, platinum analogs, and fluoropyrimidine) may improve the prognosis of this cancer.

© 2013 Baishideng. All rights reserved.

Key words: Unresectable; Biliary tract cancer; Gemcitabine; Platinum analogs; Fluoropyrimidine

Sasaki T, Isayama H, Nakai Y, Takahara N, Sasahira N, Kogure H, Mizuno S, Yagioka H, Ito Y, Yamamoto N, Hirano K, Toda N, Tada M, Omata M, Koike K. Improvement of prognosis for unresectable biliary tract cancer. *World J Gastroenterol* 2013; 19(1): 72-77 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i1/72.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i1.72

INTRODUCTION

Biliary tract cancer (BTC) is the sixth leading cause of cancer-related death in Japan according to "Cancer Statistics in Japan (2009)". Surgery is still the only treatment that can cure this life-threatening disease. However, many patients are diagnosed at an advanced stage of disease. Therefore, chemotherapy is indispensable for the treatment of advanced BTC.

In the 21st century, many clinical trials have been conducted to investigate the use of chemotherapy for advanced BTC[1]. Regimens involving 5-fluorouracil were widely used around the year 2000. However, gemcitabine has now become a key drug based on the results of several retrospective analyses[2,3]. In Japan, gemcitabine and S-1 (an oral fluoropyrimidine) have been approved and are widely used for the treatment of BTC[4-8]. Combination therapy using gemcitabine and S-1 has produced promising results in phase Ⅱ studies and is considered as one of the regimen for advanced BTC, particularly in Japan[9,10]. Meanwhile, combination therapy with gemcitabine and cisplatin has become a standard chemotherapy regimen based on the results of recent randomized controlled studies reported in 2009[11,12].

BTC include the cancers of several biliary sites, therefore the results might have strongly been affected by the patient characteristics enrolled in the clinical trial. Moreover, most of these past trials also included both unresectable and recurrent cases; however, the prognoses of these two conditions are extremely different[12]. One of the reasons for this difference might be that the tumor volume of recurrent cases was usually smaller than that of unresectable cases. We have previously reported that tumor volume affects both the tumor response and prognosis in advanced BTC patients who received chemotherapy[13]. Another reason might be that the tolerance of chemotherapeutic agents was usually poorer in recurrent cases than in unresectable cases. It is sometimes difficult to treat recurrent cases with the same treatment dose and schedule as unresectable cases because metabolism of the anti-cancer agent is often influenced by major surgery[14]. Therefore, it is better to separate these two conditions when the treatment outcomes of chemotherapy for advanced BTC are analyzed. Thus, we conducted a retrospective study to clarify the treatment outcomes of chemotherapy and confirm the recent improvement of prognosis for the treatment of unresectable BTC patients.

MATERIALS AND METHODS

Patients and methods

A total of 186 patients with unresectable BTC, who had been treated with chemotherapy between 2000 and 2009 at five institutions in Japan, were retrospectively analyzed. The patients were divided into three groups: the patients who started chemotherapy between 2000 and 2003 were enrolled as Group A, the patients who started chemotherapy between 2004 and 2006 were enrolled as Group B and the patients who started chemotherapy between 2007 and 2009 were enrolled as Group C. The eligibility criteria of this retrospective study were as follows: (1) no previous chemotherapy; (2) an Eastern Cooperative Oncology Group performance score of 0 to 3; (3) adequate bone marrow function (white blood cell count > 3000/mm^3, hemoglobin > 9.0 g/dL and platelet count > 100 000/mm^3), liver function [total bilirubin < three times the upper limit of normal (ULN) and aspartate/alanine transaminase levels < five times the ULN] and renal function (creatinine < 1.2 mg/dL or creatinine clearance > 50 mL/min); (4) no serious complications; (5) no uncontrolled infections; and (6) written informed consent from the patient.

Treatment regimen

First-line chemotherapy: Because there was no standard chemotherapy or efficacious key drug for advanced BTC, several 5-fluorouracil-based chemotherapies were selected between 2000 and 2003 (Group A). Chemoradiation was primarily selected in cases of locally advanced BTC. The treatment regimen was chosen by the attending doctor during this period. Starting in the middle of 2004, gemcitabine monotherapy (1000 mg/m^2, days 1, 8, 15, q 4 wk) was introduced. Several clinical trials have been conducted since 2005. First, a feasibility study of S-1 monotherapy (80 mg/m^2, days 1-28, q 6 wk) was started in 2005[7]. A multicenter phase Ⅱ study of gemcitabine and S-1 combination therapy (gemcitabine: 1000 mg/m^2, days 1, 15; S-1: 80 mg/m^2, days 1-14, q 4 wk) started to enroll participants in 2007[9]. Furthermore, a randomized phase Ⅱ study comparing gemcitabine and S-1 combination therapy (gemcitabine: 1000 mg/m^2, days 1, 15; S-1: 80 mg/m^2, days 1-14, q 4 wk) with gemcitabine monotherapy (1000 mg/m^2, days 1, 8, 15, q 4 wk) started to enroll participants at the end of 2008. After the completion of this randomized study, gemcitabine and S-1 combination therapy became the first choice for clinical practice.

Second-line chemotherapy: Only certain patients were treated with second-line chemotherapy between 2000 and 2003 (Group A). Several clinical trials of second-line chemotherapy have also been conducted since 2007. A multicenter phase Ⅱ study of S-1 monotherapy (80 mg/m^2,

Table 1 Patient characteristics *n* (%)

	Group A	Group B	Group C	*P* value
Number of patients	25	54	107	
Age (yr)				0.08
Median	64	66	68	
Range	47-81	35-84	24-89	
Gender				0.20
Male	11 (44)	28 (52)	66 (62)	
Female	14 (56)	26 (48)	41 (38)	
ECOG performance score				0.12
0	10 (40)	13 (24)	46 (43)	
1	12 (48)	37 (69)	50 (47)	
2-3	3 (12)	4 (7)	11 (10)	
Primary biliary site				0.47
Gallbladder	14 (56)	22 (41)	46 (43)	
Intra-hepatic bile duct	5 (20)	21 (39)	40 (37)	
Extra-hepatic bile duct	6 (24)	9 (16)	20 (19)	
Ampulla of Vater	0 (0)	2 (4)	1 (1)	
Disease status				0.76
Locally advanced	6 (24)	15 (28)	24 (22)	
Metastatic	19 (76)	39 (72)	83 (78)	

ECOG: Eastern Cooperative Oncology Group.

days 1-28, q 6 wk) as a second-line chemotherapy started to enroll participants in 2007[8]. Moreover, a feasibility study of gemcitabine and cisplatin combination therapy (gemcitabine: 1000 mg/m^2, days 1, 8; cisplatin: 25 mg/m^2, days 1, 8, q 3 wk) for refractory BTC was started in 2008[15]. Recently, gemcitabine and cisplatin combination therapy are chosen as second-line chemotherapy in clinical trial.

Statistical analysis

The Mann-Whitney *U* test was used to compare quantitative variables. The time to progression and overall survival were calculated using the Kaplan-Meier method. The time to progression was calculated from the start of the treatment to the first date of documented disease progression. The overall survival was defined as the time from the initiation of therapy to the final follow-up or until death from any cause. The final analysis was conducted using the follow-up data through the end of December 2011 to get enough follow-up periods. The JMP 8.0 statistical software program (SAS Institute Inc., Cary, NC, United States) was used for all statistical analyses.

RESULTS

Patient characteristics

A total of 186 patients with unresectable BTC were treated with chemotherapy between January 2000 and December 2009. Patient characteristics are shown in Table 1. The number of patients in Group A (2000-2003), B (2004-2006) and C (2007-2009) was 25, 54 and 107, respectively. No patient characteristics were significantly different among the three groups, although the median age of each group became slightly higher with time. There were no differences in either the primary biliary site or disease status. The gallbladder was involved in about half of the patients in each group and metastatic BTC was present in three quarters of the enrollees in each group.

Table 2 Regimens for first-line and second-line treatment

First-line treatment	
Group A (*n* = 25)	
5-fluorouracil + radiation	5
5-fluorouracil ia + radiation	2
5-fluorouracil + cisplatin + radiation	1
5-fluorouracil ia	1
5-fluorouracil + doxorubicin + mitomycin C ia	1
5-fluorouracil + cisplatin	3
5-fluorouracil + doxorubicin + mitomycin C	1
Uracil-tegafur	2
Gemcitabine	9
Group B (*n* = 54)	
5-fluorouracil + radiation	2
5-fluorouracil ia + radiation	2
Uracil-tegafur + radiation	1
5-fluorouracil	1
5-fluorouracil + doxorubicin + mitomycin C	2
Gemcitabine	25
S-1	20
Gemcitabine + cisplatin	1
Group C (*n* = 107)	
Gemcitabine + S-1	62
Gemcitabine	44
S-1	1
Second-line treatment	
Group A (*n* = 6)	
Gemcitabine	2
Gemcitabine ia	1
5-fluorouracil + radiation	1
S-1	1
Uracil-tegafur	1
Group B (*n* = 27)	
Gemcitabine	14
S-1	9
S-1 + interferon-α	1
5-fluorouracil	2
Mitomycin C	1
Group C (*n* = 51)	
Gemcitabine + cisplatin	28
Gemcitabine	6
S-1	13
Cisplatin ia	1
5-fluorouracil + interferon-α	1
Irinotecan	1
Uracil-tegafur	1

Chemotherapy

Group A (2000-2003): The regimens for first-line and second-line chemotherapy are listed in Table 2. As first-line chemotherapy, 5-fluorouracil-based chemotherapies were primarily selected during this period (sixteen patients; 64%). Eight patients (32%) were treated with chemoradiation and intra-arterial infusions were delivered to four patients (16%). In this treatment group, all of the patients progressed with first-line chemotherapy. Only six patients (24%) were treated with second-line chemotherapy; two patients were treated with gemcitabine monotherapy and one patient was treated with S-1

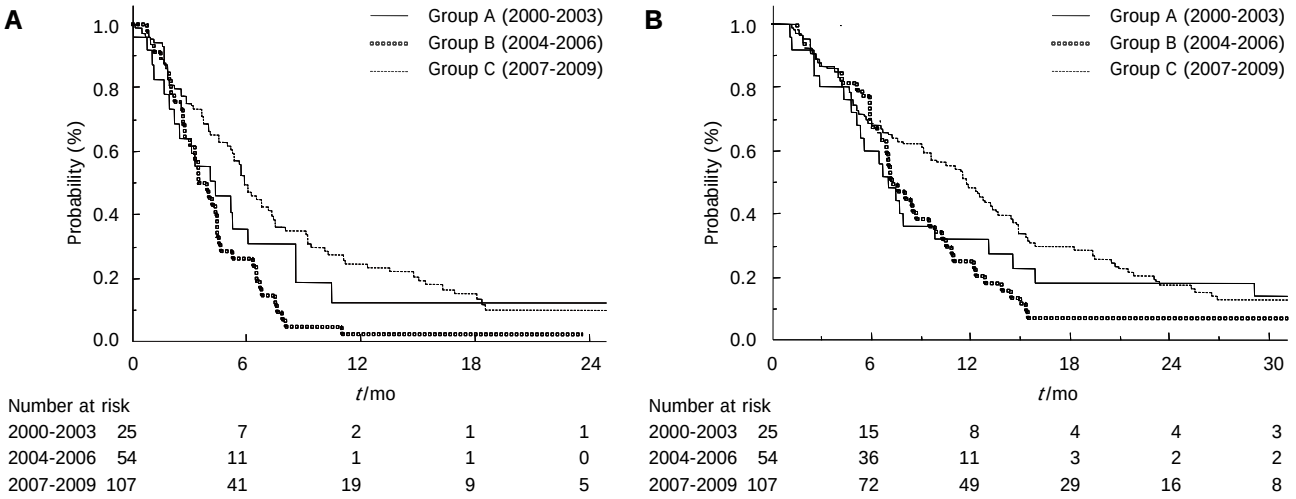


Figure 1 Time-to-progression curves (A) and overall survival curves (B) of patients with unresectable biliary tract cancer. A: The median time to progression was 4.4 mo, 3.5 mo and 5.9 mo in Groups A, B and C, respectively (log-rank test; $P < 0.01$). There was no difference between Groups A and B (log-rank test; $P = 0.20$); B: The median overall survival was 7.1 mo, 7.3 mo and 11.7 mo in Groups A, B and C, respectively (log-rank test; $P = 0.03$). There was no difference between Groups A and B (log-rank test; $P = 0.41$).

monotherapy, uracil-ftorafur monotherapy, 5-fluorouracil-based chemoradiation and intra-arterial infusion of gemcitabine. Of all, only one patient (4%) was treated with all three effective drugs (gemcitabine, platinum analogs and fluoropyrimidine) in this group.

Group B (2004-2006): During this period, gemcitabine or S-1 monotherapy was primarily selected for first-line chemotherapy (83%); twenty-five patients (46%) underwent gemcitabine monotherapy and twenty patients (37%) underwent S-1 monotherapy. In this treatment group, fifty-three patients progressed with first-line chemotherapy. Of the patients who were refractory to first-line chemotherapy, twenty-seven patients (51%) were treated with second-line chemotherapy, fourteen patients were treated with gemcitabine monotherapy and nine patients were treated with S-1 monotherapy. Sequential monotherapy of gemcitabine and S-1 was introduced during this period. Of all, only one patient (2%) was treated with all three effective drugs (gemcitabine, platinum analogs and fluoropyrimidine) in this group.

Group C (2007-2009): As first-line chemotherapy, combination therapy with gemcitabine and S-1 was introduced and used predominantly during this period (in 58% of patients). In this treatment group, ninety-nine patients progressed with first-line chemotherapy. Of these patients, fifty-two (53%) were treated with second-line chemotherapy; twenty-eight patients were treated with gemcitabine and cisplatin combination therapy, six patients were treated with gemcitabine monotherapy and thirteen patients were treated with S-1 monotherapy. During this period, sequential combination therapy using gemcitabine, S-1 and cisplatin was primarily prescribed. Of all, twenty-nine patients (27%) were treated with all three effective drugs (gemcitabine, platinum analogs and fluoropyrimidine) in this group.

Time-to-progression and overall survival

The median times-to-progression in Groups A, B and C were 4.4 mo (95% CI, 2.0-8.6 mo), 3.5 mo (95% CI, 2.8-4.5 mo) and 5.9 mo (95% CI, 5.2-7.3 mo), respectively (log-rank test; $P < 0.01$) (Figure 1A). There was no difference between Groups A and B (log-rank test; $P = 0.20$). The median overall survival times in Groups A, B and C were 7.1 mo (95% CI, 5.1-13.1 mo), 7.3 mo (95% CI, 6.6-9.5 mo) and 11.7 mo (95% CI, 9.2-13.7 mo), respectively (log-rank test; $P = 0.03$) (Figure 1B). There was also no difference between Groups A and B (log-rank test; $P = 0.41$).

After assessing the treatment outcomes of each primary biliary site, the median overall survival for patients with gallbladder cancer ($n = 46$), intrahepatic cholangiocarcinoma ($n = 40$) and extrahepatic cholangiocarcinoma ($n = 20$) in Group C (2007-2009) was 9.1 mo (95% CI, 5.2-12.5 mo), 12.4 mo (95% CI, 6.7-19.4 mo) and 14.9 mo (95% CI, 11.1 mo - not reached), respectively (log-rank test; $P = 0.049$).

DISCUSSION

Many clinical trials investigating the use of chemotherapy for advanced BTC have been reported since 2000, although most of these previous trials included both unresectable and recurrent cases[16]. However, the prognoses of these two conditions are extremely different[12]. In BT-22 study, the median overall survivals of unresectable and recurrent BTC, who were treated with the combination therapy of gemcitabine and cisplatin, were 9.4 mo and 16.1 mo, respectively. Thus, the patient characteristics strongly affect the results of clinical trial for advanced BTC, though most previous trial did not report the detail about the prognosis of unresectable BTC. In our retrospective study, we ascertained that the prognosis for unresectable BTC has actually improved in recent years

(Group C; 2007-2009) and the median overall survival has reached approximately one year. We also confirmed that the prognoses for unresectable BTC at several biliary sites are actually different and that the prognosis for unresectable gallbladder cancer is still extremely poor.

The median overall survival of Group C (2007-2009) was 11.7 mo (95% CI, 9.2-13.7 mo). The difference between the median overall survival and the median time to progression increased with time (Group A, 2.7 mo; Group B, 3.8 mo; Group C, 5.8 mo). The rate of second-line chemotherapy also increased in Group B (51%) and Group C (53%). However, greater improvement was observed only in Group C, which may have been caused by the use of three effective agents (gemcitabine, platinum analogs and fluoropyrimidine). In fact, induction rates of these three effective drugs in Groups A, B and C were 4%, 2% and 27% ($P < 0.01$). Therefore, using three effective drugs (gemcitabine, platinum analogs and fluoropyrimidine) may improve the prognosis of unresectable BTC.

One limitation of this retrospective study is that the regimens in each group were not uniform. However, the improvement of prognosis for unresectable BTC in clinical settings was confirmed because all of the patients who underwent chemotherapy in the past ten years were included in this retrospective study. Another limitation is that gemcitabine and S-1 had mainly been used for first-line chemotherapy because the efficacy of gemcitabine and cisplatin combination therapy was reported in 2009.

In conclusion, the use of chemotherapy for unresectable BTC has made recent advances and the median overall survival has reached approximately one year. However, the prognosis of unresectable gallbladder cancer is still exceedingly poor. Using three effective drugs (gemcitabine, platinum analogs and fluoropyrimidine) may improve the prognosis of unresectable BTC. Future assessments are needed to establish the best strategy for using these three effective drugs (gemcitabine, platinum analogs and fluoropyrimidine) for the treatment of unresectable BTC.

ACKNOWLEDGEMENT

In addition to the authors listed in the author field, following are the authors who contributed equally to this study. Kouji Miyabayashi, Keisuke Yamamoto, Dai Mohri, Kazumichi Kawakubo, Osamu Togawa and Saburo Matsubara from Department of Gastroenterology, Graduate School of Medicine, The University of Tokyo, Tokyo, Japan; Makoto Okamoto from Department of Gastroenterology, JR Tokyo General Hospital, Tokyo, Japan; Rie Uchino, Tateo Kawase from Department of Gastroenterology, Kanto Central Hospital, Tokyo, Japan; Tsuyoshi Hamada, Yoko Yashima, Takeshi Tsujino, Ryo Nakata from Department of Gastroenterology, Japanese Red Cross Medical Center, Tokyo, Japan; Toshihiko Arizumi, Kazumi Tagawa from Department of Gastroenterology, Mitsui Memorial Hospital, Tokyo, Japan.

COMMENTS

Background

Biliary tract cancer (BTC) is a rare cancer worldwide. Obstructive jaundice and infection to the biliary system usually become obstacles to introduce chemotherapy. Thus, enrollment of clinical trial for advanced biliary tract cancer is more difficult than other common cancers.

Research frontiers

Previous clinical trials investigating the efficacy of chemotherapy for advanced biliary tract cancer enrolled both unresectable and recurrent cases. Recent reports showed the differences of prognoses between these two conditions. However, the details about the prognoses of each condition have not been evaluated sufficiently.

Innovations and breakthroughs

Although the prognoses of unresectable and recurrent cases were extremely different, previous studies included both conditions. Moreover, most of these previous reports only showed the overall efficacies. Therefore, the treatment outcomes of unresectable biliary tract cancer were not fully evaluated. In our retrospective analysis, they only included unresectable biliary tract cancer and confirmed the recent improvement of prognosis in patients receiving chemotherapy.

Applications

The prognosis of unresectable biliary tract cancer has improved recently and the median overall survival has reached approximately one year. Using three effective drugs (gemcitabine, platinum analogs, and fluoropyrimidine) may improve the prognosis of unresectable biliary tract cancer. Future assessments are needed to establish the best strategy for using these three effective drugs (gemcitabine, platinum analogs, and fluoropyrimidine) for the treatment of unresectable biliary tract cancer.

Terminology

Biliary tract cancer includes cholangiocarcinoma, gallbladder cancer, and ampullary carcinoma. 5-fluorouracil is a fluoropyrimidine analog used for chemotherapy. Gemcitabine is a nucleoside analog used for chemotherapy. S-1 is an oral fluoropyrimidine widely used in Japan. Cisplatin is a platinum alalog used for chemotherapy.

Peer review

This paper evaluates the chemotherapeutic outcomes of patients affected by unresectable BTC. The study retrospectively analyzed 186 consecutive patients with unresectable BTC between 2000 and 2009 at five institutions in Japan. The Authors concluded that the prognosis of unresectable BTC has improved during the years and reached approximately one year. Using three effective drugs in combination (gemcitabine, platinum analogs and fluoropyrimidine) could improve the prognosis of unresectable BTC. The authors possess an expertise about the topic since published some interesting papers about the treatment of biliary and pancreatic cancer. Moreover, this paper is well written and also the number of patients is relevant.

REFERENCES

1 **Tada M**, Nakai Y, Sasaki T, Hamada T, Nagano R, Mohri D, Miyabayashi K, Yamamoto K, Kogure H, Kawakubo K, Ito Y, Yamamoto N, Sasahira N, Hirano K, Ijichi H, Tateishi K, Isayama H, Omata M, Koike K. Recent progress and limitations of chemotherapy for pancreatic and biliary tract cancers. *World J Clin Oncol* 2011; **2**: 158-163 [PMID: 21611090 DOI: 10.5306/wjco.v2.i3.158]

2 **Eckel F**, Schmid RM. Chemotherapy in advanced biliary tract carcinoma: a pooled analysis of clinical trials. *Br J Cancer* 2007; **96**: 896-902 [PMID: 17325704 DOI: 10.1038/sj.bjc.6603648]

3 **Yonemoto N**, Furuse J, Okusaka T, Yamao K, Funakoshi A, Ohkawa S, Boku N, Tanaka K, Nagase M, Saisho H, Sato T. A multi-center retrospective analysis of survival benefits of chemotherapy for unresectable biliary tract cancer. *Jpn J Clin Oncol* 2007; **37**: 843-851 [PMID: 17942578 DOI: 10.1093/jjco/hym116]

4 **Okusaka T**, Ishii H, Funakoshi A, Yamao K, Ohkawa S, Saito S, Saito H, Tsuyuguchi T. Phase II study of single-

agent gemcitabine in patients with advanced biliary tract cancer. *Cancer Chemother Pharmacol* 2006; **57**: 647-653 [PMID: 16142487 DOI: 10.1007/s00280-005-0095-3]

5 **Ueno H**, Okusaka T, Ikeda M, Takezako Y, Morizane C. Phase II study of S-1 in patients with advanced biliary tract cancer. *Br J Cancer* 2004; **91**: 1769-1774 [PMID: 15505626 DOI: 10.1038/sj.bjc.6602208]

6 **Furuse J**, Okusaka T, Boku N, Ohkawa S, Sawaki A, Masumoto T, Funakoshi A. S-1 monotherapy as first-line treatment in patients with advanced biliary tract cancer: a multicenter phase II study. *Cancer Chemother Pharmacol* 2008; **62**: 849-855 [PMID: 18214482 DOI: 10.1007/s00280-007-0673-7]

7 **Sasaki T**, Isayama H, Yashima Y, Yagioka H, Kogure H, Arizumi T, Togawa O, Matsubara S, Ito Y, Nakai Y, Sasahira N, Hirano K, Tsujino T, Tada M, Kawabe T, Omata M. S-1 monotherapy in patients with advanced biliary tract cancer. *Oncology* 2009; **77**: 71-74 [PMID: 19556812 DOI: 10.1159/000226214]

8 **Sasaki T**, Isayama H, Nakai Y, Mizuno S, Yamamoto K, Yagioka H, Yashima Y, Kawakubo K, Kogure H, Togawa O, Matsubara S, Ito Y, Sasahira N, Hirano K, Tsujino T, Toda N, Tada M, Omata M, Koike K. Multicenter phase II study of S-1 monotherapy as second-line chemotherapy for advanced biliary tract cancer refractory to gemcitabine. *Invest New Drugs* 2012; **30**: 708-713 [PMID: 20924641 DOI: 10.1007/s10637-010-9553-9]

9 **Sasaki T**, Isayama H, Nakai Y, Ito Y, Kogure H, Togawa O, Toda N, Yasuda I, Hasebe O, Maetani I, Sasahira N, Hirano K, Tsujino T, Tada M, Omata M. Multicenter, phase II study of gemcitabine and S-1 combination chemotherapy in patients with advanced biliary tract cancer. *Cancer Chemother Pharmacol* 2010; **65**: 1101-1107 [PMID: 19707761 DOI: 10.1007/s00280-009-1115-5]

10 **Kanai M**, Yoshimura K, Tsumura T, Asada M, Suzuki C, Niimi M, Matsumoto S, Nishimura T, Nitta T, Yasuchika K, Taura K, Mori Y, Hamada A, Inoue N, Tada S, Yanagihara K, Yazumi S, Osaki Y, Chiba T, Ikai I, Fukushima M, Uemoto S, Hatano E. A multi-institution phase II study of gemcitabine/S-1 combination chemotherapy for patients with advanced biliary tract cancer. *Cancer Chemother Pharmacol* 2011; **67**: 1429-1434 [PMID: 20811895 DOI: 10.1007/s00280-010-1443-5]

11 **Valle J**, Wasan H, Palmer DH, Cunningham D, Anthoney A, Maraveyas A, Madhusudan S, Iveson T, Hughes S, Pereira SP, Roughton M, Bridgewater J. Cisplatin plus gemcitabine versus gemcitabine for biliary tract cancer. *N Engl J Med* 2010; **362**: 1273-1281 [PMID: 20375404 DOI: 10.1056/NEJMoa0908721]

12 **Okusaka T**, Nakachi K, Fukutomi A, Mizuno N, Ohkawa S, Funakoshi A, Nagino M, Kondo S, Nagaoka S, Funai J, Koshiji M, Nambu Y, Furuse J, Miyazaki M, Nimura Y. Gemcitabine alone or in combination with cisplatin in patients with biliary tract cancer: a comparative multicentre study in Japan. *Br J Cancer* 2010; **103**: 469-474 [PMID: 20628385 DOI: 10.1038/sj.bjc.6605779]

13 **Sasaki T**, Isayama H, Nakai Y, Togawa O, Kogure H, Ito Y, Yamamoto K, Mizuno S, Yagioka H, Yashima Y, Kawakubo K, Arizumi T, Matsubara S, Sasahira N, Hirano K, Tsujino T, Toda N, Tada M, Omata M, Koike K. Prognostic factors in patients with advanced biliary tract cancer receiving chemotherapy. *Cancer Chemother Pharmacol* 2011; **67**: 847-853 [PMID: 20563811 DOI: 10.1007/s00280-010-1360-7]

14 **Muneoka K**, Shirai Y, Sasaki M, Wakai T, Sakata J, Kanda J, Wakabayashi H, Hatakeyama K. [Effect of pylorus-preserving pancreaticoduodenectomy on serum levels of 5-fluorouracil during S-1 treatment for pancreaticobiliary malignancy]. *Gan To Kagaku Ryoho* 2010; **37**: 1503-1506 [PMID: 20716875]

15 **Sasaki T**, Isayama H, Nakai Y, Mizuno S, Yamamoto K, Yagioka H, Yashima Y, Kawakubo K, Kogure H, Togawa O, Matsubara S, Sasahira N, Hirano K, Tsujino T, Tada M, Omata M, Koike K. Feasibility study of gemcitabine and cisplatin combination chemotherapy for patients with refractory biliary tract cancer. *Invest New Drugs* 2011; **29**: 1488-1493 [PMID: 20607585 DOI: 10.1007/s10637-010-9485-4]

16 **Hezel AF**, Zhu AX. Systemic therapy for biliary tract cancers. *Oncologist* 2008; **13**: 415-423 [PMID: 18448556 DOI: 10.1634/theoncologist.2007-0252]

P- Reviewers Fava G, Nath G **S- Editor** Gou SX
L- Editor A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i1.78

World J Gastroenterol 2013 January 7; 19(1): 78-85
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Alcohol, postprandial plasma glucose, and prognosis of hepatocellular carcinoma

Hiroshi Abe, Yuta Aida, Haruya Ishiguro, Kai Yoshizawa, Tamihiro Miyazaki, Munenori Itagaki, Satoshi Sutoh, Yoshio Aizawa

Hiroshi Abe, Yuta Aida, Haruya Ishiguro, Kai Yoshizawa, Tamihiro Miyazaki, Munenori Itagaki, Satoshi Sutoh, Yoshio Aizawa, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Jikei University School of Medicine Katsushika Medical Center, Tokyo 125-8506, Japan
Author contributions: Abe H, Yoshizawa K, Sutoh S and Aizawa Y designed the research; Abe H, Aida Y, Ishiguro H, Miyazaki T and Itagaki M analyzed the data; Abe H, Yoshizawa K and Aizawa Y wrote the paper.
Correspondence to: Hiroshi Abe, MD, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Jikei University School of Medicine Katsushika Medical Center, 6-41-2 Aoto, Katsushika-ku, Tokyo 125-8506, Japan. hiroshiabe222@aol.com
Telephone: +81-3-36032111 Fax: +81-3-38389944
Received: August 8, 2012 Revised: September 17, 2012
Accepted: September 29, 2012
Published online: January 7, 2013

Abstract

AIM: To identify factors associated with prognosis of hepatocellular carcinoma (HCC) after initial therapy.

METHODS: A total of 377 HCC patients who were newly treated at Katsushika Medical Center, Japan from January 2000 to December 2009 and followed up for > 2 years, or died during follow-up, were enrolled. The factors related to survival were first analyzed in 377 patients with HCC tumor stage T1-T4 using multivariate Cox proportional hazards regression analysis. A similar analysis was performed in 282 patients with tumor stage T1-T3. Additionally, factors associated with the period between initial and subsequent therapy were examined in 144 patients who did not show local recurrence. Finally, 214 HCC stage T1-T3 patients who died during the observation period were classified into four groups according to their alcohol consumption and postprandial glucose levels, and differences in their causes of death were examined.

RESULTS: On multivariate Cox proportional hazards regression analysis, the following were significantly associated with survival: underlying liver disease stage [non-cirrhosis/Child-Pugh A *vs* B/C, hazard ratio (HR): 0.603, 95% CI: 0.417-0.874, $P = 0.0079$], HCC stage (T1/T2 *vs* T3/T4, HR: 0.447, 95% CI: 0.347-0.576, $P < 0.0001$), and mean postprandial plasma glucose after initial therapy (< 200 *vs* ⩾ 200 mg/dL, HR: 0.181, 95% CI: 0.067-0.488, $P = 0.0008$). In T1-T3 patients, uninterrupted alcohol consumption after initial therapy (no *vs* yes, HR: 0.641, 95% CI: 0.469-0.877, $P = 0.0055$) was significant in addition to underlying liver disease stage (non-cirrhosis/Child-Pugh A *vs* B/C, HR: 0649, 95% CI: 0.476-0.885, $P = 0.0068$), HCC stage (T1 *vs* T2/T3, HR: 0.788, 95% CI: 0.653-0.945, $P = 0.0108$), and mean postprandial plasma glucose after initial therapy (< 200 mg/dL *vs* ⩾ 200 mg/dL, HR: 0.502, 95% CI: 0.337-0.747, $P = 0.0005$). In patients without local recurrence, time from initial to subsequent therapy for newly emerging HCC was significantly longer in the "postprandial glucose within 200 mg/dL group" than the "postprandial glucose > 200 mg/dL group" (log-rank test, $P < 0.05$), whereas there was no difference in the period between the "non-alcohol group" (patients who did not drink regularly or those who could reduce their daily consumption to < 20 g) and the "continuation group" (drinkers who continued to drink > 20 g daily). Of 214 T1-T3 patients who died during the observation period, death caused by other than HCC progression was significantly more frequent in "group AL" (patients in the continuation and postprandial glucose within 200 mg/dL groups) than "group N" (patients in the non-alcohol and postprandial glucose within 200 mg/dL groups) ($P = 0.0016$).

CONCLUSION: This study found that abstinence from habitual alcohol consumption and intensive care for diabetes mellitus were related to improved prognosis in HCC patients.

© 2013 Baishideng. All rights reserved.

Key words: Hepatocellular carcinoma; Prognosis; Alcohol consumption; Diabetes mellitus; Postprandial plasma glucose level; Initial therapy; Local recurrence; Survival

Abe H, Aida Y, Ishiguro H, Yoshizawa K, Miyazaki T, Itagaki M, Sutoh S, Aizawa Y. Alcohol, postprandial plasma glucose, and prognosis of hepatocellular carcinoma. *World J Gastroenterol* 2013; 19(1): 78-85 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i1/78.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i1.78

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most important diseases, with increasing numbers of patients worldwide in recent years[1]. The number of deaths from HCC has also increased steadily. In Japan, chronic viral infection, such as hepatitis C virus (HCV) or hepatitis B virus (HBV), is considered to be the most significant risk factor for the development of HCC[2]. It has been recognized that chronic infection with these viruses induces continuous or recurrent necroinflammatory reactions in the liver that lead to cirrhosis; in most cases, the cirrhotic stage facilitates the development of HCC[3].

In Japan, hepatitis B surface antigen (HBs-Ag)-positive or anti-HCV (HCV-Ab)-positive subjects have accounted for > 80% of HCC patients in the last decade[4]. Meanwhile, 5%-15% of patients with HCC have neither HBs-Ag nor HCV-Ab (non-B non-C)[5-7], and the proportion of non-B non-C HCC has been increasing in newly diagnosed subjects[8].

Alcoholic liver disease[9] and nonalcoholic fatty liver disease[10] have been proposed as possible major backgrounds associated with non-B non-C HCC. Moreover, diabetes mellitus (DM), which is frequently present in association with nonalcoholic steatohepatitis or alcoholic liver disease, has been identified as a risk factor for developing HCC[11-15].

As mentioned above, factors associated with the development of HCC have been well recognized. However, factors affecting the prognosis after treatment for HCC have not been extensively examined, except for HCC tumor stage and underlying liver function.

A large majority of HCC patients have been treated according to the "Japanese Guidelines for HCC"[16]. There have been numerous therapeutic strategies against HCC, including surgical resection, percutaneous ethanol injection therapy (PEIT), percutaneous radiofrequency ablation (RFA), and transhepatic arterial chemoembolization (TACE). The therapeutic strategy is determined based on liver function and HCC tumor stage, and aims to achieve the best prognosis[16]. However, there have often been obvious differences in clinical course even when HCC has been treated according to the "Japanese Guidelines for HCC"[16].

A relationship between HCC recurrence and the presence of DM in patients who are negative for markers of HBV and HCV[17] has been reported. The prognostic factors of HCC other than HCC tumor stage and underlying liver function have been evaluated[18]. However, there is no information about the relationship between HCC prognosis and general health management, including abstinence from alcohol drinking and intensive control of plasma glucose levels after therapy for HCC.

In the present study, the impact of plasma glucose levels and continuation of alcohol consumption on the prognosis after initial therapy for HCC was investigated.

MATERIALS AND METHODS

Patients

Of 475 consecutive HCC patients who were newly diagnosed and treated at the Jikei University Katsushika Medical Center from January 2000 to December 2009, we enrolled 377 who were treated based on the Japanese Guidelines for HCC[16]. Initial therapy consisted of surgical resection in five of 377 HCC patients, PEIT in 46 patients, RFA in 111 patients, TACE in 146 patients, transhepatic arterial infusion in 54 patients, and supportive care without therapy for HCC in 15 patients. Only 1.3% of patients underwent surgical resection, because patients who did not wish to undergo an operation tended to consult our department. Patients who received liver transplantation were not included in this study, because the number of deceased donors is small in Japan.

The diagnosis of HCC was based on the presence of characteristic imaging findings on computed tomography, magnetic resonance imaging, or ultrasonography. In subjects in whom the definite diagnosis of HCC could not be made by imaging, the diagnosis was confirmed by ultrasonography-guided tumor biopsy.

The characteristics of the 377 patients at the time of initial diagnosis of HCC, including age, sex, serum aspartate aminotransferase (AST)/alanine aminotransferase/α-fetoprotein (AFP)/protein induced by vitamin K absence or antagonist-Ⅱ (PIVKA-Ⅱ) level at the time of diagnosis, stage of underlying liver disease (cirrhotic or non-cirrhotic, Child-Pugh staging in cirrhotic patients), obesity [body mass index (BMI) > 25 kg/m^2], presence of DM, existence of malignant tumors other than HCC, habitual use of alcohol, and HCC tumor stage according to the tumor-node-metastasis staging classification system[19], are summarized in Table 1.

The presence or absence of serum HCV-Ab and HBs-Ag was determined in all patients using an enzyme immunoassay kit (AxSYM Abbott HCV; Axsym Co. Ltd., Tokyo, Japan, for HCV-Ab and AxSYM Abbott HBs-Ag; Axsym, for HBs-Ag).

The diagnosis of cirrhosis was made based on imaging findings, histological findings of liver biopsy specimens, and a combination of laboratory data[20]. Cirrhotic patients were then subdivided into A-C according to the Child-Pugh classification[21].

Habitual alcohol consumption was defined as cumulative consumption ≥ 320 kg ethanol in females or ≥ 480 kg in males, with daily alcohol consumption ≥ 20 g

ethanol. A history of alcohol consumption was obtained from interviews with patients, their families, or neighbors.

The definition of the presence of DM was as follows: fasting plasma glucose level > 126 mg/dL or postprandial plasma glucose > 200 mg/dL on at least two occasions, or the need for subcutaneous injection of insulin or oral hypoglycemic drugs to control glucose levels.

Enrolled patients were followed up after initial therapy for HCC for at least 2 years or until death during follow-up. No patients took drugs or foods containing carcinogenic substances. HCV-positive HCC patients did not undergo antiviral therapy after therapy for HCC, while all of the HBs-Ag-positive HCC patients were treated with nucleoside and/or nucleotide analogs including lamivudine, adefovir, dipivoxil, and entecavir.

Follow-up of patients after initial therapy for HCC

Survival after initial therapy and the period from initial therapy to subsequent therapy were recorded. Continuation of alcohol consumption and the mean postprandial plasma glucose values during almost monthly follow-up were examined.

The manner of drinking after initial therapy for HCC was classified into two groups: patients who did not drink regularly or habitual alcohol drinkers who were able to reduce their quantity of daily alcohol consumption to < 20 g ethanol (non-alcohol group), and habitual alcohol drinkers who continued to drink > 20 g ethanol daily (continuation group).

Patients defined as having DM were classified into the "postprandial glucose within 200 mg/dL group" or the "postprandial glucose > 200 mg/dL group". The postprandial glucose within 200 mg/dL group was defined as having a mean monthly postprandial plasma glucose level (plasma glucose measured about 2 h after taking the meal) ≤ 200 mg/dL during follow-up. Patients without DM were included in the postprandial glucose within 200 mg/dL group. The other HCC patients whose mean postprandial plasma glucose level was > 200 mg/dL despite treatment for DM were included in the postprandial glucose > 200 mg/dL group.

Education about abstinence from drinking was given to all habitual drinkers. For all patients with DM, education about appropriate caloric intake and standard drug therapy for DM, including insulin injection, was provided. This study complied with the standards of the Declaration of Helsinki and current ethical guidelines.

Statistical analysis

Data are expressed as means ± SD, and the parameters were categorized into two groups by the median value, except for the stage of underlying liver disease and the T stage of HCC. Factors associated with survival were examined by Cox proportional hazards regression analysis to elucidate factors associated with HCC prognosis. The differences in the survival period and in the period from initial therapy to subsequent therapy were evaluated by the Kaplan-Meier method with the log-rank test. Differences in the causes of death were evaluated by the χ^2 test with Yates's correction. All tests of significance were two-tailed, and a $P < 0.05$ was considered significant. All statistical analyses were carried out using Statistica for Windows Version 6.

RESULTS

Characteristics of HCC patients

The patients' median age was 69 years. The most common stage of underlying liver disease was Child-Pugh class A cirrhosis. The frequencies of HBs-Ag and HCV-Ab were 10.1% and 67.9%, respectively. The prevalence of habitual drinkers was 53.8%, and 21.1% had a BMI > 25 kg/m^2. DM was present in 35.0% of HCC patients (Table 1). The median survival period after initial therapy for HCC was 23 mo.

Factors related to survival after initial therapy for HCC

The factors associated with survival after initial therapy for HCC were investigated in 377 patients. On univariate analysis, the following 11 factors were significantly related to survival: stage of underlying liver disease (non-cirrhosis, Child A *vs* B, C cirrhosis, $P = 0.0001$), T stage of HCC (T1, 2 *vs* 3, 4, $P < 0.0001$), number of HCCs (solitary *vs* multiple, $P < 0.0001$), maximum size of HCCs (< 20 *vs* ≥ 20 mm, $P < 0.0001$), vascular invasion of HCCs (no *vs* yes, $P < 0.0001$), method of therapy (curative: surgical resection, PEIT, and RFA *vs* non-curative: other than surgical resection, PEIT, and RFA, $P = 0.0004$), serum AST (< 63 *vs* ≥ 63 IU/L, $P = 0.0089$), serum AFP (< 41 *vs* ≥ 41 ng/mL, $P < 0.0001$), serum PIVKA-Ⅱ (< 83 *vs* ≥ 83 mAU/mL, $P < 0.0001$), uninterrupted habitual drinking after initial therapy for HCC (no *vs* yes, $P = 0.0135$), and the mean postprandial plasma glucose level after initial therapy for HCC (< 200 *vs* ≥ 200 mg/dL, $P < 0.0001$).

These parameters were entered into the multivariate Cox proportional hazards regression analysis. Stage of underlying liver disease (non-cirrhosis, Child A *vs* B, C cirrhosis, $P = 0.0079$), T stage of HCC (T1, 2 *vs* 3, 4; $P < 0.0001$), and the mean postprandial plasma glucose level after initial therapy for HCC (< 200 *vs* ≥ 200 mg/dL, $P = 0.0008$) were extracted as independent factors affecting survival. However, uninterrupted habitual alcohol consumption was not related to survival (Table 2).

Factors related to period of survival after initial therapy for HCC in patients with stage T1-T3

The survival period is quite short in T4 stage HCC, therefore, T4 stage HCC was excluded to clarify the factors related to survival (Table 3). Univariate analysis of 282 patients with T1-T3 stage HCC identified the following seven factors as related to survival after initial therapy for HCC: stage of underlying liver disease (non-cirrhosis, Child A *vs* B, C, $P = 0.0009$), T stage of HCC (T1 *vs* 2, 3, $P = 0.0023$), number of HCCs (solitary *vs* multiple, $P = 0.0082$), serum AFP (< 41 *vs* ≥ 41 ng/mL,

Table 1 Clinical characteristics of hepatocellular carcinoma patients

Characteristics	Data
Age (yr)	67 ± 9.4
Sex (M:F)	276:101
Stage of liver disease	
Non LC	57 (15.1)
LC	
Child A	213 (56.5)
Child B	86 (22.8)
Child C	21 (5.6)
T stage in the TNM system	
1	101 (26.8)
2	125 (33.2)
3	56 (14.9)
4	95 (25.2)
HBs-Ag-positive	38 (10.1)
HCV-Ab-positive	256 (67.9)
Serum AST (IU/L)	88 ± 119.1
Serum ALT (IU/L)	66 ± 62.9
Serum AFP (ng/mL)	11 623 ± 48 270
Serum PIVKA-Ⅱ (mAU/mL)	15 270 ± 96 781
Habitual alcohol drinkers	203 (53.8)
Obesity (BMI > 25 kg/m^2)	80 (21.1)
Presence of DM	132 (35.0)

M: Male; F: Female; LC: Liver cirrhosis; Child: Child-Pugh classification; HBs-Ag: Surface antigen of the hepatitis B virus; HCV-Ab: Hepatitis C antibody; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; AFP: α-fetoprotein; PIVKA-Ⅱ: Proteins induced by vitamin KAbsense of antagonist-Ⅱ; BMI: Body mass index; DM: Diabetes mellitus; TNM: Tumor, node, metastasis.

Table 2 Univariate analysis of factors related to survival period after diagnosis of stage T1-T4 hepatocellular carcinoma

	HR	95% CI	*P* value
Age (< 69/≥ 69 yr)	0.899	0.587-1.376	0.6232
Sex (M/F)	1.218	0.760-1.952	0.4132
Stage of underlying liver disease (non-cirrhosis, Child-Pugh A/Child-Pugh B, C)	0.537	0.394-0.733	0.0001
T stage in the TNM system (T1,T2/T3, T4)	0.419	0.330-0.531	< 0.0001
Number of HCCs (solitary/multiple)	0.192	0.119-0.309	< 0.0001
Maximum size of HCCs (< 20/≥ 20 mm)	0.403	0.260-0.623	< 0.0001
Vascular invasion of HCCs (no/yes)	0.105	0.051-0.216	< 0.0001
Method of therapy (curative/non curative)[1]	0.432	0.272-0.687	0.0004
HBs-Ag (negative/positive)	1.078	0.538-2.163	0.8317
HCV-Ab (negative/positive)	1.362	0.858-2.162	0.1907
AST (< 63/≥ 63 IU/L)	0.554	0.357-0.861	0.0089
ALT (< 49/≥ 49 IU/L)	0.939	0.609-1.448	0.7750
AFP (< 41/≥ 41 ng/mL)	0.260	0.155-0.439	< 0.0001
PIVKA-Ⅱ (< 83/≥ 83 mAU/mL)	0.200	0.103-0.386	< 0.0001
Habitual alcohol drinkers (yes/no)	1.130	0.740-1.726	0.5722
Uninterrupted alcohol consumption after initial therapy for HCC (no/yes)	0.489	0.278-0.861	0.0135
BMI (≥ 25/< 25 kg/m^2)	0.885	0.530-1.476	0.6398
Presence of DM (yes/no)	1.141	0.731-1.782	0.5623
Mean postprandial plasma glucose level after initial therapy for HCC (< 200/≥ 200 mg/dL)	0.131	0.051-0.336	< 0.0001

[1]Curative therapies are surgical resection, percutaneous ethanol injection therapy, and percutaneous percutaneous radiofrequency ablation. M: Male; F: Female; HCV: Hepatitis C virus; HBs-Ag: Surface antigen of the hepatitis B virus; HCV-Ab: Hepatitis C antibody; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; AFP: α-fetoprotein; PIVKA-Ⅱ: Proteins induced by vitamin KAbsense of antagonist-Ⅱ; BMI: Body mass index; DM: Diabetes mellitus; HR: Hazard ratio.

Table 3 Survival period according to the T stage of hepatocellular carcinoma

T stage of hepatocellular carcinoma	Survival period (mo)
T1	43.3 ± 31.3
T2	39.8 ± 30.8
T3	23.6 ± 17.2
T4	8.7 ± 11.8

P = 0.0007), serum PIVKA-Ⅱ (< 83 *vs* ≥ 83 mAU/mL, *P* = 0.0101), uninterrupted alcohol consumption after initial therapy for HCC (no *vs* yes, *P* = 0.0002), and the mean postprandial plasma glucose level (< 200 *vs* ≥ 200 mg/dL, *P* = 0.0001).

On multivariate regression analysis using a stepwise Cox's hazard model, stage of underlying liver disease (non-cirrhosis, Child A *vs* B, C cirrhosis, *P* = 0.0068), T stage of HCC (T1 *vs* 2, 3, *P* = 0.0108), uninterrupted habitual alcohol consumption (no *vs* yes, *P* = 0.0055), and the mean postprandial plasma glucose level (< 200 *vs* ≥ 200 mg/dL, *P* = 0.0005) were extracted as independent factors related to survival after initial therapy for HCC (Table 4).

Period from initial curative to subsequent therapy for newly emerging HCC in patients without local recurrence

Of the 377 HCC patients, 144 (38.2%) patients did not show local recurrence after initial curative therapy for HCC. Of the 144 HCC patients who did not show local recurrence, 100 (69.4%) died during the observation period. Of the patients who did not show local recurrence, the period from initial curative therapy to subsequent therapy for newly emerging HCC was significantly longer in the postprandial glucose within 200 mg/dL group than the postprandial glucose > 200 mg/dL group (log-rank test, *P* < 0.05), although there was no difference in the period to second therapy between the non-alcohol group and continuation group (Figure 1).

Cause of death in HCC patients with stage T1-T3

A total of 214 T1-T3 stage HCC patients who died during the observation period were classified into four groups: "Group N", patients belonging to both the non-alcohol group and the postprandial glucose within 200 mg/dL group; "Group AL", patients belonging to both the continuation group and the postprandial glucose within 200 mg/dL group; "Group DM", patients belonging to both the daily alcohol < 20 g ethanol and the postprandial glucose > 200 mg/dL group; and "Group AD", patients belonging to both the continuation group and the postprandial glucose > 200 mg/dL group (Table 5). Death caused by other than HCC progression (hepatic failure, gastrointestinal bleeding, ischemic heart disease, cerebral vessel disease, bacterial infection, *etc.*) was significantly more frequent in "Group AL" than in "Group N" (*P* = 0.0016). On the other hand, the cause of death

Table 4 Univariate and multivariate regression analysis of factors related to survival after diagnosis of stage T1-T3 hepatocellular carcinoma

	HR	95% CI	*P* value
Univariate analysis			
Age (< 69/≥ 69 yr)	0.846	0.527-1.356	0.4854
Sex (male/female)	1.148	0.687-1.919	0.5975
Stage of underlying liver disease (non cirrhosis, Child-Pugh A/Child-Pugh B, C)	0.363	0.200-0.658	0.0009
T stage in the TNM system (T1/T2, T3)	0.352	0.181-0.687	0.0023
Number of HCCs (solitary/multiple)	0.397	0.232-0.677	0.0082
Maximum size of HCCs (< 20/≥ 20 mm)	0.833	0.520-1.335	0.4469
Vascular invasion of HCCs (no/yes)	0.382	0.119-1.220	0.1039
Method of therapy (curative/non-curative)[1]	0.770	0.472-1.255	0.2918
HBs-Ag (negative/positive)	1.596	0.696-3.664	0.2686
HCV-Ab (negative/positive)	0.972	0.568-1.664	0.9157
AST (< 63/≥ 63 IU/L)	0.683	0.418-1.115	0.1269
ALT (< 49/≥ 49 IU/L)	0.968	0.596-1.570	0.8935
AFP (< 41/≥ 41 ng/mL)	0.365	0.205-0.649	0.0007
PIVKA-Ⅱ (< 83/≥ 83 mAU/mL)	0.381	0.183-0.792	0.0101
Habitual alcohol drinkers (yes/no)	1.065	0.665-1.704	0.7939
Uninterrupted alcohol consumption after initial therapy for HCC (no/yes)	0.319	0.172-0.589	0.0002
BMI (< 25/≥ 25 kg/m^2)	0.826	0.473-1.441	0.4989
Presence of diabetes mellitus (yes/no)	0.681	0.416-1.115	0.1258
Mean postprandial plasma glucose level after initial therapy for HCC (< 200/≥ 200 mg/dL)	0.143	0.054-0.381	0.0001
Multivariate regression analysis			
A stepwise Cox's hazard model			
Stage of underlying liver disease			
Non-cirrhosis, Child-Pugh A	1.0		
Child-Pugh B, C	0.649	0.476-0.885	0.0068
T stage in the TNM system			
T1		1.0	
T2/T3	0.788	0.653-0.945	0.0108
Uninterrupted alcohol consumption after initial therapy for HCC			
No		1.0	
Yes	0.641	0.469-0.877	0.0055
Mean postprandial plasma glucose level after initial therapy for HCC			
< 200 mg/dL	1.0		
≥ 200 mg/dL	0.502	0.337-0.747	0.0005

[1]Curative therapies are surgical resection, percutaneous ethanol injection therapy, and percutaneous percutaneous radiofrequency ablation. TNM: Tumor, node, metastasis; HCC: Hepatocellular carcinoma; HCV: Hepatitis C virus; HBs-Ag: Surface antigen of the hepatitis B virus; HCV-Ab: Hepatitis C antibody; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; AFP: alpha-fetoprotein; PIVKA-Ⅱ: Proteins induced by vitamin KAbsense of antagonist-Ⅱ; BMI: Body mass index; HR: Hazard ratio.

in "Group DM" was the same as that in "Group N". Similarly, the cause of death was not different between "Group AD" and "Group AL" (Figure 2).

DISCUSSION

In the present study, stage of underlying liver disease, T stage of HCC, and the mean postprandial plasma glucose level after initial therapy for HCC despite standard treatment for DM were extracted as independent factors related to survival after initial therapy in 377 HCC patients who were newly diagnosed and treated according

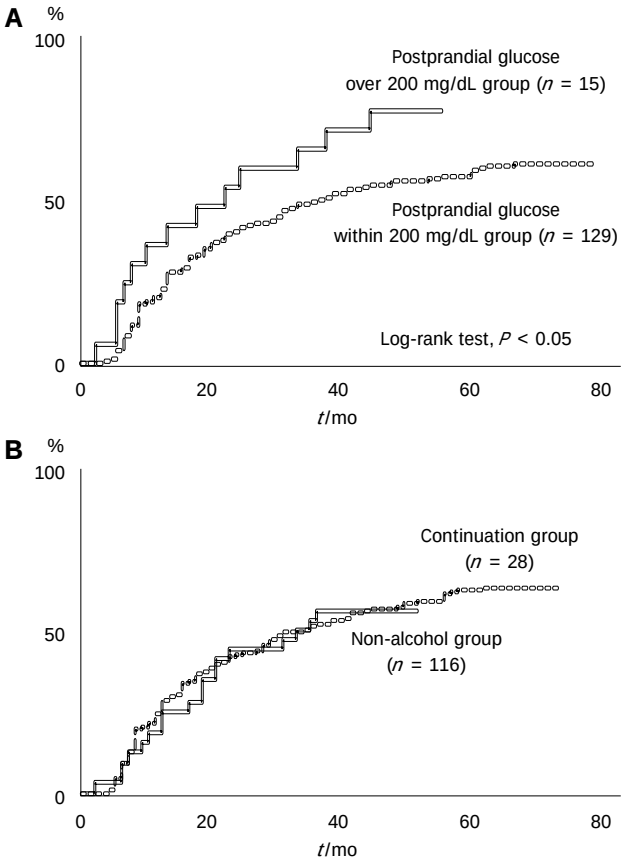


Figure 1 Duration from initial curative therapy for hepatocellular carcinoma to hepatocellular carcinoma recurrence in the different region. A: The period until developing a new lesion in a remote location of the liver was significantly longer in the postprandial glucose within 200 mg/dL group with diabetes mellitus than in the postprandial glucose > 200 mg/dL group without local recurrence after initial therapy for hepatocellular carcinoma (HCC) (log-rank test, $P < 0.05$); B: There was no difference in the period between the non-alcohol group with a history of drinking and the continuation group without local recurrence after initial curative therapy for HCC.

to the Japanese Guidelines for HCC[16]. Then, after T4 stage HCC patients were excluded, uninterrupted excess alcohol consumption was extracted as an independent factor along with the three factors described above.

Alcohol abuse provokes inflammatory and innate immune responses of Kupffer cells due to elevation of gut-derived endotoxin and increases reactive-oxygen-species-induced damage and profibrogenic factors such as acetaldehyde or lipid peroxidation products, which contribute to activation of hepatic stellate cells and progression of liver fibrosis[22]. Therefore, uninterrupted alcohol abuse may induce rapid worsening of hepatic dysfunction. Meanwhile, the presence of DM is related to HCC recurrence after potentially curative therapy in patients with non-B non-C HCC[17]. Furthermore, type 2 DM has consistently been identified as an independent predictor of hepatic fibrosis[10,23,24], faster fibrosis progression[24], and increased mortality[25]. In type 2 DM, insulin resistance may be observed in the majority of patients. Therefore, high levels of circulating insulin in type 2 DM may stimulate the proliferation of hepatic stellate cells and induce production of collagen, resulting in he-

Table 5 Profile of T1-T3 stage hepatocellular carcinoma patients who died during observation *n* (%)

	Group N	Group AL	Group DM	Group AD
Age (yr)	69.1 ± 8.1	68.2 ± 8.8	68.9 ± 6.5	67.4 ± 9.6
Sex (M:F)	88:52	38:1	7:10	1:17
Stage of liver disease				
Non LC	13	6	2	2
LC child				
A	91	23	6	8
B	31	7	9	8
C	5	3	0	0
T stage in the TNM system				
1	54	10	5	4
2	60	18	5	8
3	26	11	7	6
Habitual alcohol drinkers	47 (33.6)	39 (100)	0 (0.0)	18 (100)
Presence of diabetes mellitus	41 (29.3)	8 (20.5)	17 (100)	18 (100)

TNM: Tumor, node, metastasis; M: Male; F: Female; LC: Liver cirrhosis; DM: Diabetes mellitus.

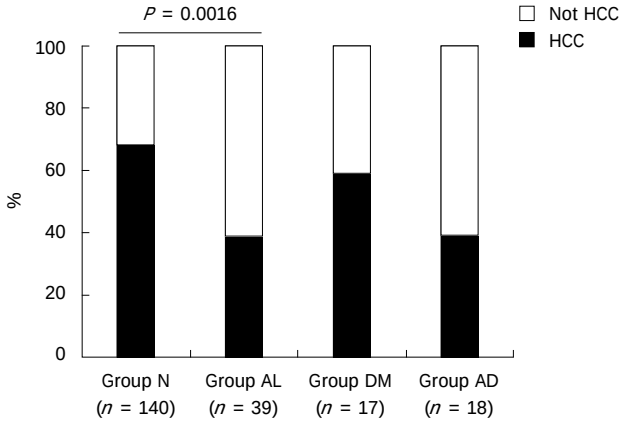


Figure 2 Cause of death in T1-T3 stage hepatocellular carcinoma. The rate of death from causes other than hepatocellular carcinoma (HCC), including hepatic failure, gastrointestinal bleeding, ischemic heart disease, cerebral vessel disease, and bacterial infection, was significantly higher in Group AL than Group N ($P = 0.0016$). The cause of death in Group diabetes mellitus (DM) was similar to that in Group N, and Group AD was similar to Group AL. Group N, patients belonging to both the non-alcohol group and postprandial glucose within 200 mg/dL group; Group AL, patients belonging to both the continuation group and postprandial glucose within 200 mg/dL group; Group DM, patients belonging to both the daily alcohol intake < 20 g ethanol and postprandial glucose > 200 mg/dL group; Group AD, patients belonging to both the continuation group and postprandial glucose > 200 mg/dL group.

patic fibrosis, as well as carcinogenesis of hepatocytes[26]. Therefore, the presence of DM may cause deterioration of hepatic function and carcinogenesis of the liver.

In the present study, the methods of initial therapy for HCC were varied, including surgical resection, PEIT, RFA and TACE. Therefore, selection bias of the therapeutic method could have affected the prognosis of HCC. However, we chose the therapeutic method according to the Japanese Guidelines for HCC[16], which guaranteed the equality of the therapeutic value of the various methods. Thus, it is not difficult to elucidate the factors related to the prognosis of HCC even though patients were treated with different methods, as long as the treatment was performed based on the Japanese Guidelines for HCC[16]. Therefore, patients treated in accordance with these guidelines were included in this study.

Although HCV and/or HBV infection is considered to be a strong carcinogenic factor, it was not extracted as a factor related to the period of survival after initial therapy for HCC. This finding suggested that the prognosis of non-B non-C HCC is no better than that of virus-related HCC, and that the prognosis is influenced by the T stage of HCC or the stage of underlying liver disease rather than the etiology of the liver disease. Non-B non-C HCC would be discovered at an advanced stage[7] compared with virus-related HCC because of the lack of periodic medical assessments[8]. Therefore, detection by means of periodic medical assessments in patients with chronic liver damage, even without HCV and/or HBV infection to diagnose early stage non-B non-C HCC, is crucial for long-term survival. Recently, the prevalence of non-B non-C HCC has been increasing. Thus, along with intensive surveillance of HCC, investigation of factors affecting the prognosis of non-B non-C HCC is urgently needed.

In the present study, the cause of death differed between patients with uninterrupted habitual alcohol consumption and those with higher postprandial plasma glucose levels. The most frequent cause of death in the postprandial glucose > 200 mg/dL group was recurrence of HCC in other locations of the liver, while hepatic failure without HCC recurrence was the most frequent cause of death in the continuation group among habitual alcohol drinkers. However, the rate of recurrence of HCC in other locations of the liver as a cause of death in the postprandial glucose > 200 mg/dL group was similar to that in Group N. These findings suggest that abstinence from alcohol did not affect the prevalence of new HCC but was related to rapid deterioration of hepatic function. Meanwhile, higher postprandial plasma glucose levels are considered to lead to both deterioration of liver function and enhanced carcinogenesis of the liver.

As shown in the period from initial curative therapy to subsequent therapy for HCC in patients without local recurrence, the period was significantly longer in the postprandial glucose within 200 mg/dL group than in the postprandial glucose > 200 mg/dL group. Therefore, the potential for carcinogenesis was considered to be enhanced in the postprandial glucose > 200 mg/dL group. In the postprandial glucose > 200 mg/dL group, the dose of insulin and the use of various oral hypoglycemic drugs, including drugs stimulating insulin secretion, tended to increase. Therefore, a higher level of circulating insulin may have an effect on the emergence of new HCC.

Control of glucose levels after therapy for HCC may be an important factor to improve the prognosis of HCC. However, it is unclear whether increasing the dose of insulin or the use of various drugs for good control of plasma glucose levels improves the prognosis of

HCC. Large prospective studies are needed to establish whether the prognosis of HCC after initial therapy is associated with the postprandial plasma glucose level in itself or other factors related to plasma glucose, including the type of hypoglycemic drug used, because the use of hypoglycemic drugs stimulating insulin secretion may affect the prognosis of HCC[27].

In conclusion, the present study provided clear evidence that uninterrupted alcohol drinking and high postprandial glucose levels after initial therapy for HCC worsen the prognosis of HCC patients. Education about abstinence from alcohol in the group having a history of habitual drinking and achieving good control of plasma glucose in the group having DM may improve the clinical course of HCC.

COMMENTS

Background

There are many factors other than hepatitis C virus or hepatitis B virus identified as risk factors for developing hepatocellular carcinoma (HCC). Among them, diabetes mellitus (DM) and habitual alcohol consumption have been considered significant risk factors for developing HCC. Meanwhile, factors affecting the prognosis after initial therapy for HCC have not been extensively examined, except for HCC tumor stage and underlying liver function.

Research frontiers

In T1-T3 stage HCC patients, uninterrupted alcohol consumption and the mean postprandial plasma glucose level (< 200 *vs* ⩾ 200 mg/dL) after initial therapy for HCC were independent significant factors affecting HCC prognosis. Uninterrupted alcohol consumption was associated with rapid deterioration of hepatic function, while the postprandial plasma glucose level was related to both early development of new HCC and rapid deterioration of hepatic function.

Innovations and breakthroughs

It is known from experience that continuation of alcohol consumption and postprandial plasma glucose levels after initial therapy for HCC greatly affect the prognosis of HCC. To the best of our knowledge, however, there have been few studies that have shown these findings.

Applications

Education about abstinence from alcohol in the group having a history of habitual drinking, and achieving good control of plasma glucose in the group having DM after HCC diagnosis may be critical for improving the clinical course of HCC.

Peer review

The authors investigated whether continuation of alcohol consumption and postprandial plasma glucose levels after initial therapy for HCC were related to the prognosis of HCC. The outcome of the study is interesting and important for the care of HCC patients after initial therapy for HCC.

REFERENCES

1 **Seeff LB**, Hoofnagle JH. Epidemiology of hepatocellular carcinoma in areas of low hepatitis B and hepatitis C endemicity. *Oncogene* 2006; **25**: 3771-3777 [PMID: 16799618 DOI: 10.1038/sj.onc.1209560]

2 **Ikeda K**, Saitoh S, Koida I, Arase Y, Tsubota A, Chayama K, Kumada H, Kawanishi M. A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 patients with viral and alcoholic cirrhosis. *Hepatology* 1993; **18**: 47-53 [PMID: 7686879 DOI: 10.1002/hep.1840180109]

3 **Bruix J**, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodés J. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; **35**: 421-430 [PMID: 11592607 DOI: 10.1016/S0168-8278(01)00130-1]

4 **Ikai I**, Itai Y, Okita K, Omata M, Kojiro M, Kobayashi K, Nakanuma Y, Futagawa S, Makuuchi M, Yamaoka Y. Report of the 15th follow-up survey of primary liver cancer. *Hepatol Res* 2004; **28**: 21-29 [PMID: 14734147 DOI: 10.1016/j.hepres.2003.08.002]

5 **Noguchi K**, Nakashima O, Nakashima Y, Shiota K, Nawata H, Kojiro M. Clinicopathologic study on hepatocellular carcinoma negative for hepatitis B surface antigen and antibody to hepatitis C virus. *Int J Mol Med* 2000; **6**: 661-665 [PMID: 11078825]

6 **Koike Y**, Shiratori Y, Sato S, Obi S, Teratani T, Imamura M, Hamamura K, Imai Y, Yoshida H, Shiina S, Omata M. Risk factors for recurring hepatocellular carcinoma differ according to infected hepatitis virus-an analysis of 236 consecutive patients with a single lesion. *Hepatology* 2000; **32**: 1216-1223 [PMID: 11093727 DOI: 10.1053/jhep.2000.20237]

7 **Yotsuyanagi H**, Shintani Y, Moriya K, Fujie H, Tsutsumi T, Kato T, Nishioka K, Takayama T, Makuuchi M, Iino S, Kimura S, Koike K. Virologic analysis of non-B, non-C hepatocellular carcinoma in Japan: frequent involvement of hepatitis B virus. *J Infect Dis* 2000; **181**: 1920-1928 [PMID: 10837171 DOI: 10.1086/315512]

8 **Abe H**, Yoshizawa K, Kitahara T, Aizawa R, Matsuoka M, Aizawa Y. Etiology of non-B non-C hepatocellular carcinoma in the eastern district of Tokyo. *J Gastroenterol* 2008; **43**: 967-974 [PMID: 19107341 DOI: 10.1007/s00535-008-2264-8]

9 **Horie Y**, Yamagishi Y, Kajihara M, Kato S, Ishii H. National survey of hepatocellular carcinoma in heavy drinkers in Japan. *Alcohol Clin Exp Res* 2003; **27**: 32S-36S [PMID: 12960504 DOI: 10.1097/01.ALC.0000078605.33391.20]

10 **Angulo P**, Keach JC, Batts KP, Lindor KD. Independent predictors of liver fibrosis in patients with nonalcoholic steatohepatitis. *Hepatology* 1999; **30**: 1356-1362 [PMID: 10573511 DOI: 10.1002/hep.510300604]

11 **Lagiou P**, Kuper H, Stuver SO, Tzonou A, Trichopoulos D, Adami HO. Role of diabetes mellitus in the etiology of hepatocellular carcinoma. *J Natl Cancer Inst* 2000; **92**: 1096-1099 [PMID: 10880555 DOI: 10.1093/jnci/92.13.1096]

12 **Fujino Y**, Mizoue T, Tokui N, Yoshimura T. Prospective study of diabetes mellitus and liver cancer in Japan. *Diabetes Metab Res Rev* 2001; **17**: 374-379 [PMID: 11747142 DOI: 10.1002/dmrr.214]

13 **El-Serag HB**, Richardson PA, Everhart JE. The role of diabetes in hepatocellular carcinoma: a case-control study among United States Veterans. *Am J Gastroenterol* 2001; **96**: 2462-2467 [PMID: 11513191 DOI: 10.1111/j.1572-0241.2001.04054.x]

14 **Tazawa J**, Maeda M, Nakagawa M, Ohbayashi H, Kusano F, Yamane M, Sakai Y, Suzuki K. Diabetes mellitus may be associated with hepatocarcinogenesis in patients with chronic hepatitis C. *Dig Dis Sci* 2002; **47**: 710-715 [PMID: 11991597]

15 **Hassan MM**, Hwang LY, Hatten CJ, Swaim M, Li D, Abbruzzese JL, Beasley P, Patt YZ. Risk factors for hepatocellular carcinoma: synergism of alcohol with viral hepatitis and diabetes mellitus. *Hepatology* 2002; **36**: 1206-1213 [PMID: 12395331 DOI: 10.1053/jhep.2002.36780]

16 **Makuuchi M**, Kokudo N, Arii S, Futagawa S, Kaneko S, Kawasaki S, Matsuyama Y, Okazaki M, Okita K, Omata M, Saida Y, Takayama T, Yamaoka Y. Development of evidence-based clinical guidelines for the diagnosis and treatment of hepatocellular carcinoma in Japan. *Hepatol Res* 2008; **38**: 37-51 [PMID: 18039202 DOI: 10.1111/j.1872-034X.2007.00216.x]

17 **Kawamura Y**, Ikeda K, Arase Y, Yatsuji H, Sezaki H, Hosaka T, Akuta N, Kobayashi M, Saitoh S, Suzuki F, Suzuki Y, Kumada H. Diabetes mellitus worsens the recurrence rate after potentially curative therapy in patients with hepatocellular carcinoma associated with nonviral hepatitis. *J Gastroenterol Hepatol* 2008; **23**: 1739-1746 [PMID: 18713301 DOI: 10.1111/j.1440-1746.2008.05436.x]

18 **Kudo M**, Chung H, Haji S, Osaki Y, Oka H, Seki T, Kasugai H, Sasaki Y, Matsunaga T. Validation of a new prognostic staging system for hepatocellular carcinoma: the JIS score compared with the CLIP score. *Hepatology* 2004; **40**: 1396-1405 [PMID: 15565571 DOI: 10.1002/hep.20486]

19 **Fleming ID,** Cooper JS, Henson DE, Hutter RVP, Kennedy BJ, Murphy GP, editors. AJCC cancer staging manual. 5th ed. Philadelphia: Lippincott-Raven, 1997: 225-230

20 **Ikeda K**, Saitoh S, Kobayashi M, Suzuki Y, Tsubota A, Suzuki F, Arase Y, Murashima N, Chayama K, Kumada H. Distinction between chronic hepatitis and liver cirrhosis in patients with hepatitis C virus infection. Practical discriminant function using common laboratory data. *Hepatol Res* 2000; **18**: 252-266 [PMID: 11058829 DOI: 10.1016/S1386-6346(00)00074-7]

21 **Pugh RN**, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973; **60**: 646-649 [PMID: 4541913 DOI: 10.1002/bjs.1800600817]

22 **Cubero FJ**, Urtasun R, Nieto N. Alcohol and liver fibrosis. *Semin Liver Dis* 2009; **29**: 211-221 [PMID: 19387920 DOI: 10.1055/s-0029-1214376]

23 **de Lédinghen V**, Ratziu V, Causse X, Le Bail B, Capron D, Renou C, Pilette C, Oules V, Gelsi E, Oberti F, Vallet-Pichard A, Le Provost N, Cadranel JF. Diagnostic and predictive factors of significant liver fibrosis and minimal lesions in patients with persistent unexplained elevated transaminases. A prospective multicenter study. *J Hepatol* 2006; **45**: 592-599 [PMID: 16837098 DOI: 10.1016/j.jhep.2006.05.008]

24 **Adams LA**, Sanderson S, Lindor KD, Angulo P. The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. *J Hepatol* 2005; **42**: 132-138 [PMID: 15629518 DOI: 10.1016/j.jhep.2004.09.012]

25 **Adams LA**, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, Angulo P. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology* 2005; **129**: 113-121 [PMID: 16012941 DOI: 10.1053/j.gastro.2005.04.014]

26 **Svegliati-Baroni G**, Ridolfi F, Di Sario A, Casini A, Marucci L, Gaggiotti G, Orlandoni P, Macarri G, Perego L, Benedetti A, Folli F. Insulin and insulin-like growth factor-1 stimulate proliferation and type I collagen accumulation by human hepatic stellate cells: differential effects on signal transduction pathways. *Hepatology* 1999; **29**: 1743-1751 [PMID: 10347117 DOI: 10.1002/hep.510290632]

27 **Hassan MM**, Curley SA, Li D, Kaseb A, Davila M, Abdalla EK, Javle M, Moghazy DM, Lozano RD, Abbruzzese JL, Vauthey JN. Association of diabetes duration and diabetes treatment with the risk of hepatocellular carcinoma. *Cancer* 2010; **116**: 1938-1946 [PMID: 20166205 DOI: 10.1002/cncr.24982]

P- Reviewers Kietzmann T, TakumaY **S- Editor** Gou SX
L- Editor Kerr C **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i1.86

World J Gastroenterol 2013 January 7; 19(1): 86-91
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Short and long-term outcomes of endoscopic balloon dilatation for Crohn's disease strictures

Katsuya Endo, Seiichi Takahashi, Hisashi Shiga, Yoichi Kakuta, Yoshitaka Kinouchi, Tooru Shimosegawa

Katsuya Endo, Seiichi Takahashi, Hisashi Shiga, Yoichi Kakuta, Yoshitaka Kinouchi, Tooru Shimosegawa, Division of Gastroenterology, Tohoku University Graduate School of Medicine, Sendai 980-8574, Japan
Author contributions: Endo K designed the research; Endo K, Takahashi S, Shiga H, Kakuta Y, Kinouchi Y and Shimosegawa T performed the research; Endo K analyzed the data; and Endo K wrote the paper.
Correspondence to: Katsuya Endo, MD, Division of Gastroenterology, Tohoku University Graduate School of Medicine, 1-1 Seiryo, Aoba-ku, Sendai 980-8574, Japan. kendo@med.tohoku.ac.jp
Telephone: +81-22-7177171 Fax: +81-22-7177177
Received: August 27, 2012 Revised: October 8, 2012
Accepted: October 30, 2012
Published online: January 7, 2013

Abstract

AIM: To investigate the short and long-term outcomes of endoscopic balloon dilatation (EBD) for Crohn's disease (CD) strictures.

METHODS: Between January 1995 and December 2011, 47 EBD procedures were performed in 30 patients (8 females and 22 males) with CD. All patients had strictures through which an endoscope could not pass, and symptoms of these strictures included abdominal pain, abdominal fullness, nausea, and/or vomiting. The 47 strictures included 17 anastomotic and 30 *de novo* strictures. Endoscopy and dilatation were performed under conscious sedation with intravenous diazepam or flunitrazepam. The dilatations were all performed using through-the-scope balloons with diameters from 8 mm to 20 mm on inflation and lengths of 30-80 mm. Each dilatation session consisted of two to four, 3-min multistep inflations of the balloon, repeated at intervals of 1 wk until adequate dilatation (up to 15-20 mm in diameter) was achieved. The follow-up data were collected from medical records and analyzed retrospectively. Primary success was defined as passage of the scope through the stricture after EBD. Long-term outcomes were analyzed focusing on intervention-free survival and surgery-free survival demonstrated by the Kaplan-Meier method. (Intervention-free meant cases in which neither endoscopic balloon re-dilatation nor surgery was needed after the first dilatation during the observation period). The log rank test was used to evaluate the difference in long-term outcomes between anastomotic and *de novo* stricture cases.

RESULTS: Primary success was achieved in 44 of the 47 strictures (93.6%). Balloon dilatations failed in 3 cases (6.4%). In 1 case, EBD was a technical failure because the guide-wire could not be passed through the stricture which showed severe adhesion and was a flexural lesion of the intestine. In 2 cases, unexpected perforations occurred immediately after balloon dilatation. Of the 47 treatments, complications occurred in 5 (10.6%). All 5 patients had *de novo* strictures. One suffered bleeding, two high fever and there were colorectal perforations. One of the patients with a colorectal perforation was treated surgically, the other was managed conservatively. These 2 cases correspond to the two aforementioned EBD failures. Long-term outcomes were evaluated for the 44 successfully-treated strictures after a median follow-up of 26 mo (range, 2-172 mo). During the observation period, re-strictures after EBDs occurred in 26 cases (60.5%). Fourteen of these 26 re-stricture cases underwent EBD again, but in two EBD failed and surgery was ultimately performed in both cases. Twelve of the 26 re-stricture cases were initially treated surgically when the re-strictures occurred. Finally, 30 of the 47 strictures (63.8%) were successfully managed with EBD, allowing surgery to be avoided. Intervention-free survival evaluated by the Kaplan-Meier method was 75% at 12 mo, 58% at 24 mo, and 43% at 36 mo. There was no significant difference between the anastomotic strictures (n = 16) and *de novo* strictures (n = 28) in the intervention-free survival as evaluated by the log-rank test. Surgery-free survival evaluated by the Kaplan-Meier method was

90% at 12 mo, 75% at 24 mo, and 53% at 36 mo. The 16 anastomotic strictures were associated with significantly better surgery-free survivals than the 28 *de novo* strictures (log-rank test: $P < 0.05$).

CONCLUSION: Anastomotic strictures were associated with better long-term outcomes than *de novo* strictures, indicating that stricture type might be useful for predicting the long-term outcomes of EBD.

© 2013 Baishideng. All rights reserved.

Key words: Crohn's disease; Endoscopic balloon dilatation; Stricture; Anastomotic; *De novo*; Outcome

Endo K, Takahashi S, Shiga H, Kakuta Y, Kinouchi Y, Shimosegawa T. Short and long-term outcomes of endoscopic balloon dilatation for Crohn's disease strictures. *World J Gastroenterol* 2013; 19(1): 86-91 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i1/86.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i1.86

INTRODUCTION

Crohn's disease (CD) is characterized by chronic or recurrent acute inflammation of the intestinal tract resulting in acute inflammatory changes of the intestinal wall, strictures and/or fistulas. Up to one-third of patients develop a stricture within 10 years after the diagnosis[1-3]. These strictures are mainly treated surgically. It is well known that up to 80% of CD patients will eventually require at least one surgical resection within 10 years of being diagnosed[4,5]. Although surgical resection can relieve the symptoms caused by these strictures, the strictures often recur after resection, sometimes necessitating another surgery and producing short bowel syndrome[6,7]. Therefore, surgical resection must be avoided whenever possible.

In recent years, endoscopic balloon dilatation (EBD) has offered a therapeutic alternative to surgery[8-12]. A recent systematic review estimated immediate success rates to be approximately 86%, and that long-term clinical efficacy was achieved in 58% of patients[9]. EBD has been regarded as a safe and effective procedure in the management of CD strictures. Many previous reports have shown short-term success rates of EBD to generally be high (80%-100%), while the long-term outcomes vary among studies. The factors which affect long-term outcomes of EBD have not been sufficiently clarified. A recent report by Mueller *et al*[12] showed that long-segment *de novo* strictures in the terminal ileum had poor long-term responses to EBD, raising the possibility that long-term outcomes of EBD could be affected by the characteristics of strictures. To assess this possibility, we hypothesized that long-term outcomes of EBD might differ between two types of strictures, anastomotic and *de novo*.

The aim of the present study was to evaluate the short- and long-term outcomes of EBD in our hospital and to compare the long-term outcomes of EBD for these two types of strictures affecting CD patients, anastomotic and *de novo* strictures.

MATERIALS AND METHODS

Patients

Between January 1995 and December 2011, 47 EBD procedures were performed in 30 patients (8 females and 22 males) with CD. The ages at dilatation ranged from 18 to 72 years (mean age, 32.5 ± 10.5). The average duration of CD was 12.8 (range, 1-35) years. Characteristics of these patients are summarized in Table 1.

All patients had strictures through which an endoscope could not pass, and symptoms of these strictures included abdominal pain, abdominal fullness, nausea, and/or vomiting.

The 47 strictures included 17 anastomotic and 30 *de novo* strictures. The locations of the anastomotic strictures were 1 at the gastro-jejunal, 5 at the ileo-ileal, 9 at the ileocolonic, and 2 at the ileorectal anastomosis. The locations of *de novo* strictures were 8 at the pyloric ring of the stomach, 1 in the duodenum, 8 in the ileum or at the ileocaecal valve, 7 in the colon, and 6 in the rectum.

Inclusion and exclusion criteria for EBD

EBD were performed on demand only when the patients presented with obstructive symptoms. Prior to endoscopy, all the patients received radiological assessment to confirm the clinically suspected stricture, to exclude intestinal fistulas and angulated strictures and to measure the length of the strictures. Detailed inclusion criteria for EBD were: (1) clinical symptoms of obstruction (abdominal pain, abdominal fullness, nausea, vomiting); (2) evidence of gut stenosis, detected by radiological examination, through which a standard colonoscope or gastrointestinal scope or enteroscope could not be passed; and (3) no or low inflammatory disease activity.

Exclusion criteria were strictures longer than 3 cm and those complicated by intestinal fistula, active ulceration, or abscess.

Dilatation protocol

Informed consent was obtained from all patients before the dilatation procedure. Endoscopy and dilatation were performed under conscious sedation with intravenous diazepam or flunitrazepam. Dilatation was performed using a standard gastrointestinal scope for 10 upper gastrointestinal strictures, a standard colonoscope for 36 lower gastrointestinal strictures, and an entroscope (double-balloon enteroscopy) for 1 ileo-ileal anstomotic stricture. The dilatations were all performed using through-the-scope balloons (Rigiiflex TTS™, or Controlled Radial Expansion Wireguided Balloon Dilatation Catheter™, Boston Scientific, Boston, MA, United States), with diameters from 8 mm to 20 mm on inflation and lengths of 30-80 mm. The balloon was introduced into the stricture using a guide-wire under radiological control. Each dilatation session consisted of two to four, 3-min multistep in-

Table 1 Patients characteristics

Number of patients	*n* = 30
Number of dilatations	47
Gender: male/female	22/8
Age at EBD (mean ± SD), yr	32.5 ± 10.5
Disease duration (mean ± SD), yr	12.8 ± 8.0
Median follow-up	26 mo (range, 2-172 mo)
Medical therapy at first dilatation (%)	
None	0
5-aminosalicylic acid:	86.7
Steroids:	13.3
Azathioprine/6-mercaptoprine:	20.0
Infliximab	13.3
Elemental diet:	33.3
Total parental nutrition:	16.7

EBD: Endoscopic balloon dilatation.

flations of the balloon, repeated at intervals of 1 wk until adequate dilatation (up to 15-20 mm in diameter) was achieved. Passage of the scope through the stricture was attempted in all patients and was used to define primary therapeutic success.

After the successful dilatation, patients were followed in our hospital as out-patients. Repeat dilatations were performed on demand when the patients had obstructive symptoms due to re-strictures confirmed by both radiological assessment and endoscopy.

Statistical analysis

The follow-up data were collected from medical records and analyzed retrospectively.

Primary success was defined as passage of the scope through the stricture after EBD. Long-term outcomes were analyzed focusing on intervention-free survival and surgery-free survival demonstrated by the Kaplan-Meier method. (Intervention-free meant cases in which neither endoscopic balloon re-dilatation nor surgery was needed after the first dilatation during the observation period). The log rank test was used to evaluate the difference in long-term outcomes between anastomotic and *de novo* stricture cases. A two-sided P value of < 0.05 was considered significant.

RESULTS

Rates of primary success

Primary success was achieved in 44 of the 47 strictures (93.6%). Balloon dilatations failed in 3 cases (6.4%). In 1 case, EBD was a technical failure because the guidewire could not be passed through the stricture which showed severe adhesion and was a flexural lesion of the intestine. In 2 cases, unexpected perforations occurred immediately after balloon dilatation.

Complications

Of the 47 treatments, complications occurred in 5 (10.6%). All 5 patients had *de novo* strictures. One suffered bleeding, two high fever and there were colorectal perforations. One of the patients with a colorectal perforation was treated surgically, the other was managed conservatively. These 2 cases correspond to the two aforementioned EBD failures.

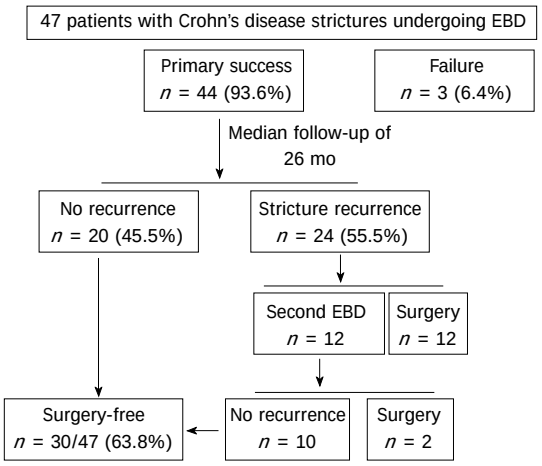


Figure 1 Overview of treatment outcomes for 47 strictures. Long-term outcomes were evaluated for the 44 successfully-treated strictures after a median follow-up of 26 mo (range, 2-172 mo). During the observation period, re-strictures after endoscopic balloon dilatations (EBDs) occurred in 26 cases (60.5%). Fourteen of these 26 re-stricture cases underwent EBD again, but in two EBD failed and surgery was ultimately performed in both cases. Twelve of the 26 re-stricture cases were initially treated surgically when the re-strictures occurred. Finally, 30 of the 47 strictures (63.8%) were successfully managed with EBD, allowing surgery to be avoided.

Long-term outcomes

An overview of the treatment outcomes for the 47 strictures is shown in Figure 1. Long-term outcomes were evaluated for the 44 successfully-treated strictures after a median follow-up of 26 mo (range, 2-172 mo). During the observation period, re-strictures after EBDs occurred in 26 cases (60.5%). Fourteen of these 26 re-stricture cases underwent EBD again, but in two EBD failed and surgery was ultimately performed in both cases. Twelve of the 26 re-stricture cases were initially treated surgically when the re-strictures occurred. Finally, 30 of the 47 strictures (63.8%) were successfully managed with EBD, allowing surgery to be avoided.

Intervention-free survival evaluated by the Kaplan-Meier method was 75% at 12 mo, 58% at 24 mo, and 43% at 36 mo (Figure 2). There was no significant difference between the anastomotic strictures ($n = 16$) and *de novo* strictures ($n = 28$) in the intervention-free survival as evaluated by the log-rank test (data not shown).

Surgery-free survival evaluated by the Kaplan-Meier method was 90% at 12 mo, 75% at 24 mo, and 53% at 36 mo (Figure 2). The 16 anastomotic strictures were associated with significantly better surgery-free survivals than the 28 *de novo* strictures (log-rank test: $P < 0.05$) (Figure 3).

DISCUSSION

EBD has become an alternative to surgery in the treatment of CD strictures in recent years. Many reports have shown good short-term success rates of EBD, while

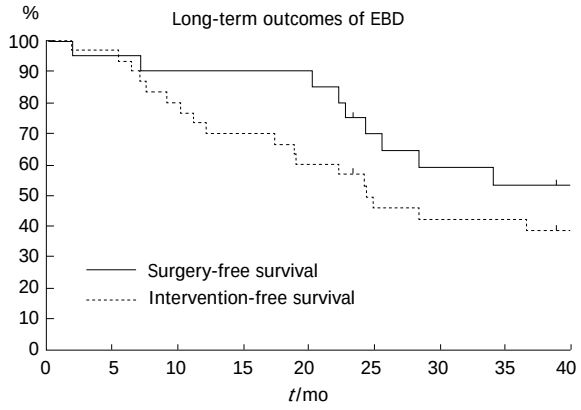


Figure 2 Long-term outcomes of endoscopic balloon dilatation by the Kaplan-Meier method. Intervention-free survival evaluated by the Kaplan-Meier method was 75% at 12 mo, 58% at 24 mo, and 43% at 36 mo. Surgery-free survival evaluated by the Kaplan-Meier method was 90% at 12 mo, 75% at 24 mo, and 53% at 36 mo. EBD: Endoscopic balloon dilatation.

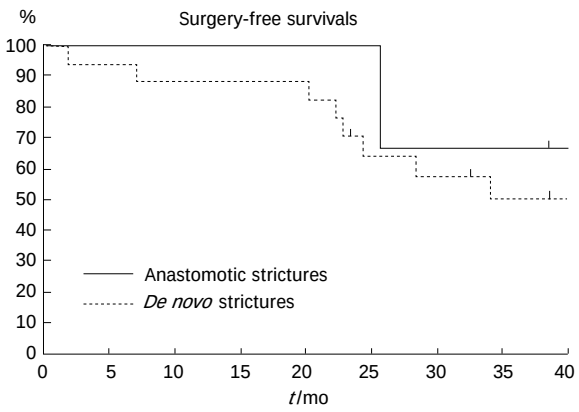


Figure 3 Surgery-free survivals-anastomotic *vs de novo* strictures. The 16 anastomotic strictures were associated with significantly better surgery-free survivals than the 28 *de novo* strictures (log-rank test: $P < 0.05$).

the long-term outcomes have varied among studies. Whether or not the stricture type, such as anastomotic or *de novo*, impacts long-term outcomes of EBD remains an open question. In this study, we evaluated short- and long-term outcomes of EBD for strictures associated with CD, in our hospital, and analyzed differences in long-term outcomes between anastomotic and de novo strictures.

As for short-term outcomes, primary success was achieved in 44 of the 47 strictures treated. EBD failed in only 3 cases. In 1 case, EBD was technically impossible and in 2 others unexpected perforations occurred immediately after EBD. Therefore, the primary success rate was 93.6% (44/47) in our study. The primary success rate was 86% in a systematic review[9] and 97% in a recent single-center cohort study[13]. Our primary success rate was thus similar to those reported previously. An important issue is the type of stricture, i.e., anastomotic versus de novo. In the aforementioned systematic review[9], 68% of the strictures treated by EBD were located at anastomotic sites, as were 84% of those in the recent single-center cohort study[13]. On the other hand, there were several more de novo (n = 28, 63.6%) than anastomotic strictures (n = 16, 36.4%) in our study. Despite the rather large percentage of *de novo* strictures, the primary success rate was very high. Mueller *et al*[12] also reported a primary success rate of 95% despite the majority of their patients presenting with de novo strictures (69%). These findings suggest that short-term outcomes might be not affected by the type of stricture.

In this study, we successfully performed EBD using double-balloon enteroscopy for one patient who had an ileo-ileal anastomtic stricture. In recent years, a few reports described the usefulness and efficacy of EBD for small intestinal strictures in CD patients using double-balloon enteroscopy[11,14]. This procedure may be potentially of significant benefit and should be considered as a useful and effective alternative to surgery. Further investigation should be necessary to confirm the benefit of this procedure.

In this study, 5 patients each experienced one complication (10.6%). All 5 patients had *de novo* strictures. The *de novo* strictures thus appeared to be associated with an increased risk of complications as compared with anastomotic strictures. One patient experienced bleeding (2.1%), 2 had high fever (4.3%), and 2 had colorectal perforations (4.3%). The perforation rate in this study was thus very similar to those in the literature[9,11,12,15-21] which have been in the 0%-10% range. We believe that it is important to perform multistep inflation with a gradually increasing diameter under minimal conscious sedation. This technique may have contributed to the low perforation rate in our study. A more aggressive endoscopic technique, general anesthesia, and using excessively large dilatation balloons are considered to be risk factors for perforation[8,22,23].

As for long-term outcomes, re-strictures after the first EBD occurred in 26 cases (60.5%) out of 44 successfully-treated strictures. Re-strictures did not seem to depend on the diameter of the first dilatation because dilatations were evenly achieved up to 15-20 mm in diameter in most of the successfully-treated strictures. Fourteen of these 26 cases were successfully treated with a second EBD during follow-up for re-strictures and surgery was avoided. Ultimately, 14 cases (31.8%) required surgery to treat re-strictures. These results compare favorably with those of the aforementioned systematic review which described 144 of 347 patients (42%) as ultimately needing surgery to treat re-strictures after EBD. In this study, we also estimated intervention-free and surgery-free survivals. Intervention-free survival was 75% at 12 mo, 58% at 24 mo, and 43% at 36 mo. Surgery-free survival was 90% at 12 mo, 75% at 24 mo, and 53% at 36 mo. These results are also similar to those of previous reports[12,13,22].

Little is known about prognostic factors that affect long-term outcomes after EBD. Smoking has been regarded as a prognostic factor increasing the risk of recurrence[20,24]. Hoffmann *et al*[24] indicated the presence of ulcers in strictures to be associated with poorer long-term outcomes, whereas Thienpont *et al*[13] reported that there

was no association between endoscopic activity, mainly determined by the sizes and number of ulcerations. Thienpont *et al*[13] also reported that increased levels of C-reactive protein did not influence long-term outcomes. As for the characteristics of strictures, Mueller *et al*[12] recently described the length and the location of strictures as affecting long-term outcomes. In their report, the patients who required surgery after EBD had longer strictures than those who did not, and all of the surgical patients had *de novo* strictures in the terminal ileum. In the present comparison of anastomotic and *de novo* strictures, we found the anastomotic type to be associated with significantly better surgery-free survival rates. Both our results and those of Mueller *et al*[12] suggested stricture type (anastomotic or *de novo*) to be useful for predicting long-term outcomes of EBD.

The effects of concurrent or subsequent therapy aimed at increasing the efficacy of EBD remain unclear. Local steroid injection into the strictures after EBD is controversial. Some studies have found this technique to improve outcomes of EBD[17,19,21-23,25,26], and a recent randomized double blind controlled trial showed this technique to effectively reduce both re-dilatation and surgery in pediatric CD patients with strictures[27]. However, opposite results were also obtained in a placebo controlled trial, although the number of patients was limited[28]. Further study is needed to clarify the utility of this technique. Little is known about the efficacy of subsequent administration of medications such as azathioprine or infliximab, and this topic also requires further study.

In summary, we examined short- and long-term effects of EDB for CD strictures. Although the complication rate was relatively low, *de novo* strictures may carry an increased risk of perforation. Our results also showed anastomotic strictures to be associated with better long-term outcomes than *de novo* strictures, indicating that stricture type (anastomotic *vs de novo*) might be useful for predicting the long-term outcomes of EBD.

COMMENTS

Background

In recent years, the efficacy of endoscopic balloon dilatation (EBD) for Crohn's disease (CD) strictures has been confirmed. Although EBD has been regarded as a safe and effective procedure in the management of CD strictures, the factors which affect long-term outcomes of EBD have not been sufficiently clarified.

Research frontiers

The authors hypothesized that outcomes of EBD might differ between two types of strictures, anastomotic and *de novo*. In this study, the authors evaluated the short- and long-term outcomes of EBD in the hospital and compared the outcomes of EBD for these two types of strictures affecting CD patients, anastomotic and *de novo* strictures.

Innovations and breakthroughs

The results showed that patients with anastomotic strictures had better long-term outcomes than those with *de novo* strictures. This is the first report to clarify that types of the strictures affect the long-term outcomes of EBD in CD patients.

Applications

When considering treatment for CD strictures, this study may indicate that the type of the stricture (anastomotic *vs de novo*) might be useful for predicting long-term outcomes of EBD.

Peer review

The study reported that EBD might be useful to treat the stricture of CD. The paper also observed the short and long-term outcomes of EBD for two different strictures in CD. The argument of the paper is new.

REFERENCES

1 **Cosnes J**, Cattan S, Blain A, Beaugerie L, Carbonnel F, Parc R, Gendre JP. Long-term evolution of disease behavior of Crohn's disease. *Inflamm Bowel Dis* 2002; **8**: 244-250 [PMID: 12131607 DOI: 10.1097/00054725-200207000-00002]

2 **Louis E**, Collard A, Oger AF, Degroote E, Aboul Nasr El Yafi FA, Belaiche J. Behaviour of Crohn's disease according to the Vienna classification: changing pattern over the course of the disease. *Gut* 2001; **49**: 777-782 [PMID: 11709511 DOI: 10.1136/gut.49.6.777]

3 **Van Assche G**, Geboes K, Rutgeerts P. Medical therapy for Crohn's disease strictures. *Inflamm Bowel Dis* 2004; **10**: 55-60 [PMID: 15058528 DOI: 10.1097/00054725-200401000-00009]

4 **Bernell O**, Lapidus A, Hellers G. Risk factors for surgery and postoperative recurrence in Crohn's disease. *Ann Surg* 2000; **231**: 38-45 [PMID: 10636100 DOI: 10.1097/00000658-200001000-00006]

5 **Farmer RG**, Whelan G, Fazio VW. Long-term follow-up of patients with Crohn's disease. Relationship between the clinical pattern and prognosis. *Gastroenterology* 1985; **88**: 1818-1825 [PMID: 3922845]

6 **Stebbing JF**, Jewell DP, Kettlewell MG, Mortensen NJ. Recurrence and reoperation after strictureplasty for obstructive Crohn's disease: long-term results [corrected]. *Br J Surg* 1995; **82**: 1471-1474 [PMID: 8535795 DOI: 10.1002/bjs.1800821108]

7 **Rutgeerts P**, Geboes K, Vantrappen G, Kerremans R, Coenegrachts JL, Coremans G. Natural history of recurrent Crohn's disease at the ileocolonic anastomosis after curative surgery. *Gut* 1984; **25**: 665-672 [PMID: 6735250 DOI: 10.1136/gut.25.6.665]

8 **Nomura E**, Takagi S, Kikuchi T, Negoro K, Takahashi S, Kinouchi Y, Hiwatashi N, Shimosegawa T. Efficacy and safety of endoscopic balloon dilation for Crohn's strictures. *Dis Colon Rectum* 2006; **49**: S59-S67 [PMID: 17106817]

9 **Hassan C**, Zullo A, De Francesco V, Ierardi E, Giustini M, Pitidis A, Taggi F, Winn S, Morini S. Systematic review: Endoscopic dilatation in Crohn's disease. *Aliment Pharmacol Ther* 2007; **26**: 1457-1464 [PMID: 17903236 DOI: 10.1111/j.1365-2036.2007.03532.x]

10 **Stienecker K**, Gleichmann D, Neumayer U, Glaser HJ, Tonus C. Long-term results of endoscopic balloon dilatation of lower gastrointestinal tract strictures in Crohn's disease: a prospective study. *World J Gastroenterol* 2009; **15**: 2623-2627 [PMID: 19496192 DOI: 10.3748/wjg.15.2623]

11 **Hirai F**, Beppu T, Sou S, Seki T, Yao K, Matsui T. Endoscopic balloon dilatation using double-balloon endoscopy is a useful and safe treatment for small intestinal strictures in Crohn's disease. *Dig Endosc* 2010; **22**: 200-204 [PMID: 20642609 DOI: 10.1111/j.1443-1661.2010.00984.x]

12 **Mueller T**, Rieder B, Bechtner G, Pfeiffer A. The response of Crohn's strictures to endoscopic balloon dilation. *Aliment Pharmacol Ther* 2010; **31**: 634-639 [PMID: 20047581 DOI: 10.1111/j.1365-2036.2009.04225.x]

13 **Thienpont C**, D'Hoore A, Vermeire S, Demedts I, Bisschops R, Coremans G, Rutgeerts P, Van Assche G. Long-term outcome of endoscopic dilatation in patients with Crohn's disease is not affected by disease activity or medical therapy. *Gut* 2010; **59**: 320-324 [PMID: 19840991]

14 **Despott EJ**, Gupta A, Burling D, Tripoli E, Konieczko K, Hart A, Fraser C. Effective dilation of small-bowel strictures by double-balloon enteroscopy in patients with symptomatic Crohn's disease (with video). *Gastrointest Endosc* 2009; **70**: 1030-1036 [PMID: 19640518 DOI: 10.1016/j.gie.2009.05.005]

15 **Blomberg B**, Rolny P, Järnerot G. Endoscopic treatment of anastomotic strictures in Crohn's disease. *Endoscopy* 1991; **23**: 195-198 [PMID: 1915133 DOI: 10.1055/s-2007-1010654]

16 **Junge U**, Züchner H. [Endoscopic balloon dilatation of symptomatic strictures in Crohn's disease]. *Dtsch Med Wochenschr* 1994; **119**: 1377-1382 [PMID: 7924946]

17 **Ramboer C**, Verhamme M, Dhondt E, Huys S, Van Eygen K, Vermeire L. Endoscopic treatment of stenosis in recurrent Crohn's disease with balloon dilation combined with local corticosteroid injection. *Gastrointest Endosc* 1995; **42**: 252-255 [PMID: 7498692 DOI: 10.1016/S0016-5107(95)70101-X]

18 **Dear KL**, Hunter JO. Colonoscopic hydrostatic balloon dilatation of Crohn's strictures. *J Clin Gastroenterol* 2001; **33**: 315-318 [PMID: 11588547 DOI: 10.1097/00004836-200110000-00012]

19 **Thomas-Gibson S**, Brooker JC, Hayward CM, Shah SG, Williams CB, Saunders BP. Colonoscopic balloon dilation of Crohn's strictures: a review of long-term outcomes. *Eur J Gastroenterol Hepatol* 2003; **15**: 485-488 [PMID: 12702904]

20 **Sabaté JM**, Villarejo J, Bouhnik Y, Allez M, Gornet JM, Vahedi K, Modigliani R, Lémann M. Hydrostatic balloon dilatation of Crohn's strictures. *Aliment Pharmacol Ther* 2003; **18**: 409-413 [PMID: 12940926 DOI: 10.1046/j.1365-2036.2003.01715.x]

21 **Ferlitsch A**, Reinisch W, Püspök A, Dejaco C, Schillinger M, Schöfl R, Pötzi R, Gangl A, Vogelsang H. Safety and efficacy of endoscopic balloon dilation for treatment of Crohn's disease strictures. *Endoscopy* 2006; **38**: 483-487 [PMID: 16767583 DOI: 10.1055/s-2006-924999]

22 **Couckuyt H**, Gevers AM, Coremans G, Hiele M, Rutgeerts P. Efficacy and safety of hydrostatic balloon dilatation of ileocolonic Crohn's strictures: a prospective longterm analysis. *Gut* 1995; **36**: 577-580 [PMID: 7737567 DOI: 10.1136/gut.36.4.577]

23 **Singh VV**, Draganov P, Valentine J. Efficacy and safety of endoscopic balloon dilation of symptomatic upper and lower gastrointestinal Crohn's disease strictures. *J Clin Gastroenterol* 2005; **39**: 284-290 [PMID: 15758621 DOI: 10.1097/01.mcg.0000155128.31208.44]

24 **Hoffmann JC**, Heller F, Faiss S, von Lampe B, Kroesen AJ, Wahnschaffe U, Schulzke JD, Zeitz M, Bojarski C. Through the endoscope balloon dilation of ileocolonic strictures: prognostic factors, complications, and effectiveness. *Int J Colorectal Dis* 2008; **23**: 689-696 [PMID: 18338175 DOI: 10.1007/s00384-008-0461-9]

25 **Matsui T**, Hatakeyama S, Ikeda K, Yao T, Takenaka K, Sakurai T. Long-term outcome of endoscopic balloon dilation in obstructive gastroduodenal Crohn's disease. *Endoscopy* 1997; **29**: 640-645 [PMID: 9360875 DOI: 10.1055/s-2007-1004271]

26 **Brooker JC**, Beckett CG, Saunders BP, Benson MJ. Long-acting steroid injection after endoscopic dilation of anastomotic Crohn's strictures may improve the outcome: a retrospective case series. *Endoscopy* 2003; **35**: 333-337 [PMID: 12664391 DOI: 10.1055/s-2003-38145]

27 **Di Nardo G**, Oliva S, Passariello M, Pallotta N, Civitelli F, Frediani S, Gualdi G, Gandullia P, Mallardo S, Cucchiara S. Intralesional steroid injection after endoscopic balloon dilation in pediatric Crohn's disease with stricture: a prospective, randomized, double-blind, controlled trial. *Gastrointest Endosc* 2010; **72**: 1201-1208 [PMID: 20951986 DOI: 10.1016/j.gie.2010.08.003]

28 **East JE**, Brooker JC, Rutter MD, Saunders BP. A pilot study of intrastricture steroid versus placebo injection after balloon dilatation of Crohn's strictures. *Clin Gastroenterol Hepatol* 2007; **5**: 1065-1069 [PMID: 17627903 DOI: 10.1016/j.cgh.2007.04.013]

P- Reviewers Yu CG, Riccioni ME **S- Editor** Song XX
L- Editor A **E- Editor** Li JY



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i1.92

World J Gastroenterol 2013 January 7; 19(1): 92-96
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Index of convexity: A novel liver function index using Tc-GSA scintigraphy

Kenji Miki, Yuichi Matsui, Masanori Teruya, Michio Kaminishi, Norihiro Kokudo

Kenji Miki, Yuichi Matsui, Masanori Teruya, Michio Kaminishi, Department of Gastrointestinal Surgery, Showa General Hospital, Tokyo187-8510, Japan
Norihiro Kokudo, Hepato-Biliary-Pancreatic Surgery Division, Artificial Organ and Transplantation Division, Department of Surgery, Graduate School of Medicine, University of Tokyo, Tokyo 113-8655, Japan
Author contributions: Miki K designed the study, analyzed and interpreted the data, and wrote the manuscript; Matui Y collected the data; Teruya M, Kaminishi M, and Kokudo N revised the manuscript for important intellectual content pertaining to the study concept, and analysis and interpretation of data.
Correspondence to: Kenji Miki, MD, Department of Gastrointestinal Surgery, Showa General Hospital, 8-1-1 Hanakoganei, Kodaira, Tokyo 187-8510, Japan. miki-kenji@umin.ac.jp
Telephone: +81-42-4610052 Fax: +81-42-4647912
Received: August 19, 2012 Revised: October 20, 2012
Accepted: November 14, 2012
Published online: January 7, 2013

Abstract

AIM: To investigate the clinical usefulness of a newly developed index, the "index of convexity (IOC)", for evaluating liver functional reserve using technetium-99m-diethylenetriaminepentaacetic acid-galactosyl-human serum albumin (Tc-GSA scintigraphy).

METHODS: In total, 349 patients underwent Tc-GSA scintigraphy. Dynamic planner images were obtained, and time activity curves of the liver and heart were generated and analyzed. Our focus was on the convex shape of the liver accumulation curve. We developed a method for evaluating the extent of convexity and calculated an index that we named the IOC. Clearance index and receptor index were also calculated. The correlations between each GSA index with other liver function tests and liver histopathology were evaluated.

RESULTS: Among the 3 indices generated by Tc-GSA, the IOC had the highest correlation with all other liver function tests (indocyanine green R15, albumin, prothrombin time, cholinesterase level, platelet count, and total bilirubin level). IOC can also differentiate between normal liver, chronic hepatitis, and liver cirrhosis with highest *F* ratio among GSA indices as determined by one-way analysis of variance. Receiver operating characteristic analysis demonstrated high diagnostic performance of IOC in the diagnosis of cirrhosis.

CONCLUSION: IOC is a very simple and reliable index for assessing liver functional reserve, which may prove to be useful in combination with the indocyanine green test for preoperative assessment of hepatic resection.

© 2013 Baishideng. All rights reserved.

Key words: Liver functional reserve; Preoperative assessment; Technetium-99m-diethylenetriaminepentaacetic acid-galactosyl-human serum albumin; Liver cirrhosis; Hepatectomy

Miki K, Matsui Y, Teruya M, Kaminishi M, Kokudo N. Index of convexity: A novel liver function index using Tc-GSA scintigraphy. *World J Gastroenterol* 2013; 19(1): 92-96 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i1/92.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i1.92

INTRODUCTION

Preoperative assessment of liver functional reserve is crucial for hepatobiliary surgeons. Technetium-99m-diethylenetriaminepentaacetic acid-galactosyl-human serum albumin (Tc-GSA) is a radiopharmaceutical developed for evaluating liver function[1,2] and has been widely used for preoperative assessments[3,4]. This asialoglycoprotein (ASGP) analogue is specifically taken up by hepatocytes[5], and its rate of accumulation in the liver can be evaluated using a gamma camera[6]. The liver accumulation process can be assessed by generating a time activ-

ity curve (TAC) from dynamic planer images. Several indices of liver function have been reported through the analysis of dynamic planer images[7-11]. Clearance index (HH15) and receptor index (LHL15) have been widely used for almost 20 years[7,8], as they are easy to calculate. On the other hand, R_{max} or R_{total} are calculated using kinetic model analysis[9,10], which is highly complex and therefore not widely used. Index LU15 requires measurement of the radioactivity of the injection syringe[11], and the K_L index requires curve-fitting software[4]. The use of both of these methods is limited. Although HH15 and LHL15 have been widely used for a long time, both indices have several problems[12,13]. HH15 uses only the radioactivity counts of the heart pool, which is very low compared to liver counts. This index can thus be influenced by counts in the liver through scatter effects or body movements. Index LHL15 is directly influenced by the manner in which the heart regions of interest (ROI) is drawn, generating inter-operator or inter-institutional differences[13,14].

To overcome these problems, we focus on the shape of the liver TAC. Liver TACs have a convex shape in patients with good liver function. In contrast, liver TACs increase in a linear manner in patients with poor liver function. We developed a method for evaluating the convexity of the liver TAC and calculated an index that we have named the index of convexity (IOC).

In this study, we evaluated the significance and usefulness of this index for the assessment of the liver functional reserve.

MATERIALS AND METHODS

Patients

This study included 349 patients who received Tc-GSA scintigraphy at the University of Tokyo Hospital or Showa General Hospital between 1998 and 2011. Of these patients, 239 had hepatocellular carcinomas, 34 had metastatic liver tumors, and 39 had cholangiocarcinomas. Others were diagnosed with miscellaneous diseases. Liver histology in 236 patients confirmed a diagnosis of chronic hepatitis (48 patients), normal liver (45 patients), and liver cirrhosis (143 patients).

Tc-GSA scintigraphy

All patients received 3 mg of Tc-GSA (185 MBq) intravenously. After the injection, dynamic images were obtained with the patient in a supine position using a large-field view gamma camera. Digital images were acquired at 30 s intervals for 30 min. TACs were generated by drawing ROI over the whole liver [L(t)] and heart [H(t)] on the digital images.

Conventional parameters

The HH15 and LHL15 were defined as follows: HH15 = H(15)/H(3); LHL15 = L(15)/(L(15) + L(3).

IOC

The convexity of the liver TAC was evaluated by an index defined as follows: ΔABC was drawn over the liver TAC, where A and B were placed on the TAC at the data points corresponding to 3 min and 27 min after injection, respectively. Therefore, the coordinates of A, B, and C were A[3, L(3)], B[27, L(27)], and C[3, L(27)], respectively. D and E were set as the mid points of lines AB and BC, respectively. F was defined as the intersection of line DE and arc AB (Figure 1).

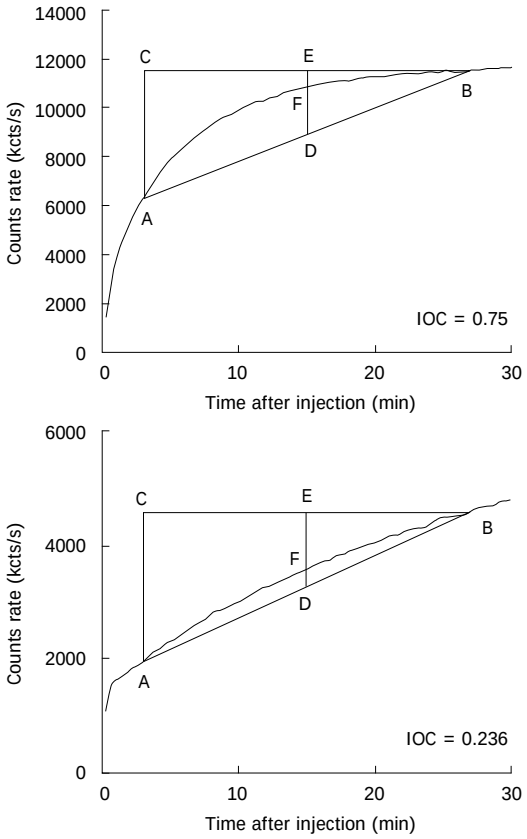


Figure 1 Representative time activity curves of a liver with good function (top) and poor function (bottom). ΔABC was drawn over the liver curve between the data points at 3 min and 27 min. D, E: The midpoints of lines AB and BC, respectively; F: The intersection of arc AB and line DE. The ratio of the length of DF to the length of DE is defined as index of convexity (IOC). High IOC values indicate good liver function.

IOC was defined as the ratio of the length of DF to the length of DE, where it is calculated as follows: IOC = [L(15) × 2 - L(3) - L(27)]/[L(27) - L(3)].

Possible values for this index range from 0 to 1.0. The better the liver function, the higher is the index value (Figure 1).

Other liver function tests

The indocyanine green (ICG) test was carried out, and 2 parameters (ICG R15 and ICG K value) were calculated using the standard methods. Serum albumin level (mg/dL), choline esterase level (IU/L), total bilirubin concentration (mg/dL), platelet counts (× 10^3/mm^3), and prothrombin time (%) were also measured.

Statistical analysis

Correlations between the 3 parameters of the Tc-GSA study (HH15, LHL15 and IOC) and other liver function

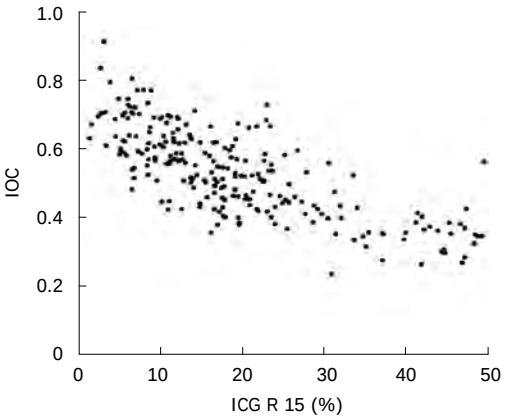


Figure 2 Scatter diagram of index of convexity and indocyanine green retention rate at 15 min. A strong correlation between the 2 parameters is observed (r = -0.761). IOC: Index of convexity; ICG R15: Indocyanine green retention rate at 15 min.

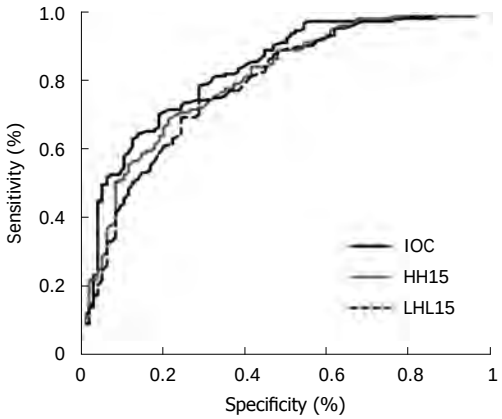


Figure 3 Receiver operating characteristic curves of index of convexity, clearance index, and receptor index for the diagnosis of cirrhosis. IOC: Index of convexity; HH15: Clearance index; LHL15: Receptor index.

Table 1 Spearman's rank correlation coefficients

	IOC	HH15	LHL15	ICG R15
ICG R15	-0.761	-0.666	-0.627	1
Albumin	0.611	0.56	-0.582	-0.538
PT	0.556	0.555	-0.521	-0.549
Platelets	0.607	0.571	-0.572	-0.465
ChE	0.548	0.481	-0.496	-0.481
T. Bil	-0.552	-0.508	0.51	0.53

ICG R15: Indocyanine green retention rate at 15 min; PT: Prothrombin time; ChE: Choline esterase level; T. Bil: Total bilirubin concentration; IOC: Index of convexity; HH15: Clearance index; LHL15: Receptor index.

Table 2 Comparison of each index by liver histology

Index	Patient group (mean ± SD)			*F* ratio[1]	*P* value[2]
	Normal liver	Chronic hepatitis	Liver cirrhosis		
IOC	0.64 ± 0.09	0.60 ± 0.12	0.46 ± 0.12	57.8	< 0.0001
HH15	0.53 ± 0.08	0.57 ± 0.09	0.66 ± 0.10	48.2	< 0.0001
LHL15	0.94 ± 0.02	0.93 ± 0.03	0.89 ± 0.08	21.7	< 0.0001
ICG R15	11.7 ± 8.8	14.3 ± 8.3	23.7 ± 13.8	20.3	< 0.0001

[1]*F* ratio obtained by one way analysis of variance (ANOVA); [2]Probability value for the differences among the 3 groups, obtained by one-way ANOVA. IOC: Index of Convexity; ICG R15: Indocyanine green retention rate at 15 min; HH15: Clearance index; LHL15: Receptor index.

tests were estimated as Spearman's rank correlations. The differences in each Tc-GSA parameter between the 3 groups (normal liver, chronic hepatitis, and liver cirrhosis) were evaluated by one-way analysis of variance (ANOVA). The diagnostic performance for the diagnosis of cirrhosis was evaluated by receiver operating characteristic (ROC) analysis.

RESULTS

Spearman's rank correlation coefficients are listed in Table 1. IOC showed higher correlations than the other conventional indices of HH15 and LHL15. In particular, the IOC has a strong correlation to ICG R15 values compared to HH15 or LHL15 (Figure 2). The correlation coefficient between IOC and ICG R15 was statistically higher than that between HH15 and ICG R15 and that between LHL15 and ICG R15.

The results of one-way ANOVA also showed that IOC was significantly different between background liver groups with the highest *F* ratio (Table 2).

We performed ROC analysis to evaluate the diagnostic performance of IOC in diagnosing cirrhosis (Figure 3). The area under the curve (AUC) of ROC curves for IOC, HH15 and LHL15 were 0.851, 0.801 and 0.821, respectively. However, these differences were not statistically significant.

DISCUSSION

In this study, we developed a novel index to evaluate liver function using the dynamic images of Tc-GSA scintigraphy. This index is very simple to calculate and has stronger correlations to other liver function tests than do conventional indices such as HH15 or LHL15. This index also has a high diagnostic performance rating for diagnosing liver cirrhosis. IOC would thus be very useful to hepatobiliary surgeons in assessing preoperative liver function.

Tc-GSA is an ASGP analogue labeled with a radioisotope and developed for the assessment of liver functional reserve. Tc-GSA scintigraphy for assessing liver function has several benefits to consider. First, accumulation of Tc-GSA in the liver can be monitored continuously through dynamic planer images. The kinetics of ASGP have been investigated in detail in animals and humans[10,15,16], and several kinetic parameters have been proposed as candidates for indices of liver function[6,9,10]. Second, regional accumulation of Tc-GSA in the liver was measured separately to evaluate regional liver function *via* single photon emission computed tomography images[17-19], which can be used for the assessment of regional liver function after portal vein embolization[20,21]. Although many parameters of Tc-GSA scintigraphy have been reported, only HH15 and LHL15 are used widely.

Despite their widespread use, we found that both of these indices showed only moderate correlations to other liver functions, and we encountered some cases with a discrepancy between the values of both indices and their liver histology or liver functions[12], Since the TAC of the heart is influenced by background radiation or radioactivity in the liver adjacent to the heart, indicated Tc-GSA concentrations may deviate from the actual blood concentrations[22]. HH15, using only TAC of the heart and ignoring liver accumulation, is not suitable for the ideal index. LHL15 has been shown to be directly influenced by the manner in which the ROI is drawn over the heart[13,14]. The LHL15 index value was also found to be influenced by the shape of the liver and the size of the heart. These observations suggest that HH15 and LHL15 indices are prone to several measurement errors.

To overcome these problems, we focused on the shape of the liver accumulation curve. The shape of the liver accumulation curve seems universally consistent, even if the patients are stout or their liver is deformed.

The convexity of the liver curve seems to be a specific feature of good liver function. The liver curve increased rapidly and afterwards reaches a plateau in the case of good liver function. In contrast, the liver curve increases linearly throughout the examination period, with no plateau, in patients with poor liver function. In order to evaluate the convexity of the liver curve, we calculated the ratio of the length of line DF to line DE as described in Figure 1. IOC decreases as liver function deteriorates. This index correlated very strongly to other liver function tests, including ICG. IOC can be calculated quite easily as calculations require liver radioactivity data at only 3 time points. Results of one-way ANOVA indicate that IOC has a higher correlation to liver histology than does HH15 or LHL15. In fact, IOC seemed to be superior to HH15 or LHL15 in the diagnosis of liver cirrhosis as indicated by ROC analysis, although the differences between the AUC of ROC curves for IOC, HH15, and LHL15 were not statistically significant.

Makuuchi *et al* proposed that the criteria determining the extent of liver resection include ascites volume, bilirubin levels, and ICG tests[23]. Since then, the ICG test has been widely used in Japan for the preoperative assessment of liver functional reserve. It is well known that hyperbilirubinemia and large port-systemic shunts interfere with the results of ICG tests[24,25]. In addition, some patients are intolerant of ICG[26]. We have encountered many cases with large discrepancies between ICG results and conventional liver function tests. In these cases, Tc-GSA scintigraphy plays a complementary role in deciding on surgical procedures. In this study, IOC showed a stronger correlation with conventional liver function tests than did the ICG test. IOC is a simple and reliable index that is superior to HH15 and LHL15 indices and is a valuable parameter exceeding ICG tests in the preoperative assessment of liver functional reserve. We analyzed the Tc-GSA data retrospectively in this study. In order to prove the usefulness of this index, further prospective studies should be undertaken.

COMMENTS

Background

Technetium-99m-diethylenetriaminepentaacetic acid-galactosyl-human serum albumin (Tc-GSA) was a radiopharmaceutical developed in Japan to assess liver functional reserve. The liver uptake of Tc-GSA can be evaluated from dynamic images obtained using a gamma camera. Among the indices for the Tc-GSA scintigraphic analysis of liver functional reserve, only the receptor index (LHL15) and clearance indices (HH15) have been widely used. However, both indices are prone to measurement errors. To date, no appropriate methods have been developed that are superior to LHL15 and HH15 in terms of reliability and accuracy in the preoperative assessment of liver functional reserve.

Research frontiers

Several methods of evaluating liver function using Tc-GSA scintigraphy have been reported. In particular, methods using kinetic model analysis are highly complex, and another method requires radioactivity measurements of the injection syringe. Therefore, to overcome the limitations of these methods and increase the use of Tc-GSA scintigraphy for the assessment of hepatobiliary surgical outcomes, it is necessary to develop a simple but reliable index that is superior to LHL15 and HH15.

Innovations and breakthroughs

The authors focused on the shape of the liver time activity curve of Tc-GSA and found that the extent of the convexity correlated with liver function, Developing a new index, the index of convexity (IOC), that is very easy to calculate. The authors demonstrated that this index predicts liver functional reserve better than the conventional LHL15 and HH15 indices.

Applications

The IOC is a very simple but reliable index for assessing liver functional reserve, which is, in turn, very useful for preoperative assessment of hepatic resection. Therefore, it can be introduced in any institute without the need for special software.

Terminology

Tc-GSA is a radiopharmaceutical developed for evaluating liver function. This asialoglycoprotein analogue is specifically absorbed by hepatocytes, and its rate of accumulation in the liver is influenced by the liver functional reserve, which can be evaluated using a gamma camera.

Peer review

This is a very interesting and well-performed study evaluating the usefulness of a novel index of liver functional reserve based on dynamic Tc-GSA scintigraphic images. The authors developed a new method for analyzing the results of Tc-GSA scintigraphy based on the shape of the liver TAC and introduced the "IOC". They found that IOC had a strong correlation with conventional liver function test results and a good performance in the diagnosis of cirrhosis. The interesting conclusions of the study were that IOC is a simple but reliable index for assessing liver functional reserve and that it would be useful in deciding on surgical procedures. The study is well designed and written, with clinically relevant conclusions and appropriate data supporting the results.

REFERENCES

1 **Torizuka K**, Ha-Kawa SK, Ikekubo K, Suga Y, Tanaka Y, Hino M, Ito H, Yamamoto K, Yonekura Y. [Phase I clinical study on 99mTc-GSA, a new agent for functional imaging of the liver]. *Kaku Igaku* 1991; **28**: 1321-1331 [PMID: 1770647]

2 **Torizuka K**, Ha-Kawa SK, Kudo M, Kitagawa S, Kubota Y, Tanaka Y, Hino M, Ikekubo K. [Phase II clinical study on 99mTc-GSA, a new agent for functional imaging of the liver]. *Kaku Igaku* 1992; **29**: 85-95 [PMID: 1578824]

3 **Kwon AH**, Ha-Kawa SK, Uetsuji S, Kamiyama Y, Tanaka Y. Use of technetium 99m diethylenetriamine-pentaacetic acid-galactosyl-human serum albumin liver scintigraphy in the evaluation of preoperative and postoperative hepatic functional reserve for hepatectomy. *Surgery* 1995; **117**: 429-434 [PMID: 7716725 DOI: 10.1016/S0039-6060(05)80063-7]

4 **Mitsumori A**, Nagaya I, Kimoto S, Akaki S, Togami I, Takeda Y, Joja I, Hiraki Y. Preoperative evaluation of hepatic functional reserve following hepatectomy by technetium-99m galactosyl human serum albumin liver scintigraphy and

computed tomography. *Eur J Nucl Med* 1998; **25**: 1377-1382 [PMID: 9818276 DOI: 10.1007/s002590050311]
5 **Morell AG**, Gregoriadis G, Scheinberg IH, Hickman J, Ashwell G. The role of sialic acid in determining the survival of glycoproteins in the circulation. *J Biol Chem* 1971; **246**: 1461-1467 [PMID: 5545089]
6 **Stadalnik RC**, Vera DR, Woodle ES, Trudeau WL, Porter BA, Ward RE, Krohn KA, O'Grady LF. Technetium-99m NGA functional hepatic imaging: preliminary clinical experience. *J Nucl Med* 1985; **26**: 1233-1242 [PMID: 2997417]
7 **Kudo M**, Todo A, Ikekubo K, Hino M, Yonekura Y, Yamamoto K, Torizuka K. Functional hepatic imaging with receptor-binding radiopharmaceutical: clinical potential as a measure of functioning hepatocyte mass. *Gastroenterol Jpn* 1991; **26**: 734-741 [PMID: 1662653]
8 **Kudo M**, Todo A, Ikekubo K, Hino M. Receptor index via hepatic asialoglycoprotein receptor imaging: correlation with chronic hepatocellular damage. *Am J Gastroenterol* 1992; **87**: 865-870 [PMID: 1615940]
9 **Ha-Kawa SK**, Tanaka Y. A quantitative model of technetium-99m-DTPA-galactosyl-HSA for the assessment of hepatic blood flow and hepatic binding receptor. *J Nucl Med* 1991; **32**: 2233-2240 [PMID: 1744708]
10 **Miki K**, Kubota K, Kokudo N, Inoue Y, Bandai Y, Makuuchi M. Asialoglycoprotein receptor and hepatic blood flow using technetium-99m-DTPA-galactosyl human serum albumin. *J Nucl Med* 1997; **38**: 1798-1807 [PMID: 9374359]
11 **Koizumi K**, Uchiyama G, Arai T, Ainoda T, Yoda Y. A new liver functional study using Tc-99m DTPA-galactosyl human serum albumin: evaluation of the validity of several functional parameters. *Ann Nucl Med* 1992; **6**: 83-87 [PMID: 1320389]
12 **Kokudo N**, Vera DR, Makuuchi M. Clinical application of TcGSA. *Nucl Med Biol* 2003; **30**: 845-849 [PMID: 14698788]
13 **Nakajima K**, Kinuya K, Mizutani Y, Hwang EH, Michigishi T, Tonami N, Kobayashi K. Simple scintigraphic parameters with Tc-99m galactosyl human serum albumin for clinical staging of chronic hepatocellular dysfunction. *Ann Nucl Med* 1999; **13**: 5-11 [PMID: 10202942]
14 **Koizumi M**, Yamada Y, Nomura E, Takiguchi T, Kokudo N. An easy and reproducible semi-automatic method for the evaluation of 99mTc-galactosyl human serum albumin. *Ann Nucl Med* 1997; **11**: 345-348 [PMID: 9460529]
15 **Schwartz AL**, Fridovich SE, Lodish HF. Kinetics of internalization and recycling of the asialoglycoprotein receptor in a hepatoma cell line. *J Biol Chem* 1982; **257**: 4230-4237 [PMID: 6279629]
16 **Sawamura T**, Nakada H, Hazama H, Shiozaki Y, Sameshima Y, Tashiro Y. Hyperasialoglycoproteinemia in patients with chronic liver diseases and/or liver cell carcinoma. Asialoglycoprotein receptor in cirrhosis and liver cell carcinoma. *Gastroenterology* 1984; **87**: 1217-1221 [PMID: 6092193]
17 **Matsuzaki S**, Onda M, Tajiri T, Kim DY. Hepatic lobar differences in progression of chronic liver disease: correlation of asialoglycoprotein scintigraphy and hepatic functional reserve. *Hepatology* 1997; **25**: 828-832 [PMID: 9096583 DOI: 10.1002/hep.510250407]
18 **Akaki S**, Mitsumori A, Kanazawa S, Togami I, Takeda Y, Hiraki Y. Lobar decrease in 99mTc-GSA accumulation in hilar cholangiocarcinoma. *J Nucl Med* 1999; **40**: 394-398 [PMID: 10086701]
19 **Hwang EH**, Taki J, Shuke N, Nakajima K, Kinuya S, Konishi S, Michigishi T, Aburano T, Tonami N. Preoperative assessment of residual hepatic functional reserve using 99mTc-DTPA-galactosyl-human serum albumin dynamic SPECT. *J Nucl Med* 1999; **40**: 1644-1651 [PMID: 10520704]
20 **Sugai Y**, Komatani A, Hosoya T, Yamaguchi K. Response to percutaneous transhepatic portal embolization: new proposed parameters by 99mTc-GSA SPECT and their usefulness in prognostic estimation after hepatectomy. *J Nucl Med* 2000; **41**: 421-425 [PMID: 10716313]
21 **Beppu T**, Hayashi H, Okabe H, Masuda T, Mima K, Otao R, Chikamoto A, Doi K, Ishiko T, Takamori H, Yoshida M, Shiraishi S, Yamashita Y, Baba H. Liver functional volumetry for portal vein embolization using a newly developed 99mTc-galactosyl human serum albumin scintigraphy SPECT-computed tomography fusion system. *J Gastroenterol* 2011; **46**: 938-943 [PMID: 21523415 DOI: 10.1007/s00535-011-0406-x]
22 **Ha-Kawa SK**, Kojima M, Suga Y, Kurokawa H, Itagaki Y, Tanaka Y. [Dose estimation of Tc-99m-DTPA-galactosyl-human serum albumin (Tc-99m-GSA) in the blood with non-linear regression method]. *Kaku Igaku* 1991; **28**: 425-428 [PMID: 1880977]
23 **Miyagawa S**, Makuuchi M, Kawasaki S, Kakazu T. Criteria for safe hepatic resection. *Am J Surg* 1995; **169**: 589-594 [PMID: 7771622 DOI: 10.1016/S0002-9610(99)80227-X]
24 **Stockmann M**, Malinowski M, Lock JF, Seehofer D, Neuhaus P. Factors influencing the indocyanine green (ICG) test: additional impact of acute cholestasis. *Hepatogastroenterology* 2009; **56**: 734-738 [PMID: 19621693]
25 **Miyamoto Y**, Oho K, Kumamoto M, Toyonaga A, Sata M. Balloon-occluded retrograde transvenous obliteration improves liver function in patients with cirrhosis and portal hypertension. *J Gastroenterol Hepatol* 2003; **18**: 934-942 [PMID: 12859723 DOI: 10.1046/j.1440-1746.2003.03087.x]
26 **Okuda K**, Ohkubo H, Musha H, Kotoda K, Abe H, Tanikawa K. Marked delay in indocyanine green plasma clearance with a near-normal bromosulphophthalein retention test: a constitutional abnormality? *Gut* 1976; **17**: 588-594 [PMID: 976796 DOI: 10.1136/gut.17.8.588]

P- Reviewers Zocco MA, Cheng KS, Tandon RK
S- Editor Jiang L **L- Editor** A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i1.97

World J Gastroenterol 2013 January 7; 19(1): 97-102
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Helicobacter pylori seropositivity in diabetic patients is associated with microalbuminuria

Goh Eun Chung, Nam Ju Heo, Min Jung Park, Su Jin Chung, Hae Yeon Kang, Seung Joo Kang

Goh Eun Chung, Nam Ju Heo, Min Jung Park, Su Jin Chung, Hae Yeon Kang, Seung Joo Kang, Department of Internal Medicine, Healthcare Research Institute, Seoul National University Hospital Healthcare System Gangnam Center, Seoul 135-984, South Korea

Author contributions: Chung GE, Heo NJ and Park MJ designed the research; Chung SJ and Kang HY contributed analytic tools; Kang SJ and Heo NJ analyzed the data; Chung GE and Chung SJ wrote the paper.

Correspondence to: Min Jung Park, MD, PhD, Department of Internal Medicine, Healthcare Research Institute, Seoul National University Hospital Healthcare System Gangnam Center, 39FL, Gangnam Finance Center 737, Yeoksam-Dong, Gangnam-Gu, Seoul 135-984, South Korea. gipmj@snuh.org
Telephone: +82-2-21125641 Fax: +82-2-21125635
Received: June 21, 2012 Revised: September 17, 2012
Accepted: September 22, 2012
Published online: January 7, 2013

Abstract

AIM: To investigate the relationship between *Helicobacter pylori* (*H. pylori*) seropositivity and the presence of microalbuminuria.

METHODS: Between December 2003 and February 2010, asymptomatic individuals who visited the Seoul National University Healthcare System Gangnam Center for a routine check-up and underwent tests for *H. pylori* immunoglobulin G antibodies and urinary albumin to creatinine ratio (UACR) were included. All study subjects completed a structured questionnaire, anthropometric measurements and laboratory tests. Anti-*H. pylori* immunoglobulin G was identified using an enzyme-linked immunosorbent assay kit. A random single-void urine sample, collected using a clean-catch technique, was obtained to determine the UACR. The presence of microalbuminuria was defined as a UACR from 30 to 300 μg/mg. The presence of diabetes mellitus (DM) was defined as either a fasting serum glucose level greater than or equal to 126 mg/dL or taking anti-diabetic medication. Multiple logistic regression analysis was performed to identify the risk factors. The dependent variable was microalbuminuria, and the independent variables were the other study variables.

RESULTS: A total of 2716 subjects (male, 71.8%; mean age, 54.9 years) were included. Among them, 224 subjects (8.2%) had microalbuminuria and 324 subjects (11.9%) had been diagnosed with DM. Subjects with microalbuminuria had a significantly higher *H. pylori* seropositivity rate than subjects without microalbuminuria (60.7% *vs* 52.8%, $P = 0.024$). Multivariate analysis after adjustment for age, body mass index (BMI), waist circumference, and glucose and triglyceride levels showed that *H. pylori* seropositivity was significantly associated with microalbuminuria [odds ratio (OR), 1.40, 95% CI, 1.05-1.89, $P = 0.024$]. After the data were stratified into cohorts by glucose levels (⩽ 100 mg/dL, 100 mg/dL < glucose < 126 mg/dL, and ⩾ 126 mg/dL or history of DM), *H. pylori* seropositivity was found to be significantly associated with microalbuminuria in diabetic subjects after adjusting for age, BMI and serum creatinine level (OR, 2.21, 95% CI, 1.20-4.08, $P = 0.011$). In addition, the subjects were divided into five groups. Those without microalbuminuria (an UACR of < 30 μg/mg) were divided into four groups in accordance with their UACR values, and subjects with microalbuminuria comprised their own group. Notably, *H. pylori* seropositivity gradually increased with an increase in UACR ($P = 0.001$) and was highest in subjects with microalbuminuria (OR, 2.41, 95% CI, 1.14-5.11). This suggests that *H. pylori* seropositivity is positively associated with microalbuminuria in diabetic subjects.

CONCLUSION: *H. pylori* seropositivity was independently associated with microalbuminuria, and the prevalence of *H. pylori* seropositivity was associated with the severity of UACR in diabetic subjects.

© 2013 Baishideng. All rights reserved.

Key words: *Helicobacter pylori*; Seropositivity; Microalbuminuria; Atherosclerosis; Diabetes

Chung GE, Heo NJ, Park MJ, Chung SJ, Kang HY, Kang SJ. *Helicobacter pylori* seropositivity in diabetic patients is associated with microalbuminuria. *World J Gastroenterol* 2013; 19(1): 97-102 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i1/97.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i1.97

INTRODUCTION

Helicobacter pylori (*H. pylori*) infection has been implicated in various extragastric conditions, including coronary heart disease and ischemic cerebrovascular disorder caused by predisposing chronic inflammation and atherosclerosis[1-5]. The association between *H. pylori* seropositivity and coronary artery calcium scores was recently reported[6]. Moreover, *H. pylori* infection was found to be associated with reduced high density lipoprotein and elevated low density lipoprotein levels in serum[7,8]. Furthermore, a prospective single-center study showed that *H. pylori* eradication had beneficial effects on insulin resistance, lipid abnormalities and low-grade inflammation[9]. These findings suggest a role for *H. pylori* infection in subjects with metabolic syndrome, including diabetes.

In contrast, microalbuminuria, which is defined as an increased urinary albumin to creatinine ratio (UACR) of 30-300 μg/mg[10], has been known to be a strong predictor of the development of diabetic nephropathy[11]. It has also been demonstrated that microalbuminuria is a risk factor for cardiovascular disease in the general and diabetic populations[12-14]. Although the mechanism linking microalbuminuria to cardiovascular morbidity remains unclear, one possible explanation is that the increased urinary leakage of albumin reflects vascular damage, i.e., endothelial dysfunction or low-grade chronic inflammation[15]. In addition, some studies have reported a relationship between microalbuminuria and metabolic syndrome, suggesting that insulin resistance underlies the pathogenesis of microalbuminuria[16-18].

Our hypothesis was that if *H. pylori* infection is involved in the pathogenesis of atherosclerosis, a significant association might exist between *H. pylori* infection and microalbuminuria, which is an early marker of atherosclerosis. Therefore, we aimed to investigate the relationship between *H. pylori* infection and microalbuminuria in subjects presenting for a routine health check-up.

MATERIALS AND METHODS

Study population

This cross-sectional study was conducted at the Seoul National University Hospital Gangnam Healthcare Center between March 2003 and February 2010. During the study period, a total of 2737 asymptomatic individuals visited our center for a routine check-up, including *H. pylori* serology and UACR tests. Among them, 21 subjects who showed macroalbuminuria exceeding 300 μg/mg were excluded. Accordingly, 2716 individuals comprised the study population. The presence of diabetes mellitus (DM) was defined as either a fasting serum glucose level greater than or equal to 126 mg/dL or taking anti-diabetic medication. This study was approved by the Institutional Review Board of Seoul National University Hospital, which waived the requirement for informed consent.

Clinical and laboratory assessments

All study subjects completed a structured questionnaire and underwent anthropometric measurements and laboratory tests. A current smoker was defined as a subject who had smoked 100 or more cigarettes during his lifetime and smoked daily at the time of the examination[19]. Height and body weight were measured using a digital scale, and body mass index (BMI) was calculated as follows: BMI = body weight (kg)/height squared (m^2). Systolic and diastolic blood pressures were measured in a sitting position after a 5-min rest, twice a day; mean values were used in the analysis. Blood samples were collected after at least a 12 h of fasting and used to determine glucose, triglyceride, high density lipoprotein cholesterol, and creatinine levels. Serum creatinine concentrations were determined using the Jaffe rate reaction. Anti-*H. pylori* immunoglobulin G was identified using an enzyme-linked immunosorbent assay kit (*H. pylori*-EIA-Well, Radim, Italy) and an automatic analyzer, Alisei® (Seac, Italy). The cut-off values were set according to the manufacturer's instructions.

A random single-void urine sample using a clean-catch technique after at least 12 h of fasting was obtained to determine the UACR (μg/mg). Urinary albumin excretion was measured using an immunoturbidimetric assay, and urinary creatinine was measured using the Jaffe rate reaction. Microalbuminuria was defined as a UACR from 30 μg/mg to 300 μg/mg.

Statistical analysis

Analyses were performed using the SPSS statistical package (Version 17.0, SPSS, Inc., Chicago, IL, United States). A Pearson χ^2 test was used to examine associations between microalbuminuria and the study variables, and P values of < 0.05 were considered statistically significant. Multiple logistic regression analysis was performed to identify risk factors. The dependent variable was microalbuminuria, and the independent variables were the other study variables. Odds ratios (OR) and the relevant 95% CI are presented for all of the potential risk factors.

RESULTS

Clinical characteristics of the study population

The mean age of the 2716 study subjects was 54.9 years, and 71.8% were men. Of the subjects, 224 (8.2%) had

Table 1 Baseline characteristics

	Normal (n = 2492)	Microalbuminuria (n = 224)	P value
Male	1 781 (71.5)	170 (75.9)	0.159
Age[1] (yr)	54.6 (9.1)	57.7 (10.5)	< 0.001
BMI[1] (kg/m^2)	24.2 (2.8)	25.5 (3.4)	< 0.001
Waist circumference[1] (cm)	86.9 (7.6)	89.8 (8.8)	< 0.001
Glucose[1] (mg/dL)	102.4 (19.5)	118.3 (33.2)	< 0.001
Triglyceride[1] (mg/dL)	129.1 (78.2)	161.2 (117.2)	< 0.001
HDL cholesterol[1] (mg/dL)	52.7 (12.3)	50.2 (12.6)	0.005
Creatinine[1] (mg/dL)	1.0 (0.2)	1.0 (0.2)	0.026
SBP[1] (mmHg)	119.0 (14.7)	119.2 (15.6)	0.899
DBP[1] (mmHg)	78.6 (11.2)	78.1 (11.8)	0.577
Hypertension	259 (10.4)	24 (10.7)	0.880
Diabetes mellitus	256 (10.3)	68 (30.4)	< 0.001
Current smoking	364 (16.6)	40 (20.4)	0.170
H. pylori seropositivity	1317 (52.8)	136 (60.7)	0.024

[1]Data are presented as the mean ± SD. BMI: Body mass index; HDL: High density lipoprotein; SBP: Systolic blood pressure; DBP: Diastolic blood pressure; *H. pylori*: *Helicobacter pylori*.

Table 2 Univariate and multivariate analysis assessing independent risk factors of microalbuminuria

Variables	Univariate analysis			Multivariate analysis		
	OR	95% CI	P value	OR	95% CI	P value
H. pylori seropositivity	1.38	1.04-1.82	0.024	1.40	1.05-1.89	0.024
Age (yr)	1.04	1.02-1.05	< 0.001	1.04	1.02-1.06	< 0.001
Sex	1.28	0.91-1.73	0.159	-	-	-
BMI	1.15	1.09-1.20	< 0.001	1.15	1.04-1.27	0.005
WC	1.05	1.03-1.07	< 0.001	0.98	0.94-1.01	0.213
Glucose	1.02	1.02-1.03	< 0.001	1.02	1.01-1.12	< 0.001
Triglyceride	1.00	1.00-1.005	< 0.001	1.00	1.00-1.00	0.001
HDL cholesterol	0.98	0.97-0.99	0.004	1.00	0.99-1.01	0.959
Creatinine	2.87	1.38-5.96	0.005	1.57	0.71-3.51	0.267
Smoking	1.29	1.86-1.87	0.171	-	-	-
Hypertension	1.04	0.67-1.61	0.880	-	-	-
SBP	1.00	0.99-1.01	0.894	-	-	-
DBP	1.00	0.98-1.01	0.558	-	-	-

H. pylori: *Helicobacter pylori*; OR: Odds ratio; BMI: Body mass index; WC: Waist circumference; HDL: High density lipoprotein; SBP: Systolic blood pressure; DBP: Diastolic blood pressure.

microalbuminuria, and 324 (11.9%) met the criteria for DM, either by taking diabetic medications or showing fasting serum glucose level in the diabetic range (≥ 126 mg/dL). BMI and the serum glucose, triglyceride and creatinine levels were found to be significantly higher in subjects with microalbuminuria than in those without microalbuminuria. In addition, subjects with microalbuminuria had a significantly higher *H. pylori* seropositivity rate than subjects without microalbuminuria (Table 1).

Risk factors of microalbuminuria

The results of the univariate and multivariate analyses of the risk factors for microalbuminuria are shown in Table 2. Multivariate analysis after adjustment for age, waist circumference, glucose, triglyceride and BMI showed that *H. pylori* seropositivity was statistically associated with microalbuminuria (OR, 1.40, 95% CI, 1.05-1.89, P = 0.024; Table 2). Because serum glucose level is the most well-known factor determining microalbuminuria, we performed the analysis after stratifying the cohort by glucose levels (≤ 100 mg/dL, 100 mg/dL < glucose < 126 mg/dL, and ≥ 126 mg/dL or history of DM) to reduce the confounding effect of serum glucose level. As a result, *H. pylori* seropositivity was significantly associated with microalbuminuria in diabetic subjects after adjusting for age, BMI and serum creatinine level (OR, 2.21, 95% CI, 1.20-4.08, P = 0.011). However, the relation between *H. pylori* seropositivity and microalbuminuria was not statistically significant in non-diabetic subjects.

Helicobacter pylori infection and microalbuminuria in diabetic subjects

The seropositivity rates for *H. pylori* in diabetic subjects and non-diabetic subjects were 50% (162/324) and 54% (1291/2392), respectively. Because *H. pylori* seropositivity was found to be significantly associated with an increased prevalence of microalbuminuria in diabetic subjects, we evaluated the relation between the severity of UACR and *H. pylori* seropositivity. The subjects were divided into five groups. Those without microalbuminuria (an UACR of < 30 μg/mg) were divided into four groups in accordance with their UACR values, and subjects with microalbuminuria comprised their own group. The percentage of *H. pylori* seropositivity was found to gradually increase with UACR (P = 0.001; Figure 1), and the highest rate was observed in subjects with microalbuminuria (OR, 2.41, 95% CI, 1.14-5.11; Table 3), suggesting that *H. pylori* seropositivity is positively associated with microalbuminuria in diabetic subjects.

DISCUSSION

This study shows that *H. pylori* seropositivity is independently associated with the presence of microalbuminuria, and the prevalence of *H. pylori* seropositivity shows a positive correlation with the severity of urine albumin creatinine ratio in diabetic subjects. These findings suggest that *H. pylori* infection might affect microvascular damage and possibly contributes to pathogenesis of early atherosclerosis in diabetes.

The mechanisms underlying increased urinary albumin excretion are complex, but endothelial cell dysfunction appears be a major pathogenic contributor[20]. Moreover, microalbuminuria is known to be associated with atherogenic risk factors, such as hypertension, hyperglycemia, central obesity and hyperinsulinemia[21]. In the present study, we consistently found that metabolic variables, such as glucose, triglyceride and BMI were significantly associated with microalbuminuria. In particular, *H. pylori* seropositivity was found to be positively related to microalbuminuria after adjusting for other variables, which suggests that *H. pylori* infection might participate in the pathogenesis of endothelial dysfunc-

Table 3 Relationship of the severity of the urine albumin to creatinine ratio to *Helicobacter pylori* seropositivity in diabetes subjects

Urine albumin-creatinine ratio (μg/mg)	*n*	*H. pylori* seropositivity (%)	OR (95% CI)	*P* for trend
Q1 (< 4.9)	64	50.0	Reference	0.001
Q2 (5.0-7.9)	63	46.0	0.86 (0.42-1.01)	
Q3 (8.0-13.9)	64	51.6	1.10 (0.36-2.23)	
Q4 (14.0-29.9)	65	61.5	1.55 (0.75-3.19)	
Microalbuminuria	68	72.1	2.41 (1.14-5.11)	

H. pylori: *Helicobacter pylori*; OR: Odds ratio.

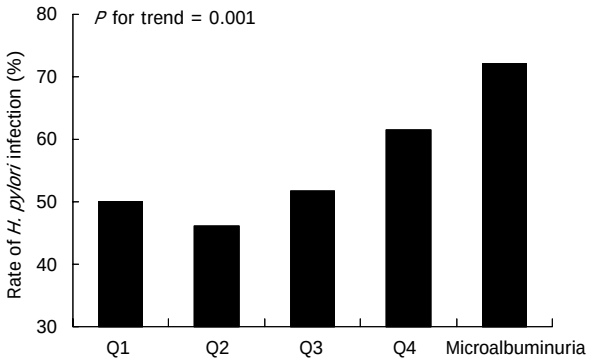


Figure 1 The rate of *Helicobacter pylori* seropositivity according to the urinary albumin to creatinine ratio in diabetic subjects. The subjects were divided into five groups: subjects without microalbuminuria were divided into four groups in accordance with their urine albumin to creatinine ratio values, and subjects with microalbuminuria comprised their own group: Q1, < 4.9 μg/mg; Q2, 5.0-7.9 μg/mg; Q3, 8.0-13.9 μg/mg; Q4, 14.0-29.9 μg/mg and microalbuminuria, > 30 μg/mg. *H. pylori*: *Helicobacter pylori*.

tion. There have been some studies suggesting the association between *H. pylori* infection and endothelial dysfunction; Oshima *et al*[22] reported that *H. pylori* seropositivity was associated with elevated C-reactive protein and soluble intercellular adhesion molecule-1. This indicates that chronic *H. pylori* infection might be involved in the pathogenesis of atherosclerosis. Prasad *et al*[23] showed that the immunoglobulin-G antibody response to pathogens was an independent risk factor for endothelial dysfunction and coronary atherosclerosis.

A possible mechanism linking *H. pylori* infection and endothelial dysfunction is the association between *H. pylori* infection and insulin resistance. Recently, a systematic review has reported a positive association between *H. pylori* infection and a homeostatic model of assessing insulin resistance[24]. *H. pylori* infection was significantly associated with metabolic syndrome in a large Japanese population[25]. Furthermore, Gunji *et al*[26] found that *H. pylori* infection significantly increases insulin resistance in the asymptomatic population. A recent prospective study showed the beneficial effect of *H. pylori* eradication on insulin resistance, serum lipid and low-grade inflammation[27]. It remains uncertain how the presence of *H. pylori* affects the pathogenic process of insulin resistance. The disturbance of proinflammatory and vasoactive substances, such as tumor necrosis factor-α, interlukin-6 and c-reactive protein, may be involved in the pathogenesis of insulin resistance[28,29]. Moreover, the reactive oxygen species caused by an *H. pylori* infection also affects insulin resistance[30,31].

Many studies have demonstrated that elevated blood pressure, poor glycemic control, older age and insulin resistance are associated with microalbuminuria in subjects with diabetes[32,33]. In this study, we stratified the study population according to glucose levels to reduce the confounding effect, and the association between *H. pylori* seropositivity and microalbuminuria was more potent in diabetic subjects than in non-diabetic and prediabetic subjects. Diabetic patients are known to have impaired function of cellular and humoral immunity, and consequently, the direct invasion of the arterial wall by bacteria might be more frequent in these patients than in non-diabetic ones. Indeed, some previous studies have found a higher prevalence of *H. pylori* infection among diabetic patients[34,35]. On the other hand, other studies did not detect an association between *H. pylori* infection and diabetes[36,37], and these contradictory results among studies might have resulted from differing methods used by the studies and from the uneven epidemiological distribution of *H. pylori* infection.

This study has several advantages. First, to the best our knowledge, this is the only study of the association between *H. pylori* infection and microalbuminuria. Second, it utilized a large study population, and we constructed a multiple regression model containing many metabolic confounding factors. Third, subjects in this study were an apparently healthy population that presented for screening, and such populations seems to approximate the general population.

Nevertheless, the study has several limitations. First, it does not provide details regarding the nature of the causative relation between *H. pylori* seropositivity and microalbuminuria because of the cross-sectional study design. Second, although the *H. pylori* seropositivity does not indicate current *H. pylori* infection, seropositivity for *H. pylori*-specific immunoglobulin G antibody was taken as a surrogate of *H. pylori* infection status in the present study. Thus, there might be some false-positive or false-negative subjects. Third, we did not evaluate *H. pylori*-induced inflammatory and virulence factors, such as the *cag A* gene, which could contribute to the pathogenesis of *H. pylori*-induced early atherosclerosis.

In conclusion, *H. pylori* seropositivity was independently associated with the presence of microalbuminuria, and the prevalence of *H. pylori* seropositivity was positively correlated with the severity of urine albumin creatinine ratio in diabetic subjects. Our findings suggest that *H. pylori* infection might be involved in the pathogenesis of early atherosclerosis in diabetes.

COMMENTS

Background

Helicobacter pylori (*H. pylori*) infection has been implicated in various extragastric conditions including coronary heart disease and ischemic cerebrovascular

disorder, caused by predisposing chronic inflammation and atherosclerosis. On the other hand, microalbuminuria is a known early marker of renal and cardiovascular diseases. Their hypothesis was that if *H. pylori* infection is involved in the pathogenesis of atherosclerosis, a significant association might exist between *H. pylori* infection and microalbuminuria, which is an early marker of atherosclerosis.

Research frontiers

Recent studies have reported the association between *H. pylori* infection and endothelial dysfunction. *H. pylori* infection significantly increases insulin resistance in the asymptomatic population and it also associated with metabolic syndrome in a large Japanese population. Moreover, the beneficial effect of *H. pylori* eradication on insulin resistance was reported.

Innovations and breakthroughs

This study showed that *H. pylori* seropositivity is independently associated with presence of microalbuminuria and the prevalence of *H. pylori* seropositivity showed a positive correlation with the severity of the urine albumin creatinine ratio in diabetic subjects.

Applications

This study suggests that *H. pylori* infection might affect microvascular damage and possibly contributes to the pathogenesis of early atherosclerosis in diabetes.

Peer review

The authors have studied the relationship between the occurrence of *H. pylori* and microalbuminuria in a healthy population. The association between *H. pylori* and microalbuminuria has only recently been reported and has not been studied in a larger setting. Thus, the paper is of importance and novel. The paper is well written and well presented. Regarding ethics, the local review board had waived the need for written consent.

REFERENCES

1 **Pellicano R**, Franceschi F, Saracco G, Fagoonee S, Roccarina D, Gasbarrini A. Helicobacters and extragastric diseases. *Helicobacter* 2009; **14** Suppl 1: 58-68 [PMID: 19712170 DOI: 10.1111/j.1523-5378.2009.00699.x]

2 **Vizzardi E**, Bonadei I, Piovanelli B, Quinzani F, Ricci C, Lanzini A, Dei Cas L. Helicobacter pylori and ischemic heart disease. *Panminerva Med* 2011; **53**: 193-202 [PMID: 21775946]

3 **Sung KC**, Rhee EJ, Ryu SH, Beck SH. Prevalence of Helicobacter pylori infection and its association with cardiovascular risk factors in Korean adults. *Int J Cardiol* 2005; **102**: 411-417 [PMID: 16004885 DOI: 10.1016/j.ijcard.2004.05.040]

4 **Danesh J**, Collins R, Peto R. Chronic infections and coronary heart disease: is there a link? *Lancet* 1997; **350**: 430-436 [PMID: 9259669 DOI: 10.1016/S0140-6736(97)03079-1]

5 **Nazmi A**, Diez-Roux AV, Jenny NS, Tsai MY, Szklo M, Aiello AE. The influence of persistent pathogens on circulating levels of inflammatory markers: a cross-sectional analysis from the Multi-Ethnic Study of Atherosclerosis. *BMC Public Health* 2010; **10**: 706 [PMID: 21083905 DOI: 10.1186/1471-2458-10-706]

6 **Park MJ**, Choi SH, Kim D, Kang SJ, Chung SJ, Choi SY, Yoon DH, Lim SH, Kim YS, Yim JY, Kim JS, Jung HC. Association between Helicobacter pylori Seropositivity and the Coronary Artery Calcium Score in a Screening Population. *Gut Liver* 2011; **5**: 321-327 [PMID: 21927661 DOI: 10.5009/gnl.2011.5.3.321]

7 **Jia EZ**, Zhao FJ, Hao B, Zhu TB, Wang LS, Chen B, Cao KJ, Huang J, Ma WZ, Yang ZJ, Zhang G. Helicobacter pylori infection is associated with decreased serum levels of high density lipoprotein, but not with the severity of coronary atherosclerosis. *Lipids Health Dis* 2009; **8**: 59 [PMID: 20030806 DOI: 10.1186/1476-511X-8-59]

8 **Kim HL**, Jeon HH, Park IY, Choi JM, Kang JS, Min KW. Helicobacter pylori infection is associated with elevated low density lipoprotein cholesterol levels in elderly Koreans. *J Korean Med Sci* 2011; **26**: 654-658 [PMID: 21532857 DOI: 10.3346/jkms.2011.26.5.654]

9 **Gen R**, Demir M, Ataseven H. Effect of Helicobacter pylori eradication on insulin resistance, serum lipids and low-grade inflammation. *South Med J* 2010; **103**: 190-196 [PMID: 20134372 DOI: 10.1097/SMJ.0b013e3181cf373f]

10 **National Kidney Foundation.** K/DOQI clinical practice guidelines for chronic kidney disease: evaluation, classification, and stratification. *Am J Kidney Dis* 2002; **39**: S1-266 [PMID: 11904577]

11 **Mogensen CE**, Chachati A, Christensen CK, Close CF, Deckert T, Hommel E, Kastrup J, Lefebvre P, Mathiesen ER, Feldt-Rasmussen B. Microalbuminuria: an early marker of renal involvement in diabetes. *Uremia Invest* 1985-1986; **9**: 85-95 [PMID: 3915933]

12 **Borch-Johnsen K**, Feldt-Rasmussen B, Strandgaard S, Schroll M, Jensen JS. Urinary albumin excretion. An independent predictor of ischemic heart disease. *Arterioscler Thromb Vasc Biol* 1999; **19**: 1992-1997 [PMID: 10446083 DOI: 10.1161/01.ATV.19.8.1992]

13 **Jørgensen L**, Jenssen T, Johnsen SH, Mathiesen EB, Heuch I, Joakimsen O, Fosse E, Jacobsen BK. Albuminuria as risk factor for initiation and progression of carotid atherosclerosis in non-diabetic persons: the Tromsø Study. *Eur Heart J* 2007; **28**: 363-369 [PMID: 17132646 DOI: 10.1093/eurheartj/ehl394]

14 **Yudkin JS**, Forrest RD, Jackson CA. Microalbuminuria as predictor of vascular disease in non-diabetic subjects. Islington Diabetes Survey. *Lancet* 1988; **2**: 530-533 [PMID: 2900920 DOI: 10.1016/S0140-6736(88)92657-8]

15 **Deckert T**, Feldt-Rasmussen B, Borch-Johnsen K, Jensen T, Kofoed-Enevoldsen A. Albuminuria reflects widespread vascular damage. The Steno hypothesis. *Diabetologia* 1989; **32**: 219-226 [PMID: 2668076 DOI: 10.1007/BF00285287]

16 **Sheng CS**, Hu BC, Fan WX, Zou J, Li Y, Wang JG. Microalbuminuria in relation to the metabolic syndrome and its components in a Chinese population. *Diabetol Metab Syndr* 2011; **3**: 6 [PMID: 21470432 DOI: 10.1186/1758-5996-3-6]

17 **Lee JE**, Huh W, Son HJ, Kim YG, Kim DJ, Lee MK, Oh HY. Association of metabolic syndrome with microalbuminuria in non-hypertensive type 2 diabetic patients. *Nephron Clin Pract* 2007; **106**: c98-103 [PMID: 17522477 DOI: 10.1159/000102996]

18 **Choi HS**, Ryu SH, Lee KB. The relationship of microalbuminuria with metabolic syndrome. *Nephron Clin Pract* 2006; **104**: c85-c93 [PMID: 16785740 DOI: 10.1159/000093995]

19 **Centers for Disease Control and Prevention (CDC).** Cigarette smoking among adults--United States, 1994. *MMWR Morb Mortal Wkly Rep* 1996; **45**: 588-590 [PMID: 9132579]

20 **Glassock RJ**. Is the presence of microalbuminuria a relevant marker of kidney disease? *Curr Hypertens Rep* 2010; **12**: 364-368 [PMID: 20686930 DOI: 10.1007/s11906-010-0133-3]

21 **Kim YI**, Kim CH, Choi CS, Chung YE, Lee MS, Lee SI, Park JY, Hong SK, Lee KU. Microalbuminuria is associated with the insulin resistance syndrome independent of hypertension and type 2 diabetes in the Korean population. *Diabetes Res Clin Pract* 2001; **52**: 145-152 [PMID: 11311969 DOI: 10.1016/S0168-8227(01)00228-5]

22 **Oshima T**, Ozono R, Yano Y, Oishi Y, Teragawa H, Higashi Y, Yoshizumi M, Kambe M. Association of Helicobacter pylori infection with systemic inflammation and endothelial dysfunction in healthy male subjects. *J Am Coll Cardiol* 2005; **45**: 1219-1222 [PMID: 15837252 DOI: 10.1016/j.jacc.2005.01.019]

23 **Prasad A**, Zhu J, Halcox JP, Waclawiw MA, Epstein SE, Quyyumi AA. Predisposition to atherosclerosis by infections: role of endothelial dysfunction. *Circulation* 2002; **106**: 184-190 [PMID: 12105156 DOI: 10.1161/01.CIR.0000021125.83697.21]

24 **Polyzos SA**, Kountouras J, Zavos C, Deretzi G. The association between Helicobacter pylori infection and insulin resistance: a systematic review. *Helicobacter* 2011; **16**: 79-88 [PMID: 21435084 DOI: 10.1111/j.1523-5378.2011.00822.x]

25 **Gunji T**, Matsuhashi N, Sato H, Fujibayashi K, Okumura M,

Sasabe N, Urabe A. Helicobacter pylori infection is significantly associated with metabolic syndrome in the Japanese population. *Am J Gastroenterol* 2008; **103**: 3005-3010 [PMID: 19086952 DOI: 10.1111/j.1572-0241.2008.02151.x]

26 **Gunji T**, Matsuhashi N, Sato H, Fujibayashi K, Okumura M, Sasabe N, Urabe A. Helicobacter pylori infection significantly increases insulin resistance in the asymptomatic Japanese population. *Helicobacter* 2009; **14**: 144-150 [PMID: 19751440 DOI: 10.1111/j.1523-5378.2009.00705.x]

27 **Kanbay M**, Gür G, Yücel M, Yilmaz U, Boyacioğlu S. Does eradication of Helicobacter pylori infection help normalize serum lipid and CRP levels? *Dig Dis Sci* 2005; **50**: 1228-1231 [PMID: 16047464]

28 **Aebischer T**, Meyer TF, Andersen LP. Inflammation, immunity, and vaccines for Helicobacter. *Helicobacter* 2010; **15** Suppl 1: 21-28 [PMID: 21054649 DOI: 10.1111/j.1523-5378.2010.00777.x]

29 **Basso D**, Plebani M, Kusters JG. Pathogenesis of Helicobacter pylori infection. *Helicobacter* 2010; **15** Suppl 1: 14-20 [PMID: 21054648 DOI: 10.1111/j.1523-5378.2010.00781.x]

30 **Aslan M**, Horoz M, Nazligul Y, Bolukbas C, Bolukbas FF, Selek S, Celik H, Erel O. Insulin resistance in H pylori infection and its association with oxidative stress. *World J Gastroenterol* 2006; **12**: 6865-6868 [PMID: 17106938]

31 **Kebapcilar L**, Sari I, Renkal AH, Alacacioglu A, Yuksel A, Ilhan E, Alkan B, Yuksel D, Kozaci DL, Gunay N. The influence of Helicobacter pylori eradication on leptin, soluble CD40 ligand, oxidative stress and body composition in patients with peptic ulcer disease. *Intern Med* 2009; **48**: 2055-2059 [PMID: 20009392 DOI: 10.2169/internalmedicine.48.2562]

32 **Remuzzi G**, Schieppati A, Ruggenenti P. Clinical practice. Nephropathy in patients with type 2 diabetes. *N Engl J Med* 2002; **346**: 1145-1151 [PMID: 11948275 DOI: 10.1056/NEJMcp011773]

33 **De Cosmo S**, Minenna A, Ludovico O, Mastroianno S, Di Giorgio A, Pirro L, Trischitta V. Increased urinary albumin excretion, insulin resistance, and related cardiovascular risk factors in patients with type 2 diabetes: evidence of a sex-specific association. *Diabetes Care* 2005; **28**: 910-915 [PMID: 15793194 DOI: 10.2337/diacare.28.4.910]

34 **Hamed SA**, Amine NF, Galal GM, Helal SR, Tag El-Din LM, Shawky OA, Ahmed EA, Abdel Rahman MS. Vascular risks and complications in diabetes mellitus: the role of helicobacter pylori infection. *J Stroke Cerebrovasc Dis* 2008; **17**: 86-94 [PMID: 18346651 DOI: 10.1016/j.jstrokecerebrovasdis.2007.10.006]

35 **Gulcelik NE**, Kaya E, Demirbas B, Culha C, Koc G, Ozkaya M, Cakal E, Serter R, Aral Y. Helicobacter pylori prevalence in diabetic patients and its relationship with dyspepsia and autonomic neuropathy. *J Endocrinol Invest* 2005; **28**: 214-217 [PMID: 15952404]

36 **Bures J**, Smahelová A, Kopácová M, Rejchrt S. [Clinical importance of Helicobacter pylori infection in patients with diabetes mellitus]. *Vnitr Lek* 2004; **50**: 350-353 [PMID: 15305630]

37 **Demir M**, Gokturk HS, Ozturk NA, Kulaksizoglu M, Serin E, Yilmaz U. Helicobacter pylori prevalence in diabetes mellitus patients with dyspeptic symptoms and its relationship to glycemic control and late complications. *Dig Dis Sci* 2008; **53**: 2646-2649 [PMID: 18320319 DOI: 10.1007/s10620-007-0185-7]

P- Reviewers Vorobjova T, Paulssen EJ, Wang JT
S- Editor Gou SX **L- Editor** A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i1.103

World J Gastroenterol 2013 January 7; 19(1): 103-107
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

A pilot study of single-use endoscopy in screening acute gastrointestinal bleeding

Jae Hee Cho, Hee Man Kim, Sangheun Lee, Yu Jin Kim, Ki Jun Han, Hyeon Geun Cho, Si Young Song

Jae Hee Cho, Hee Man Kim, Sangheun Lee, Yu Jin Kim, Ki Jun Han, Hyeon Geun Cho, Division of Gastroenterology, Department of Internal Medicine, Myongji Hospital, Kwandong University College of Medicine, Goyang 412-270, South Korea
Jae Hee Cho, Hee Man Kim, Department of Internal Medicine, Graduate School, Yonsei University College of Medicine, Seoul 120-752, South Korea
Si Young Song, Division of Gastroenterology, Department of Internal Medicine, Brain Korea 21 Project for Medical Science, Severance Biomedical Science Institute, Yonsei University College of Medicine, Seoul 120-752, South Korea
Author contributions: Cho JH, Kim HM, Han KJ, Cho HG and Song SY designed the research; Lee S and Kim YJ performed the research; Cho JH, Kim HM and Lee S analyzed the data; and Kim HM wrote the paper.
Supported by Basic Science Research Program through the National Research Foundation of Korea, the Ministry of Education, Science and Technology, No. 2011-0008901; and Research Program of Clinical Professor Research committee of Myongji Hospital, No. 20120106
Correspondence to: Hee Man Kim, MD, Division of Gastroenterology, Department of Internal Medicine, Myongji Hospital, Kwandong University College of Medicine, Goyang 412-270, South Korea. eastin@kd.ac.kr
Telephone: +82-31-8105412 Fax: +82-31-9690500
Received: April 9, 2012 Revised: September 19, 2012
Accepted: September 22, 2012
Published online: January 7, 2013

Abstract

AIM: To investigate the feasibility of a single-use endoscopy as an alternative procedure to nasogastric lavage in patients with acute gastrointestinal (GI) bleeding.

METHODS: Patients who presented with hematemesis, melena or hematochezia were enrolled in this study. EG scan™ and conventional esophagogastroduodenoscopy (EGD) were subsequently performed. Active bleeding was defined as blood in the stomach, and inactive bleeding was defined as coffee ground clots and clear fluid in the stomach. The findings were recorded and compared.

RESULTS: Between January and March, 2011, 13 patients that presented with hematemesis (n = 4), melena (n = 6), or bleeding from a previous nasogastric feeding tube (n = 3), were enrolled in this study. In 12 patients with upper GI bleeding, the EG scan device revealed that 7 patients had active bleeding and 5 patients had inactive bleeding, whereas conventional EGD revealed that 8 patients had active bleeding and 4 patients had inactive bleeding. The sensitivity and specificity of the EG scan device was 87.5% and 100% for active bleeding, with conventional EGD serving as a reference. No complication were reported during the EG scan procedures.

CONCLUSION: The EG scan is a feasible device for screening acute upper GI bleeding. It may replace nasogastric lavage for the evaluation of acute upper GI bleeding.

© 2013 Baishideng. All rights reserved.

Key words: Gastrointestinal hemorrhage; Bleeding; Endoscopy; Nasogastric tube; Lavage

Cho JH, Kim HM, Lee S, Kim YJ, Han KJ, Cho HG, Song SY. A pilot study of single-use endoscopy in screening acute gastrointestinal bleeding. *World J Gastroenterol* 2013; 19(1): 103-107 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i1/103.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i1.103

INTRODUCTION

Acute upper gastrointestinal (GI) bleeding is managed by early conventional endoscopy within 24 h of presentation[1-3]. In high-risk upper GI bleeding, urgent endoscopy reduces mortality; therefore, risk stratification is important[4,5]. Nasogastric lavage is a routine procedure used to evaluate acute upper GI bleeding and

to assess risk before performing conventional endoscopy. The findings of nasogastric lavage help doctors decide whether to perform an early conventional endoscopy[1,6-8]. However, the effect of nasogastric lavage on clinical outcomes and mortality remains controversial[3,9-12]. Nasogastric intubation and lavage is one of the most painful procedures performed in the emergency department[13,14].

A novel single-use endoscopy device (EG scan™: IntroMedic Co., Ltd., Seoul, South Korea) has been developed from the MiroCam® capsule endoscopy technology created by the IntroMedic company and Professor Si Young Song of Yonsei University College of Medicine[15,16]. The EG scan machinery (version I) is composed of an optical probe, a handle for control, and a display monitor. The optical probe is disposable (Figure 1), and the largest diameter is 6 mm, similar to a nasogastric tube with diameter of 16 French (Figure 2). The optical probe can be deflected to 60° upward or 60° downward, but not left or right. The optical probe is inserted though the mouth or nose using display monitor to visualize the process, and the probe can only be used once. The machinery of the full EG scan system is as small as a laptop computer and is portable. It can operate anywhere electric power is supplied. It is easy to use and is accessible for use by a non-GI endoscopist.

The EG scan has the potential to improve the easy and accuracy of diagnosis due to its visual monitoring capabilities for the evaluation of acute GI bleeding in decision making for early conventional upper endoscopy. It is also less painful and has a lower risk of complication than a blindly inserted nasogastric tube. We aimed to test the feasibility of the EG scan to assess GI bleeding for guidance of early conventional endoscopy in patients with acute GI bleeding as an alternative procedure to nasogastric lavage.

MATERIALS AND METHODS

Patients who were older than 18 years old and had clinical manifestations suspected of being acute GI bleeding were enrolled in this study. Acute GI bleeding was defined as presentation with hematemesis, melena, or hematochezia within 24 h. Patients who visited the emergency room for acute GI bleeding or in-patients who had been admitted to intensive care unit or general ward and presented with acute GI bleeding in Myongji Hospital were enrolled in this study. Patients with confirmed lower GI bleeding were excluded from this study.

When the patients were hemodynamically stable, an EG scan (version I) was immediately performed at the patients' bedside by one PGY-4 medical resident. The EG scan's optical probe was inserted through the mouth using the display monitor to visualize the process. The findings of the EG scan were recorded. Thereafter, conventional esophagogastroduodenoscopy (EGD) was instantly performed by one qualified endoscopist, who had not been informed of the findings from the EG scan.



Figure 1 EG scan device. The EG scan (version I) is composed of a disposable optical probe, a handle, and a display monitor.

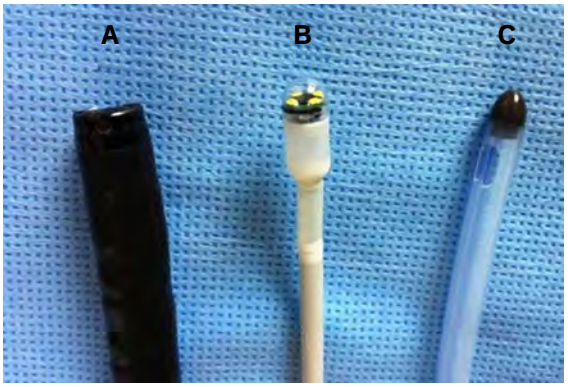


Figure 2 Comparison of diameters. A: The diameters of GIF-Q260 (Olympus) endoscopy; B: An optical probe of an EG scan device; C: An optical probe of a 16-Fr nasogastric tube.

The findings from the 2 procedures were compared.

Blood in the stomach was categorized into "active bleeding", and coffee ground clots and clear fluid were categorized into "inactive bleeding". In the EGD, high-risk lesions were defined as spurting, oozing of blood, or non-bleeding visible vessels, or by the Forrest classifications I a, I b, or II a[17,18].

Statistical analysis

To investigate the diagnostic efficacy of the EG scan device, the sensitivity, specificity, positive predictive value and negative predictive value for active bleeding were calculated, with conventional EGD serving as a reference.

RESULTS

Thirteen patients with acute GI bleeding were enrolled between January and March 2011 (Table 1). Of the 13 patients, 12 patients had symptoms suspicious of upper GI bleeding: 6 patients presented with melena, 3 patients presented with hematemesis, and 3 patients presented with bleeding from a previously located nasogastric tube for feeding or drainage. One patient presented with hematemesis, but the final focus of bleeding was identified as lung cancer.

Table 1 Clinical features and the findings of EG scan and esophagogastroduodenoscopy in all patients

No.	Age (yr)	Sex	Reason for endoscopy	Underlying disease/condition	EG scan		EGD		
					Performance location	Bleeding status	Bleeding status	Diagnosis	Risk
1	52	M	Hematemesis	Heavy alcoholics	ER	Active	Active	Mallory-Weiss syndrome	High
2	47	F	Hematemesis	Liver cirrhosis ESRD	ER	Active	Active	Gastric varix bleeding	High
3	87	M	Hematemesis	Old stroke	ER	Inactive	Active	Gastric ulcer with bleeding	High
4	79	M	Melena	Pneumonia Abdominal aorta aneurysm	ICU	Active	Active	Gastric ulcer with bleeding	High
5	88	M	Melena	Cardiac arrest Pneumonia	ICU	Active	Active	Gastric ulcer with bleeding	High
6	74	M	Melena	Old stroke	ER	Inactive	Inactive	Duodenal ulcer	Low
7	63	M	Melena	Valvular heart disease Coumadin toxicity	General ward	Inactive	Inactive	Gastric ulcer and duodenal ulcer	Low
8	85	F	Melena	Acute myocardial infarction	ICU	Active	Active	Gastric ulcer with bleeding	High
9	50	M	Melena	None	ER	Inactive	Inactive	Duodenal ulcer with visible vessel	High
10	72	F	Blood clots from NG tube for feeding	Stroke Parkinson's disease	ICU	Inactive	Inactive	Multiple gastric hematin,	Low
11	89	F	Fresh blood from NG tube for feeding	Aspiration pneumonia	ICU	Active	Active	Gastric ulcer with bleeding	High
12	80	M	Fresh blood from NG tube for drainage	Postoperative state for gastric cancer	ICU	Active	Active	Anastomosis site bleeding	High
13	73	M	Hematemesis	COPD with acute exacerbation Lung cancer	ER	Active	Active	Hemoptysis (Ingestion of blood from lung cancer)	Not related

M: Male; F: Female; NG: Nasogastric; ER: Emergency room; ICU: Intensive care unit; Active: Blood; Inactive: Coffee ground or clear; COPD: Chronic obstructive pulmonary disease; ESRD: End stage renal disease; EGD: Esophagogastroduodenoscopy.

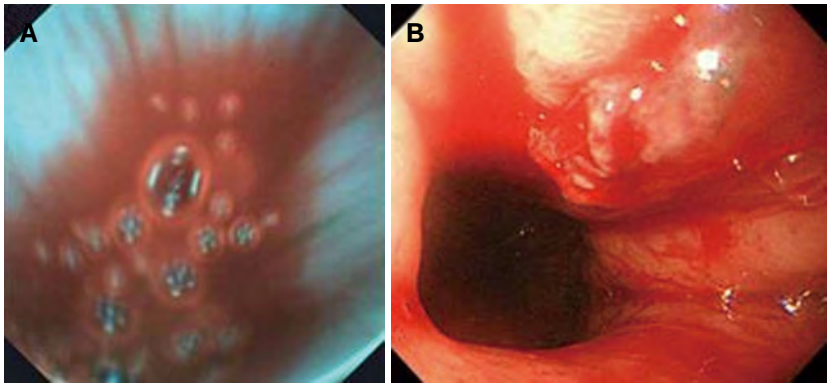


Figure 3 A concordant case of acute gastrointestinal bleeding from Mallory-Weiss syndrome. A: EG scan shows blood at the esophagogastric junction; B: Conventional esophagogastroduodenoscopy (GIF-Q260) shows oozing from the tear wound of the esophagogastric junction.

In the 12 patients with upper GI bleeding, 7 patients were diagnosed as having active bleeding by the EG scan, with the same finding by conventional EGD (Figure 3). In 5 patients diagnosed as having inactive bleeding by EG scan, 4 patients were confirmed as having inactive bleeding. The remaining patient was reported as active bleeding by conventional EGD. The sensitivity of the EG scan was 87.5% for active bleeding, and the specificity of the EG scan was 100% for inactive bleeding, with conventional EGD serving as a reference. The positive predictive value for active bleeding was 100%, and the negative predictive value for active bleeding was 80%, with conventional EGD serving as a reference. No complication were reported during the EG scan procedures.

DISCUSSION

In our small-scale pilot study, the EG scan was shown to evaluate acute upper GI bleeding before conventional EGD in a manner similar to nasogastric lavage. The sensitivity, specificity, and positive predictive values of the EG scan procedure were significant although the results are preliminary. Only one of 12 patients with upper GI bleeding had a discrepancy between the EG scan results and the EGD results. The cause of this discrepancy was possibly a gastric ulcer that had an inactive period of bleeding during the EG scan, whereas blood spurted from the ulcer during the EGD. Therefore, the result was not associated with the accuracy of the EG scan. In a large-scale study using a Canadian retrospective registry, a bloody nasogastric aspirate has a sensitivity of 48.4%, a specificity of 75.8%, and a negative predictive value of 77.9% of for high-risk endoscopic lesions[17]. In a prospective study, 73% of Greek patients with upper GI bleeding and a bloody nasogastric aspirate have active bleeding[19]. In a retrospective cohort study with GI

bleeding patients without hematemesis, the sensitivity of a positive nasogastric aspirate is 42%[10]. With respect to these findings, the EG scan is worth consideration of a large-scale controlled study as an alternative procedure to nasogastric lavage.

An EG scan probe can be inserted through a patient's mouth as it is in conventional EGD. This method can avoid pain during the nasal insertion of a nasogastric tube. The visual monitoring of the EG scan may prevent serious complications related to the blind insertion of a nasogastric tube, such as nasopharynx perforation, esophageal perforation and displacement into the trachea[11,20-22]. In addition, the EG scan's optical probe can be inserted through a patient's nose in the same manner in which a nasogastric tube is inserted. In our study, the pain related to the insertion of the EG scan was not investigated and should be part of the evaluation in a further study.

In critically ill patients with other morbidities such as acute myocardial infarction and COPD, conventional EGD has an increased complication rate[23-26]. When these patients are suspected of having acute GI bleeding, it is necessary to exactly assess the acuity, severity, and location of the GI bleeding. In these high-risk patients, the EG scan could play the role of a bridge to conventional EGD by assessing the exact status of GI bleeding. In our study, critically ill patients with acute myocardial infarction, cardiac arrest, and pneumonia received an EG scan with high performance and without any complications.

In Korea, the prices of a nasogastric tube and lavage are approximately 10 United States dollars and 20 United States dollars, respectively, whereas the price of a disposable optical probe of EG scan is approximately 100 United States dollars. The cost of performing EG scan is currently more expensive than nasogastric lavage. However, an EG scan may screen active bleeding more precisely than nasogastric lavage and may eventually reduce unnecessary emergency EGDs and costs. The cost and effectiveness of the EG scan must be confirmed through large-scale studies. Additionally, the price of an EG scan could be decreased by advancements in electronics in the future, and this technology could replace nasogastric lavage.

However, the EG scan has several weaknesses. First, EG scan version I does not provide air inflation; therefore, it is difficult to find the focus of the bleeding in the stomach. Recently, an EG scan version Ⅱ with an air inflation function has been released that could resolve this problem. Second, there is no method to clear the cover glass in front of the camera during the procedure, which means that vision can be impaired when secreted materials become stuck to the cover glass. Additionally, angulation of the head of the optical probe is limited up to 60° upward or 60° downward. Further development of the technique will compensate for these weak points when using the EG scan.

In conclusion, the EG scan was developed for single-use and as a portable device. It is feasible for screening acute upper GI bleeding with high sensitivity and specificity. If larger, controlled trials looking at the predictive value, the cost, patient discomfort, and the relative side effect profile of immediate endoscopy or nasogastric lavage in comparison to the EG scan prove positive, this device has the potential to replace nasogastric lavage or universal urgent EGD in an emergency room, ward or intensive care unit setting.

COMMENTS

Background

Nasogastric lavage is an important method to evaluate acute gastrointestinal bleeding. However, the findings of nasogastric lavage sometimes indicate false positive or false negative active bleeding, and nasogastric tube insertion is very painful to patients. A more precise and less painful procedure has been needed.

Research frontiers

The EG scan™ (IntroMedic Co., Ltd., Seoul, South Korea) is a novel disposable endoscopy tool using the mechanism of capsule endoscopy, and its machinery is small enough to be portable. It has been developed to be used in outpatient clinics quickly and comfortably.

Innovations and breakthroughs

This is the first study to investigate the diagnostic efficacy of the EG scan for screening active bleeding. The promising results suggest that the EG scan should be a candidate procedure as a substitute to nasogastric lavage, although this is a pilot study with a small sample size.

Applications

These preliminary findings suggest that the EG scan may be a substitute for nasogastric lavage. This device can also be used as esophagoscopy in outpatient clinics and as a flexible rectosigmoidoscopy. In the future, the portable nature of this device may enable EG scans to be used for patients in an emergency outside of a hospital.

Terminology

A single-use endoscopy tool is disposable after use. Early endoscopy is an endoscopic procedure used within 24 h of presentation to diagnose or treat diseases. Active bleeding is defined as the existence of blood in the stomach, and inactive bleeding is defined as the existence of coffee ground clots or clear fluid in the stomach. A high-risk ulcer is one with current bleeding or high risk of bleeding.

Peer review

This pilot study shows that the EG scan, a novel single-use endoscopy device, can screen active bleeding simply and easily. It is a portable system suitable for fast diagnosis in emergency rooms and intensive care units, although the efficacy should be confirmed in large-scale trials.

REFERENCES

1 **Barkun AN**, Bardou M, Kuipers EJ, Sung J, Hunt RH, Martel M, Sinclair P. International consensus recommendations on the management of patients with nonvariceal upper gastrointestinal bleeding. *Ann Intern Med* 2010; **152**: 101-113 [PMID: 20083829]

2 **Greenspoon J**, Barkun A, Bardou M, Chiba N, Leontiadis GI, Marshall JK, Metz DC, Romagnuolo J, Sung J. Management of patients with nonvariceal upper gastrointestinal bleeding. *Clin Gastroenterol Hepatol* 2012; **10**: 234-239 [PMID: 21820395]

3 **Bardou M**, Benhaberou-Brun D, Le Ray I, Barkun AN. Diagnosis and management of nonvariceal upper gastrointestinal bleeding. *Nat Rev Gastroenterol Hepatol* 2012; **9**: 97-104 [PMID: 22230903 DOI: 10.1038/nrgastro.2011.260]

4 **Lim LG**, Ho KY, Chan YH, Teoh PL, Khor CJ, Lim LL, Rajnakova A, Ong TZ, Yeoh KG. Urgent endoscopy is associated with lower mortality in high-risk but not low-risk nonvariceal upper gastrointestinal bleeding. *Endoscopy* 2011; **43**: 300-306 [PMID: 21360421 DOI: 10.1055/s-0030-1256110]

5 **Stanley AJ**. Update on risk scoring systems for patients with

upper gastrointestinal haemorrhage. *World J Gastroenterol* 2012; **18**: 2739-2744 [PMID: 22719181 DOI: 10.3748/wjg.v18.i22.2739]
6 **Pallin DJ**, Saltzman JR. Is nasogastric tube lavage in patients with acute upper GI bleeding indicated or antiquated? *Gastrointest Endosc* 2011; **74**: 981-984 [PMID: 22032314 DOI: 10.1016/j.gie.2011.07.007]
7 **Barkun A**, Bardou M, Marshall JK. Consensus recommendations for managing patients with nonvariceal upper gastrointestinal bleeding. *Ann Intern Med* 2003; **139**: 843-857 [PMID: 14623622]
8 **Anderson RS**, Witting MD. Nasogastric aspiration: a useful tool in some patients with gastrointestinal bleeding. *Ann Emerg Med* 2010; **55**: 364-365 [PMID: 20031264 DOI: 10.1016/j.annemergmed.2009.10.007]
9 **Huang ES**, Karsan S, Kanwal F, Singh I, Makhani M, Spiegel BM. Impact of nasogastric lavage on outcomes in acute GI bleeding. *Gastrointest Endosc* 2011; **74**: 971-980 [PMID: 21737077 DOI: 10.1016/j.gie.2011.04.045]
10 **Witting MD**, Magder L, Heins AE, Mattu A, Granja CA, Baumgarten M. Usefulness and validity of diagnostic nasogastric aspiration in patients without hematemesis. *Ann Emerg Med* 2004; **43**: 525-532 [PMID: 15039700 DOI: 10.1016/j.annemergmed.2003.09.002]
11 **Pitera A**, Sarko J. Just say no: gastric aspiration and lavage rarely provide benefit. *Ann Emerg Med* 2010; **55**: 365-366 [PMID: 20031262 DOI: 10.1016/j.annemergmed.2009.10.016]
12 **Marmo R**, Koch M, Cipolletta L, Capurso L, Pera A, Bianco MA, Rocca R, Dezi A, Fasoli R, Brunati S, Lorenzini I, Germani U, Di Matteo G, Giorgio P, Imperiali G, Minoli G, Barberani F, Boschetto S, Martorano M, Gatto G, Amuso M, Pastorelli A, Torre ES, Triossi O, Buzzi A, Cestari R, Della Casa D, Proietti M, Tanzilli A, Aragona G, Giangregorio F, Allegretta L, Tronci S, Michetti P, Romagnoli P, Nucci A, Rogai F, Piubello W, Tebaldi M, Bonfante F, Casadei A, Cortini C, Chiozzini G, Girardi L, Leoci C, Bagnalasta G, Segato S, Chianese G, Salvagnini M, Rotondano G. Predictive factors of mortality from nonvariceal upper gastrointestinal hemorrhage: a multicenter study. *Am J Gastroenterol* 2008; **103**: 1639-1647; quiz 1648 [PMID: 18564127 DOI: 10.1111/j.1572-0241.2008.01865.x]
13 **Singer AJ**, Richman PB, Kowalska A, Thode HC. Comparison of patient and practitioner assessments of pain from commonly performed emergency department procedures. *Ann Emerg Med* 1999; **33**: 652-658 [PMID: 10339680 DOI: 10.1016/j.bbr.2011.03.031]
14 **Schlager A**, Metzger YC, Adler SN. Use of surface acoustic waves to reduce pain and discomfort related to indwelling nasogastric tube. *Endoscopy* 2010; **42**: 1045-1048 [PMID: 20857371 DOI: 10.1055/s-0030-1255801]
15 **Chung JW**, Park S, Chung MJ, Park JY, Park SW, Chung JB, Song SY. A novel disposable, transnasal esophagoscope: a pilot trial of feasibility, safety, and tolerance. *Endoscopy* 2012; **44**: 206-209 [PMID: 22271030 DOI: 10.1055/s-0031-1291483]
16 **Bang S**, Park JY, Jeong S, Kim YH, Shim HB, Kim TS, Lee DH, Song SY. First clinical trial of the "MiRo" capsule endoscope by using a novel transmission technology: electric-field propagation. *Gastrointest Endosc* 2009; **69**: 253-259 [PMID: 18640676 DOI: 10.1016/j.gie.2008.04.033]
17 **Aljebreen AM**, Fallone CA, Barkun AN. Nasogastric aspirate predicts high-risk endoscopic lesions in patients with acute upper-GI bleeding. *Gastrointest Endosc* 2004; **59**: 172-178 [PMID: 14745388 DOI: 10.1016/S0016-5107(03)02543-4]
18 **Forrest JA**, Finlayson ND, Shearman DJ. Endoscopy in gastrointestinal bleeding. *Lancet* 1974; **2**: 394-397 [PMID: 4136718 DOI: 10.1016/S0140-6736(74)91770-X]
19 **Adamopoulos AB**, Baibas NM, Efstathiou SP, Tsioulos DI, Mitromaras AG, Tsami AA, Mountokalakis TD. Differentiation between patients with acute upper gastrointestinal bleeding who need early urgent upper gastrointestinal endoscopy and those who do not. A prospective study. *Eur J Gastroenterol Hepatol* 2003; **15**: 381-387 [PMID: 12655258]
20 **Tho PC**, Mordiffi S, Ang E, Chen H. Implementation of the evidence review on best practice for confirming the correct placement of nasogastric tube in patients in an acute care hospital. *Int J Evid Based Healthc* 2011; **9**: 51-60 [PMID: 21332663 DOI: 10.1111/j.1744-1609.2010.00200.x]
21 **Ronen O**, Uri N. A case of nasogastric tube perforation of the nasopharynx causing a fatal mediastinal complication. *Ear Nose Throat J* 2009; **88**: E17-E18 [PMID: 19750464]
22 **Pillai JB**, Vegas A, Brister S. Thoracic complications of nasogastric tube: review of safe practice. *Interact Cardiovasc Thorac Surg* 2005; **4**: 429-433 [PMID: 17670450]
23 **Cappell MS**. Gastrointestinal endoscopy in high-risk patients. *Dig Dis* 1996; **14**: 228-244 [PMID: 8843979 DOI: 10.1159/000171555]
24 **Yachimski P**, Hur C. Upper endoscopy in patients with acute myocardial infarction and upper gastrointestinal bleeding: results of a decision analysis. *Dig Dis Sci* 2009; **54**: 701-711 [PMID: 18661236]
25 **Cappell MS**, Iacovone FM. Safety and efficacy of esophagogastroduodenoscopy after myocardial infarction. *Am J Med* 1999; **106**: 29-35 [PMID: 10320114 DOI: 10.1016/S0002-9343(98)00363-5]
26 **Lieberman DA**, Wuerker CK, Katon RM. Cardiopulmonary risk of esophagogastroduodenoscopy. Role of endoscope diameter and systemic sedation. *Gastroenterology* 1985; **88**: 468-472 [PMID: 3965335]

P-Reviewers Misra SP, Kozarek RA **S- Editor** Gou SX
L- Editor A **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i1.108

World J Gastroenterol 2013 January 7; 19(1): 108-114
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Double guidewire technique *vs* transpancreatic precut sphincterotomy in difficult biliary cannulation

Young Wook Yoo, Sang-Woo Cha, Woong Cheul Lee, Sae Hee Kim, Anna Kim, Young Deok Cho

Young Wook Yoo, Department of Internal Medicine, Gastroenterology and Hepatobiliary Center, Cheongju St. Mary's Hospital, Cheongju 360-568, South Korea
Sang-Woo Cha, Woong Cheul Lee, Young Deok Cho, Department of Internal Medicine, Soonchunhyang University College of Medicine, Institute for Digestive Research, Digestive Disease Center, Soonchunhyang University Hospital, Seoul 140-743, South Korea
Sae Hee Kim, Anna Kim, Division of Gastroenterology, Department of Internal Medicine, Eulji University College of Medicine, Eulji University Hospital, Daejeon 302-799, South Korea
Author contributions: Yoo YW drafted of the article, analysis and interpretation of the data; Cha SW made the conception and design, analysis and interpretation of the data, critical revision of the article for important intellectual content and final approval of the article; Lee WC provided analytical tools and involved in editing the manuscript; Kim SH filled up the data; Kim A supplemented inadequate contents; Cho YD supplemented inadequate contents.
Correspondence to: Sang-Woo Cha, MD, PhD, Associate Professor of Internal Medicine, Department of Internal Medicine, Soonchunhyang University College of Medicine, Institute for Digestive Research, Digestive Disease Center, Soonchunhyang University Hospital 59, Daesagwan-ro, Younsan-gu, Seoul 140-743, South Korea. swcha@schmc.ac.kr
Telephone: +82-2-7099202 Fax: +82-2-7099696
Received: June 6, 2012 Revised: November 14, 2012
Accepted: November 24, 2012
Published online: January 7, 2013

Abstract

AIM: To compare the outcomes between double-guidewire technique (DGT) and transpancreatic precut sphincterotomy (TPS) in patients with difficult biliary cannulation.

METHODS: This was a prospective, randomized study conducted in single tertiary referral hospital in Korea. Between January 2005 and September 2010. A total of 71 patients, who bile duct cannulation was not possible and selective pancreatic duct cannulation was achieved, were randomized into DGT (n = 34) and TPS (n = 37) groups. DGT or TPS was done for selective biliary cannulation. We measured the technical success rates of biliary cannulation, median cannulation time, and procedure related complications.

RESULTS: The distribution of patients after randomization was balanced, and both groups were comparable in baseline characteristics, except the higher percentage of endoscopic nasobiliary drainage in the DGT group (55.9% *vs* 13.5%, $P < 0.001$). Successful cannulation rate and mean cannulation times in DGT and TPS groups were 91.2% *vs* 91.9% and 14.1 ± 13.2 min *vs* 15.4 ± 17.9 min, $P = 0.732$, respectively. There was no significant difference between the two groups. The overall incidence of post- endoscopic retrograde cholangiopancreatography (ERCP) pancreatitis was 38.2% *vs* 10.8%, $P < 0.011$ in the DGT group and the TPS group; post-procedure pancreatitis was significantly higher in the DGT group. But the overall incidence of post-ERCP hyperamylasemia was no significant difference between the two groups; DGT group *vs* TPS group: 14.7% *vs* 16.2%, $P < 1.0$.

CONCLUSION: When free bile duct cannulation was difficult and selective pancreatic duct cannulation was achieved, DGT and TPS facilitated biliary cannulation and showed similar success rates. However, post-procedure pancreatitis was significantly higher in the DGT group.

© 2013 Baishideng. All rights reserved.

Key words: Endoscopic retrograde cholangiopancreatography; Post-endoscopic retrograde cholangiopancreatography pancreatitis; Duoble guidewire technique; Transpancrestic precut sphincterotomy

Yoo YW, Cha SW, Lee WC, Kim SH, Kim A, Cho YD. Double guidewire technique *vs* transpancreatic precut sphincterotomy

in difficult biliary cannulation. *World J Gastroenterol* 2013; 19(1): 108-114 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i1/108.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i1.108

INTRODUCTION

Selective biliary cannulation is an important step for therapeutic endoscopic retrograde cholangiopancreatography (ERCP). In the hands of experienced endoscopists, successful biliary cannulation rates are higher than 90%[1]. However, in some cases, bile duct (BD) cannulation can be difficult because of special anatomical features, inflammatory processes, and adenomas of the papilla or periampullary diverticulum. Large prospective studies have demonstrated that difficult cannulation is an independent risk factor for post-ERCP pancreatitis[2-4]. In the past few years, various efforts have been made to develop alternative endoscopic techniques, with the goal of increasing the rate of successful biliary cannulation. The use of a guidewire to physically occupy the pancreatic duct (PD), also known as the double-guidewire technique (DGT), was initially described by Dumonceau *et al*[5]. Since its first description, this method has been used with promising results in cases of complex biliary cannulation, especially in patients with a distorted BD anatomy caused by neoplasia or atypical morphology of the ampulla[6].

Transpancreatic precut sphincterotomy (TPS) using a guidewire, another technique for difficult biliary cannulation, was first described by Goff[7] in 1995. A sphincterotomy over the guidewire in the PD helps to cannulate the biliary orifice because the cut either opens the BD or runs along the side of the duct, thus exposing the duct's anatomy.

DGT and TPS might facilitate biliary cannulation. However, post-ERCP pancreatitis has been reported to occur with DGT and TPS in 0% to 25% of patients[6,8-11]. Most previous studies compared the performance of the new techniques with standard cannulation techniques or precut sphincterotomy. However, there has been no prospective, randomized study of the performance of DGT with TPS in patients with difficult biliary cannulation. Therefore, we designed this randomized, prospective study to compare the outcomes of DGT and TPS in patients with difficult biliary cannulation.

MATERIALS AND METHODS

Study design and patients

This was a prospective, randomized study conducted in the Tertiary Referral Hospital in South Korea. Between January 2005 and September 2010, a total of 1893 ERCPs were performed at Eulji University Hospital, and 71 patients were enrolled in this study. Consecutive patients were considered for inclusion if they underwent ERCP with clear indication of biliary access. Among these patients, those in whom free cannulation of the BD was not possible and selective PD cannulation was achieved without difficulty were enrolled in this study. Patients were excluded for any of the following reasons: (1) age of less than 18 years; (2) subjects who underwent prior biliary or pancreatic sphincterotomy or dilatation or stenting of either duct; (3) acute pancreatitis at the time of the procedure; and (4) intrauterine pregnancy.

Enrolled patients were randomly assigned to either the DGT group or the TPS group. A randomization list for group allocation was generated using computer-based pseudo-random number generators. We compared both techniques for a maximum of 10 extra attempts after randomization which are common BD (CBD) cannulation by each method. Thus, we did not impose a time limit for CBD cannulation. We received approval by our hospital's institutional review board and obtained written informed consent from all 1394 patients.

Endoscopic procedures and patient care after the procedure

Benzodiazepines, anti-spasmodic agents, and non-narcotic analgesics, alone or in combination, were administered routinely before the procedure. Therapy with antibiotics and analgesics was allowed to continue. One senior endoscopist directly performed all procedures using side-viewing endoscopes (JF-240, JF-260V, and TJF-240; Olympus Optical Co., Ltd., Tokyo, Japan).

Standard cannulation techniques were used to attempt biliary cannulation. However, if BD cannulation failed with standard techniques but the PD was successfully cannulated, patients were randomly assigned to either the DGT group or the TPS group, and selective BD cannulation was continued with those techniques.

The DGT was performed as follows. First, after PD cannulation had been achieved without difficulty, a guidewire (0.035-inch Tracer Hybrid® Wire Guide; Wilson-Cook Medical, Bloomington, United States) was left in the PD. Second, another cannula or sphincterotome was passed into the same working channel of the scope alongside the guidewire using the two-devices-in-one-channel method. The tip of the device was positioned in the papilla, and another guidewire (0.035-inch Tracer Metro® Direct™ Wire Guide; Wilson-Cook Medical, Bloomington, United States) was bent over the pancreatic wire to attempt cannulation of the BD (Figure 1A and B).

TPS was performed as follows. After a guidewire (0.035-inch Tracer Hybrid® Wire Guide; Wilson-Cook Medical, Bloomington, United States) had been inserted deeply into the PD without difficulty, the tip of a standard traction sphincterotome was wedged into the pancreatic orifice, and a sphincterotomy was performed with a cutting wire along the biliary direction at 11 o'clock. The incision was made through the septum between the pancreatic and biliary duct with the aim of exposing the BD orifice. The BD orifice was exposed to the left and either below or above the pancreatic orifice. After TPS, the guidewire placed in the PD was removed. Bili-

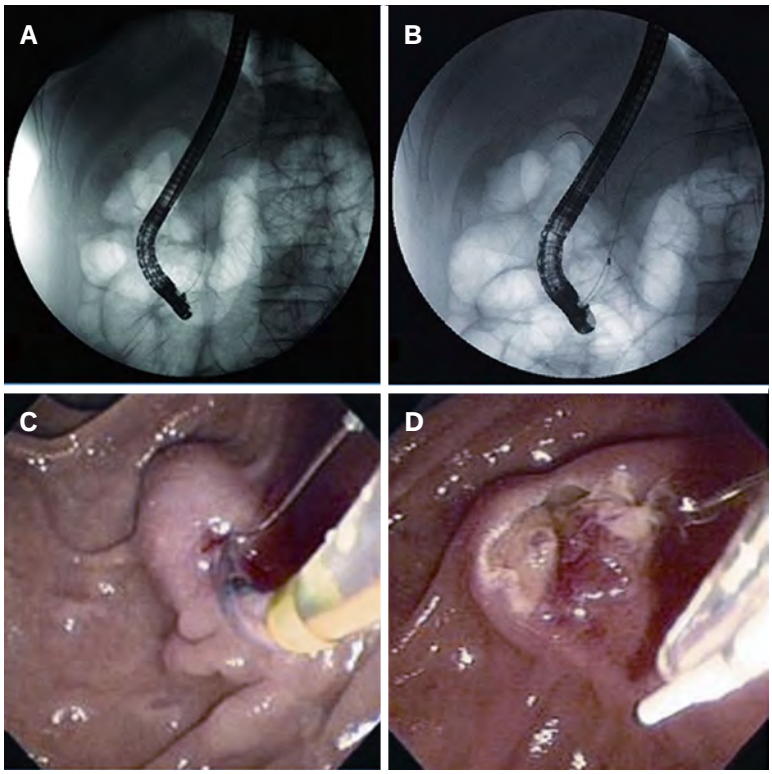


Figure 1 Radiologic images showing the use of double-guidewire technique and transpancreatic precut sphincterotomy. A: Guidewire inserted and left in the pancreatic duct (PD) [double-guidewire technique (DGT)]; B: Common bile duct cannulation with a guidewire after previous insertion of a guidewire in the PD (DGT); C: Sphincterotomy performed with a cutting wire along the biliary direction at 11 o'clock [transpancreatic precut sphincterotomy (TPS)] with guidewire inserted and left in the PD (TPS); D: The bile duct orifice exposed to the left and below the pancreatic orifice (TPS). And then common bile duct cannulation after transpancreatic sphincterotomy.

ary cannulation was then attempted using a catheter or sphincterotome, either with or without a guidewire at the discretion of the endoscopist (Figure 1C and D).

When cannulation of the BD was achieved with the DGT or TPS procedure, additional procedures were performed as necessary.

Serum amylase and lipase levels were checked before ERCP, 4 h and 24 h after ERCP, and when clinically indicated. The presence of abdominal pain attributable to the pancreas and the use and type of analgesic therapy were evaluated at those times.

Outcome measures

The primary outcome measure was the successful biliary cannulation rate. Secondary outcome measures were the median cannulation time and the rates of post-procedure-related complications, post-ERCP pancreatitis, bleeding, perforation, cholangitis, and cholecystitis.

Definition

The standard cannulation technique was defined as the use of a cannulation device (catheter or sphincterotome) preloaded with a guidewire, positioned in the ampullary orifice, and targeting the presumed entry of the CBD. Each cannulation attempt started when the device was inserted into the ampullary orifice and ended when the device was disengaged from the papilla; during the same attempt, while the cannulation device was still in contact with the papilla.

Cannulation success was determined as the achievement of deep biliary cannulation. Difficult biliary cannulation was defined as unsuccessful cannulation after 10 or more attempts with a cannula/sphincterotome or failure of cannulation after 10 min. Successful biliary cannulation at the time of DGT or TPS was considered to be an initial success. In cases in which the first biliary cannulation failed after DGT or TPS, ERCP was repeated within 2 d to 5 d with the consent of the patient, and the same cannulation technique was performed during the second ERCP attempt. Successful biliary cannulation on the first or second ERCP attempt was considered the final success.

The definition of post-ERCP pancreatitis was based on consensus criteria[12], as follows: newly developed or increased abdominal pain within 24 h after ERCP requiring analgesic agents, and the elevation of the serum amylase and/or lipase level by at least three times the normal upper limit at about 24 h after the procedure. Hyperamylasemia was defined as elevation of serum amylase levels to more than three times the normal upper limit at 4 and/or 24 h after the ERCP, without other symptoms. The severity of pancreatitis, bleeding, cholangitis, and perforation were defined as described elsewhere[12].

Sample size calculation

Our hypothesis was that the success rates of biliary cannulation were similar using either DGT or TPS; however, the post-ERCP pancreatitis rate may be lower in the TPS group than in the DGT group. Because an unrealistically large sample size would have been required to examine the biliary cannulation rates in the setting of an equivalence trial, the comparison of post-ERCP pancreatitis rates in a superiority setting was the basis for sample size calculation. Thirty-one patients in each group were needed to detect such a difference with a two-tailed χ^2 test, an a value of 0.05, and a statistical power of 80%.

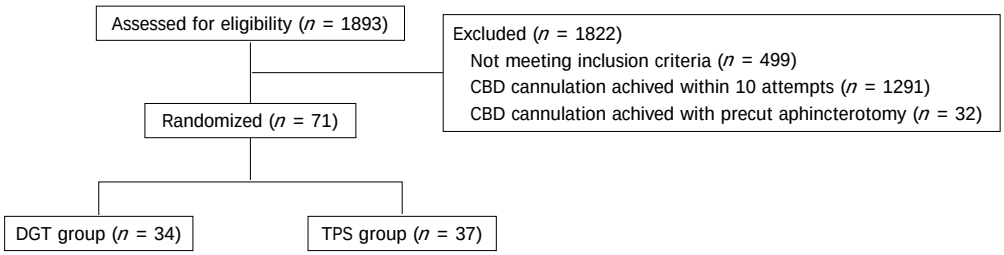


Figure 2 Subject flow in the study. CBD: Common bile duct; DGT: Double-guidewire technique; TPS: Transpancreatic sphincterotomy.

Table 1 Baseline characteristics, procedures, successful cannulation rate, median cannulation time, and procedure-related complications of the double-guidewire technique and transpancreatic precut sphincterotomy groups

	DGT group *n* = 34 (%)	TPS group *n* = 37 (%)	*P* value
Age (yr)	67.0 (11.5)	63.7 (16.5)	0.326
Sex			0.478
Male	18 (52.9)	23 (62.2)	
Female	16 (47.1)	14 (37.8)	
Indication of ERCP			0.222
CBD stone	14 (41.2)	16 (43.2)	
GB stone	7 (20.6)	6 (16.2)	
Cholangiocarcinoma	8 (23.5)	6 (16.2)	
Pancreatic cancer	1 (2.9)	3 (8.1)	
Other bile duct disease	3 (8.8)	2 (5.4)	
Other pancreatic disease	1 (2.9)	4 (10.8)	
PADD	9 (26.5)	5 (13.5)	0.426
Type Ⅰ	2 (5.9)	0 (0)	
Type Ⅱ	5 (14.7)	4 (10.8)	
Type Ⅲ	2 (5.9)	1 (2.7)	
ERCP maneuver			
Contrast injection in PD	34 (100)	33 (89.2)	0.116
EST	25 (73.5)	28 (75.7)	0.629
CBD stone extraction	13 (38.2)	12 (32.4)	0.823
EPBD	3 (9.1)	0 (0)	0.153
ENBD	19 (55.9)	5 (13.5)	< 0.001
ERBD	3 (8.8)	5 (13.5)	0.574
Successful cannulation			
First trial	27 (79.4)	29 (78.4)	0.915
Including second trial	31 (91.2)	34 (91.9)	0.914
Failure of cannulation	3 (8.8)	3 (8.1)	0.914
Median cannulation time, min (IQR)	19.0 (11.0-37.0)	20.5 (12.8-34.75)	0.732
Post-ERCP hyperamylasemia	5 (14.7)	6 (16.2)	1.000
Post-ERCP pancreatitis	13 (38.2)	4 (10.8)	0.011
Mild PEP	10 (29.4)	3 (8.1)	0.031
Moderate to severe PEP	3 (8.8)	1 (2.7)	0.344
Bleeding	1 (2.9)	2 (5.4)	1.000
Cholangitis	7 (20.6)	2 (5.4)	0.077
Cholecystitis	0	0	-
Perforation	0	0	-

ERCP: Endoscopic retrograde cholangiopancreatography; DGT: Double-guidewire technique; PD: Pancreatic duct; EST: Endoscopic sphincterotomy; CBD: Common bile duct; GB: Gallbladder; PADD: Periampullary duodenal diverticulum; EPBD: Endoscopic papillary balloon dilatation; ENBD: Endoscopic nasobiliary drainage; ERBD: Endoscopic retrograde biliary drainage; IQR: Inter-quartile range; PEP: Post endoscopic retrograde cholangiopancreatography pancreatitis; TPS: Transpancreatic sphincterotomy.

Statistical analysis

The data were analyzed with SPSS version 14.0 (SPSS Inc., Chicago, IL, United States). Continuous variables were described as median (interquartile range) and compared using Student's *t*-test. Categorical variables were tested using an χ^2 test. Statistical significance was indicated by a two-tailed *P* value of < 0.05. It is recognized that there was multiple testing of outcome data arising from individual patients. In that regard, there was no correction made to the *P* value for the comparison of post-ERCP pancreatitis rates because that comparison was considered to be the focal point when making sample size calculations. All other statistical tests of outcome results should be considered to be secondary, and their results should be taken as descriptive only.

RESULTS

Characteristics of patients

During the study period, 1893 ERCPs were performed at Eulji University Hospital. We excluded 499 patients for the following reasons: age of < 18 years (five patients); previous endoscopic sphincterotomy or endoscopic papillary balloon dilation (399 patients); acute pancreatitis before ERCP (222 patients); and pregnancy (three patients). After this exclusion, ERCP was attempted in the remaining 1394 patients with the native papilla of Vater with standard cannulation technique. In 1291 patients (92.6%), selective BD cannulation was achieved within 10 attempts and 10 min; difficult biliary cannulation occurred in 103 (7.4%) patients. Of these, PD cannulation was also not achieved in 32 patients. Finally, 71 patients in whom deep PD guidewire cannulation was achieved were enrolled in this study and randomly assigned to the DGT group (34 patients) or the TPS group (37 patients) (Figure 2).

The distribution of patients after randomization was balanced, and both groups were comparable in terms of their baseline characteristics, such as ERCP indication, devices used, ERCP findings, and maneuvers. The only significant difference was a higher percentage of endoscopic nasobiliary drainage (ENBD) in the DGT group (55.9% *vs* 13.5%, $P < 0.001$) (Table 1).

Successful BD cannulation rates and median cannulation time

Within the limit of 10 extra attempts, initial successful biliary cannulation was achieved in 27 of the 34 (79.4%) patients in the DGT group and 29 of the 37 (78.4%) patients in the TPS group. Additional successful biliary

cannulation was achieved in four and five patients using the initial technique in the second ERCP trial. Thus, the overall successful biliary cannulation rates, including the repeat ERCPs, were 91.2% (31/34) in the DGT group and 91.9% (34/37) in the TPS group. There was no significant difference in the initial and final cannulation rates of BD between the two groups (Table 1).

In patients who underwent successful biliary cannulation, the mean time of cannulation was 14.1 min in the DGT group and 15.4 min in the TPS group; the difference between the two groups was not statistically significant (Table 1).

Post-ERCP hyperamylasemia and pancreatitis

The overall incidence of post-ERCP hyperamylasemia was 14.7% (5/34) in the DGT group and 16.2% (6/37) in the TPS group. There was no significant difference between the two groups. Post-ERCP pancreatitis developed in 38.2% (13/34) of the DGT group and 10.8% (4/37) of the TPS group. Post-ERCP pancreatitis was significantly higher in the DGT group than in the TPS group ($P = 0.011$). However, most cases of pancreatitis were mild. Moderate or severe pancreatitis developed rarely in both groups (Table 1).

Other complications

One episode of bleeding occurred in the DGT group (2.9%), and two were detected in the TPS group (5.4%). Acute cholangitis developed in 20.6% (7/34) of the DGT group and 5.4% (2/37) of the TPS group. There was no statistically significant difference between the groups in the rates of procedure-related bleeding or cholangitis. However, the incidence of cholangitis in the DGT group was higher than that in the TPS group. Acute cholecystitis and perforation were not detected in any group (Table 1).

DISCUSSION

Several techniques of overcoming difficult biliary cannulation have evolved in the past few years. Needle-knife papillotomy is frequently used to open the biliary entrance and is highly successful when performed by an expert endoscopist. However, the disadvantage of this method is the higher rate of complications, including bleeding, perforation, and pancreatitis. Complication rates of 6% to more than 20% have been reported[2,12,13]. In the 1990s, new techniques for overcoming difficult biliary cannulation, such as DGT, TPS, and wire-guided biliary cannulation over the PD stent, were introduced.

The superior rate of biliary cannulation when using DGT has been attributed to the capability of the pancreatic guidewire to straighten both the PD and BD while at the same time occupying the PD, thus facilitating biliary cannulation and preventing repeated pancreatic cannulation[14,15]. One prospective randomized study reported by Maeda *et al*[8] compared DGT with standard methods, and indicated a higher cannulation success rate (93%) with DGT with no apparent added risk of post-ERCP pancreatitis. However, in the most recent prospective randomized multicenter study by Herreros de Tejada *et al*[6], DGT was not superior to standard cannulation techniques (success rates of 47% and 56%, respectively) and may have been associated with a higher risk of post-ERCP pancreatitis (17% and 8%, respectively).

TPS can facilitate cannulation of the biliary orifice because the cut either opens the BD or runs along the side of the duct, thus exposing the orifice. Akashi *et al*[16] published a prospective series in which TPS was successful in approximately 60% of the patients immediately and in 95% with repeated ERCP. However, the complication rates of TPS were significantly higher than those of standard biliary sphincterotomy (9.9% *vs* 0.8%, $P < 0.001$). In another prospective study by Kahaleh *et al*[17], the primary success rate of pancreatic sphincterotomy was 85%, which, when combined with the needle-knife technique, rose to 95%. The rate of post-ERCP pancreatitis was 8%, which was not different from that of conventional biliary sphincterotomy.

Our results show that the two techniques facilitate selective biliary cannulation with a similar success rate. The initial success rate of DGT in our study was 79.4%, and the final success rate, including the repeat ERCP, was 91.2%. This result is similar to that reported by Maeda *et al*[8] (92.6%), but higher than that reported by Herreros de Tejada *et al*[6] (47%). In the latter study, the difficult cannulation rates were as high as 49.5% and 22%. In comparison, the difficult biliary cannulation rate in our study was lower and more comparable with the experience in high-volume centers. Our difficult biliary cannulation rate was only 7.4%, which represented a truly difficult biliary cannulation group.

In our study, the initial success rate of TPS was 78.4%, and the final success rate was 91.9%. These results are similar to those reported elsewhere[16,17].

The incidence of post-ERCP pancreatitis after DGT was significantly higher than that after TPS (38.2% *vs* 10.8%, $P = 0.011$). Four patients in the DGT group needed special attention. Their total ERCP procedure time was more than 45 min. There were almost twice as many patients with periampullary duodenal diverticulum in the DGT group, but this difference was not statistically significant. Those patients needed more extensive manipulation; therefore, the longer procedure time and the more extensive manipulation might have had an effect on the development of post-ERCP pancreatitis. This may have been another reason for the higher pancreatitis rate in the DGT group. We kept the guidewire inserted into the PD for a longer period of time while attempting selective BD cannulation and removed the PD guidewire after BD cannulation. Conversely, TPS allowed for drainage of the pancreatic juice spontaneously *via* the PD opening, which may have helped decrease the rate of pancreatitis.

The overall post-ERCP pancreatitis rate in this study was 23.9% (17/71). This is similar to those of previous

studies, and most cases of pancreatitis were mild. However, in contrast to the other studies, we did not perform prophylactic PD stenting in any of the cases. The PD stent facilitates biliary cannulation and prevents post-ERCP pancreatitis, but is not usually used in Korea because the health insurance system does not cover the costs of PD stents for prevention of post-ERCP pancreatitis and the financial burden of patients is substantially increased by their use. Another difference is that we did not use any pharmacologic agents for post-ERCP pancreatitis, such as protease inhibitors, which are widely used by Japanese endoscopists. This might have lowered the frequency of post-ERCP pancreatitis in previous studies.

Problems with using a pancreatic guidewire include the potential for injuring branch ducts and the failure to place the guidewire deeply enough into a tortuous main duct. In our study, guidewire insertion into the PD was performed under the guidance of contrast and fluoroscopy to minimize branch duct injury. However, repeated PD injection of contrast is one of the risk factors of post-ERCP pancreatitis.

The incidence of cholangitis after DGT was almost four times higher than that after TPS (20.6% *vs* 5.4%, $P = 0.077$); however, this difference was not statistically significant. Patients with cholangitis had CBD stones or CBD stricture and underwent ENBD insertion. Therefore, we believe that the difference in cholangitis incidence was due to a difference not in methods, but in patients' characteristics. We believe that this is due to *post hoc* fallacy.

Our study has some limitations. First, the number of enrolled patients was relatively small, and the study was conducted in a single tertiary center. We enrolled only 71 patients with difficult cannulation from among a total of 1394 patients (5.1%) who presented for ERCP during the study period. This may have influenced the interpretation of the difference in success and post-ERCP pancreatitis rates. Therefore, a larger study is necessary to overcome this limitation. Second, information regarding the number of PD injections and patients with sphincter of Oddi dysfunction were not collected. This is one of the main limiting factors in drawing a firm conclusion regarding the role of guidewire insertion in the PD in post-ERCP pancreatitis.

In conclusion, we report here successful PD cannulation in cases of difficult BD cannulation. Both the DGT and TPS facilitated biliary cannulation and showed similar success rates. However, the incidence of post-procedure pancreatitis was significantly higher in the DGT group. Therefore, we suggest that the use of TPS when the standard biliary cannulation technique fails and PD cannulation is achieved is effective and has an acceptable complication profile. Further large-scale multicenter studies are needed.

COMMENTS

Background

Selective biliary cannulation is an important step for therapeutic endoscopic retrograde cholangiopancreatography (ERCP). In the hands of experienced endoscopists, successful biliary cannulation rates are higher than 90%. However, in some cases, bile duct (BD) cannulation can be difficult because of special anatomical features, inflammatory processes, and adenomas of the papilla or periampullary diverticulum.

Research frontiers

Double-guidewire technique (DGT) and transpancreatic precut sphincterotomy (TPS) might facilitate biliary cannulation. Most previous studies compared the performance of the new techniques with standard cannulation techniques or precut sphincterotomy. However, there has been no prospective, randomized study of the performance of DGT with TPS in patients with difficult biliary cannulation.

Innovations and breakthroughs

Authors designed this randomized, prospective study to compare the outcomes of DGT and TPS in patients with difficult biliary cannulation. They measured the technical success rates of biliary cannulation, median cannulation time, and procedure related complications.

Applications

Several techniques of overcoming difficult biliary cannulation have evolved in the past few years. Needle-knife papillotomy is frequently used to open the biliary entrance and is highly successful when performed by an expert endoscopist. The superior rate of biliary cannulation when using DGT has been attributed to the capability of the pancreatic guidewire to straighten both the pancreatic duct and BD. TPS can facilitate cannulation of the biliary orifice because the cut either opens the BD or runs along the side of the duct, thus exposing the orifice.

Peer review

Excellent prospective randomized study designed to compare the outcome between two different techniques for endoscopic biliary cannulation. Because this is a randomised controlled trial which showed differences in post ERCP pancreatitis between the 2 methods, it can be published.

REFERENCES

1 **Ramirez FC**, Dennert B, Sanowski RA. Success of repeat ERCP by the same endoscopist. *Gastrointest Endosc* 1999; **49**: 58-61 [PMID: 9869724 DOI: 10.1016/S0016-5107(99)704463]

2 **Freeman ML**, Nelson DB, Sherman S, Haber GB, Herman ME, Dorsher PJ, Moore JP, Fennerty MB, Ryan ME, Shaw MJ, Lande JD, Pheley AM. Complications of endoscopic biliary sphincterotomy. *N Engl J Med* 1996; **335**: 909-918 [PMID: 8782497 DOI: 10.1056/NEJM199609263351301]

3 **Vandervoort J**, Soetikno RM, Tham TC, Wong RC, Ferrari AP, Montes H, Roston AD, Slivka A, Lichtenstein DR, Ruymann FW, Van Dam J, Hughes M, Carr-Locke DL. Risk factors for complications after performance of ERCP. *Gastrointest Endosc* 2002; **56**: 652-656 [PMID: 12397271 DOI: 10.1067/mge.2002.129086]

4 **Masci E**, Mariani A, Curioni S, Testoni PA. Risk factors for pancreatitis following endoscopic retrograde cholangiopancreatography: a meta-analysis. *Endoscopy* 2003; **35**: 830-834 [PMID: 14551860 DOI: 10.1055/s-2003-42614]

5 **Dumonceau JM**, Devière J, Cremer M. A new method of achieving deep cannulation of the common bile duct during endoscopic retrograde cholangiopancreatography. *Endoscopy* 1998; **30**: S80 [PMID: 9826155]

6 **Herreros de Tejada A**, Calleja JL, Díaz G, Pertejo V, Espinel J, Cacho G, Jiménez J, Millán I, García F, Abreu L. Double-guidewire technique for difficult bile duct cannulation: a multicenter randomized, controlled trial. *Gastrointest Endosc* 2009; **70**: 700-709 [PMID: 19560764 DOI: 10.1016/j.gie.2009.03.031]

7 **Goff JS**. Common bile duct pre-cut sphincterotomy: transpancreatic sphincter approach. *Gastrointest Endosc* 1995; **41**: 502-505 [PMID: 7615231 DOI: 10.1016/S0016-5107(05)80011-2]

8 **Maeda S**, Hayashi H, Hosokawa O, Dohden K, Hattori M, Morita M, Kidani E, Ibe N, Tatsumi S. Prospective randomized pilot trial of selective biliary cannulation using pancreatic guide-wire placement. *Endoscopy* 2003; **35**: 721-724 [PMID: 12929017 DOI: 10.1055/s-2003-41576]

9 **Wang P**, Zhang W, Liu F, Li ZS, Ren X, Fan ZN, Zhang X, Lu NH, Sun WS, Shi RH, Li YQ, Zhao Q. Success and complication rates of two precut techniques, transpancreatic sphincterotomy and needle-knife sphincterotomy for bile duct cannulation. *J Gastrointest Surg* 2010; **14**: 697-704 [PMID: 20054659 DOI: 10.1007/s11605-009-1134-x]

10 **Halttunen J**, Keränen I, Udd M, Kylänpää L. Pancreatic sphincterotomy versus needle knife precut in difficult biliary cannulation. *Surg Endosc* 2009; **23**: 745-749 [PMID: 18649101 DOI: 10.1007/s00464-008-0056-0]

11 **Ito K**, Fujita N, Noda Y, Kobayashi G, Obana T, Horaguchi J, Takasawa O, Koshita S, Kanno Y, Ogawa T. Can pancreatic duct stenting prevent post-ERCP pancreatitis in patients who undergo pancreatic duct guidewire placement for achieving selective biliary cannulation? A prospective randomized controlled trial. *J Gastroenterol* 2010; **45**: 1183-1191 [PMID: 20607310 DOI: 10.1007/s00535-010-0268-7]

12 **Freeman ML**, Guda NM. ERCP cannulation: a review of reported techniques. *Gastrointest Endosc* 2005; **61**: 112-125 [PMID: 15672074 DOI: 10.1016/S0016-5107(04)02463-0]

13 **Freeman ML**. Complications of endoscopic biliary sphincterotomy: a review. *Endoscopy* 1997; **29**: 288-297 [PMID: 9255535 DOI: 10.1055/s-2007-1004193]

14 **Gyökeres T**, Duhl J, Varsányi M, Schwab R, Burai M, Pap A. Double guide wire placement for endoscopic pancreaticobiliary procedures. *Endoscopy* 2003; **35**: 95-96 [PMID: 12510238 DOI: 10.1055/s-2003-36403]

15 **Gotoh Y**, Tamada K, Tomiyama T, Wada S, Ohashi A, Satoh Y, Higashizawa T, Miyata T, Ido K, Sugano K. A new method for deep cannulation of the bile duct by straightening the pancreatic duct. *Gastrointest Endosc* 2001; **53**: 820-822 [PMID: 11375604 DOI: 10.1067/mge.2001.113387]

16 **Akashi R**, Kiyozumi T, Jinnouchi K, Yoshida M, Adachi Y, Sagara K. Pancreatic sphincter precutting to gain selective access to the common bile duct: a series of 172 patients. *Endoscopy* 2004; **36**: 405-410 [PMID: 15100948 DOI: 10.1055/s-2004-814315]

17 **Kahaleh M**, Tokar J, Mullick T, Bickston SJ, Yeaton P. Prospective evaluation of pancreatic sphincterotomy as a precut technique for biliary cannulation. *Clin Gastroenterol Hepatol* 2004; **2**: 971-977 [PMID: 15551249 DOI: 10.1016/S1542-3565(04)00484-7]

P- Reviewers Vege SS, Grande L **S- Editor** Gou SX
L- Editor A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i1.115

World J Gastroenterol 2013 January 7; 19(1): 115-121
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Vanishing bile duct syndrome in human immunodeficiency virus infected adults: A report of two cases

Ana Paula Oppenheimer, Christopher Koh, Mary McLaughlin, John C Williamson, Thomas D Norton, Jennifer Laudadio, Theo Heller, David E Kleiner, Kevin P High, Caryn G Morse

Ana Paula Oppenheimer, John C Williamson, Kevin P High, Section of Infectious Diseases, Department of Medicine, Wake Forest School of Medicine, Winston-Salem, NC 27109-7205, United States
Christopher Koh, Theo Heller, Liver Diseases Branch, National Institute of Diabetes, Digestive and Kidney Diseases, National Institutes of Health Clinical Center, Bethesda, MD 20892-1403, United States
Mary McLaughlin, Laboratory of Immunoregulation, National Institute of Allergy and Infectious Diseases, National Institutes of Health Clinical Center, Bethesda, MD 20892-1403, United States
Thomas D Norton, Division of Infectious Diseases and Immunology, Department of Medicine, New York University School of Medicine, New York, NY 10016, United States
Jennifer Laudadio, Laboratory of Pathology, Wake Forest School of Medicine, Winston-Salem, NC 27109-7205, United States
David E Kleiner, Laboratory of Pathology, National Cancer Institute, National Institutes of Health Clinical Center, Bethesda, MD 20892-1403, United States
Caryn G Morse, Department of Critical Care Medicine, National Institutes of Health Clinical Center, Bethesda, MD 20892-1403, United States
Author contributions: Oppenheimer AP, Koh C, McLaughlin M, Williamson JC, Norton TD, Heller T, High KP and Morse CG provided clinical support for the patients; Laudadio J and Kleiner DE performed pathological examinations; Oppenheimer AP, Williamson JC, and Morse CG analyzed the data; Oppenheimer AP, Koh C Morse CG wrote the paper; Williamson JC and High KP provided critical revision.
Supported by The Intramural Research Programs of the National Institutes of Health Clinical Center and the National Institute of Allergy and Infectious Diseases
Correspondence to: Caryn G Morse, MD, MPH, Department of Critical Care Medicine, National Institutes of Health Clinical Center, 9000 Rockville Pike, Building 10, 5A06, MSC 1403, Bethesda, MD 20892-1403, United States. cmorse@mail.nih.gov
Telephone: +1-301-4966028 Fax: +1-301-4021137
Received: March 13, 2012 Revised: July 2, 2012
Accepted: August 3, 2012
Published online: January 7, 2013

Abstract

Vanishing bile duct syndrome (VBDS) is a group of rare disorders characterized by ductopenia, the progressive destruction and disappearance of intrahepatic bile ducts leading to cholestasis. Described in association with medications, autoimmune disorders, cancer, transplantation, and infections, the specific mechanisms of disease are not known. To date, only 4 cases of VBDS have been reported in human immunodeficiency virus (HIV) infected patients. We report 2 additional cases of HIV-associated VBDS and review the features common to the HIV-associated cases. Presentation includes hyperbilirubinemia, normal liver imaging, and negative viral and autoimmune hepatitis studies. In HIV-infected subjects, VBDS occurred at a range of CD4+ T-cell counts, in some cases following initiation or change in antiretroviral therapy. Lymphoma was associated with two cases; nevirapine, antibiotics, and viral co-infection were suggested as etiologies in the other cases. In HIV-positive patients with progressive cholestasis, early identification of VBDS and referral for transplantation may improve outcomes.

© 2013 Baishideng. All rights reserved.

Key words: Human immunodeficiency virus; Antiretroviral therapy; Vanishing bile duct syndrome; Ductopenia; Liver biopsy

Oppenheimer AP, Koh C, McLaughlin M, Williamson JC, Norton TD, Laudadio J, Heller T, Kleiner DE, High KP, Morse CG. Vanishing bile duct syndrome in human immunodeficiency virus infected adults: A report of two cases. *World J Gastroenterol* 2013; 19(1): 115-121 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i1/115.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i1.115

INTRODUCTION

Vanishing bile duct syndrome (VBDS) is a term that refers to a group of rare disorders characterized by progressive destruction and disappearance of the intrahepatic bile ducts leading to intrahepatic cholestasis[1]. VBDS has been described in association with autoimmune disorders, medications, cancer, transplantation, and infection, though idiopathic cases have also been described[2]. Immune mediated mechanisms have been proposed[3]. The prognosis is poor with progression to biliary cirrhosis in the majority of cases.

To date only 4 previous cases in human immunodeficiency virus (HIV) infected adults have been reported[2-5]. Antiretroviral therapy (ART) has been implicated as causal in three of the cases[2,4,5]. Here we report two additional cases of biliary ductopenia consistent with VBDS seen in HIV-infected adults cared for at two academic hospitals in 2010. The previously reported cases are reviewed and the potential etiologies, pathogenesis, management and prognosis for VBDS in HIV-infected adults are discussed.

CASE REPORT

Case 1

A 41-year-old man with HIV infection, diagnosed in 1989, was referred for evaluation of a 9-wk history of progressive jaundice. ART was initiated in 1999 with adherence to a regimen of tenofovir, didanosine, atazanavir, and ritonavir for at least 9 mo prior to presentation. CD4+ count was 304 cells/μL and HIV-1 viral load (VL) < 75 copies/mL two months prior to symptoms. He was taking no other medications and had no history of opportunistic infections (OI) or pancreatitis.

Eight weeks prior to referral he was evaluated for a one-week history of fatigue, nausea, abdominal pain, and scleral icterus. He denied new medications, alcohol or drug use. Laboratory studies revealed elevated alkaline phosphatase (AP), aspartate aminotransferase (AST), alanine aminotransferase (ALT), and total bilirubin (TB) 4.7 mg/L. Lipase was elevated at 3571 U/L. All studies were within normal ranges two months earlier. Abdominal computed tomography (CT) scan showed pancreatic enlargement consistent with pancreatitis and a normal appearing liver. Because of concern for ART-associated pancreatitis and liver injury, ART was held and he received nothing by mouth. Magnetic resonance imaging and magnetic retrograde cholangio-pancreatography (MRI/MRCP) of the liver were normal. Subsequently, his nausea and abdominal pain improved, lipase trended down, and his diet was successfully advanced. He was discharged on the third day of hospitalization.

Five days later, he reported worsening fatigue and new generalized pruritus. Laboratory studies showed worsening hyperbilirubinemia (TB 14.3 mg/dL). He was referred to the National Institutes of Health (NIH) for further evaluation.

On admission to the NIH, he was febrile (38.2 ℃) with laboratory studies consistent with progressive cholestasis. CD4+ count was 164 cells/μL and HIV VL was 33 945 copies/mL. Viral hepatitis and autoimmune studies were negative. Epstein Barr virus (EBV) polymerase chain reaction (PCR) was low positive (660 genome equivalents/mL) and cytomegalovirus (CMV) PCR was low positive (< 250 genome equivalents/mL) in blood.

Liver biopsy revealed marked bile duct paucity associated with moderate inflammation, cholestasis, injury of residual ducts, and perivenular hepatocyte dropout with fibrosis (Figure 1B). Less than 10% of portal areas showed an intact duct, confirming the diagnosis of VBDS. No viral inclusions or granulomas were seen. Fungal and acid fast bacilli (AFB) staining were negative. Liver biopsy was negative for CMV by PCR and immunohistochemistry, and low positive for EBV by PCR, however in situ hybridization for EBV was negative. Electron microscopy showed mitochondrial injury, presumably secondary to the patient's prior ART (Figure 1D).

ART was reinitiated three weeks after admission with lamivudine, etravirine, raltegravir and enfuvirtide[6]. Four weeks later, his CD4+ count was 133 (12%) and HIV VL < 50. The patient's hospital course was complicated by pneumococcal sepsis, small bowel intussusception requiring surgical resection complicated by an anastomotic bleed, and renal failure. TB remained elevated, peaking at 45.6 mg/dL, and he was referred for liver transplant evaluation.

Due to poor nutritional and functional status, the patient was not deemed a candidate for liver transplant and was discharged to hospice care. He died two weeks later. Autopsy identified hepatomegaly with marked bile duct paucity, splenomegaly and bloody ascites. Classic Hodgkin's lymphoma, lymphocyte depleted subtype, was found in the lymph nodes of the neck and mediastinum as well as in the gallbladder and common bile ducts (Figure 1E).

Case 2

A 39-year-old African-American male with HIV, diagnosed in 1996, and a history of toxoplasmosis, disseminated *Mycobacterium avium* complex (MAC), seizure disorder, and medication non-compliance, presented with confusion. Evaluation revealed acute renal failure, hyperbilirubinemia and moderately elevated transaminases. Three months earlier, his CD4+ count was 190 cells/μL, HIV VL 136 copies/mL, AP 154 U/L, with normal serum transaminases and bilirubin. His medications included tenofovir, emtricitabine, lopinavir/ritonavir, dapsone, azithromycin, ethambutol, and divalproex. Two weeks prior, he received an unknown antibiotic for a sinus infection.

At presentation, he was awake but not following commands. Examination revealed jaundice and bibasilar rales. Laboratory studies showed pancytopenia, acute renal failure, elevated AP, AST and ALT, with TB 10 mg/dL. CT of the chest, abdomen and pelvis showed bibasilar lung infiltrates and hepatomegaly. He received

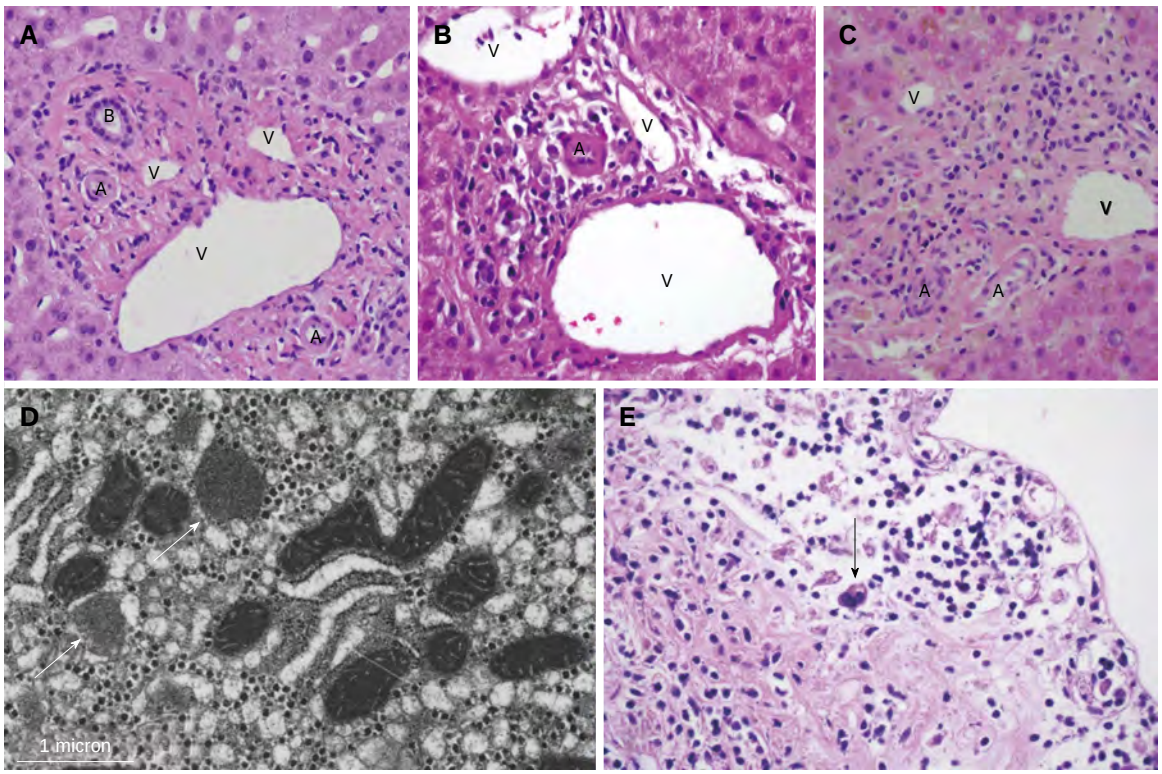


Figure 1 Liver core biopsy. A: Portal tract from normal liver for comparison; B: Portal inflammation and absence of bile duct from the portal area from case 1; C: Portal area showing absence of bile ducts and associated infiltration of lymphocytes and plasma cells from case 2 (HE, 600 ×); D: Electron microgram, case 1. Evidence of mitochondrial toxicity was noted on electron microscopy. Some mitochondria showed loss of cristae, as well as loss of inner and outer membranes, and an amorphous appearance to the mitochondrial matrix (arrows). Similar mitochondrial changes have been described previously in cases of nucleoside reverse transcriptase inhibitor-related hepatotoxicity. Courtesy of Dr. Maria Tsokos, National Cancer Institute Laboratory of Pathology; E: Involvement of the right hepatic bile duct by Hodgkin's Disease seen on autopsy from case 1. A Reed-Sternberg cell (arrow) is seen within the infiltrate beneath the autolysed bile duct (HE, 600×). V: Vein; A: Artery; B: Bile duct.

cefepime for pneumonia and hemodialysis for metabolic acidosis and uremia. His prior medications were held.

MRI/MRCP showed borderline small hepatic ducts with ductal irregularity in the right lobe without definitive obstruction. Liver biopsy showed ductopenia and intrahepatic and canalicular cholestasis. Of eleven portal tracts identified, none contained bile ducts. Where a bile duct would be expected, a mild lymphoplasmacytic infiltrate was seen without resultant expansion of the portal tract or associated piecemeal necrosis (Figure 1C). Fungal and AFB staining were negative. PCR for EBV DNA was low level positive, though no viral cytopathic effect was seen. Immunohistochemistry was negative for CMV or herpes simplex virus.

With supportive care, the patient's mental status improved. ART was reinitiated with etravirine, raltegravir, and ritonavir-boosted darunavir to improve his immune function and prevent recurrent or new OI. Secondary MAC and toxoplasmosis prophylaxis were restarted with ethambutol, azithromycin, and atovaquone. TB remained stable at 25 mg/dL. After discharge, he refused to take his medications and died 3 wk later of multisystem organ failure. Autopsy was not performed.

Summary of other reported cases

Case 3 was a 28-year-old African American female, 31-wk pregnant, who presented with jaundice four weeks after initiating ART with zidovudine, lamivudine, and nevirapine[4]. Prior to initiation of ART, CD4+ count was 234 cells/μL, and VL of 35 853 copies/mL. Liver biopsy showed marked ductopenia and cholestasis. ART was discontinued and the patient was treated with ursodeoxycholic acid. A repeat liver biopsy obtained 8 wk after presentation showed worsening cholestasis and persistent ductopenia. She was referred and listed for liver transplantation, however her cholestasis improved and she was ultimately removed from the transplant list (personal communication, Dr. Rajan Kochar, August 10, 2010). The authors felt that drug-induced liver injury from nevirapine was the likely cause of the VBDS.

Case 4 was a 39-year-old female with advanced acquired immunodeficiency syndrome (AIDS), hepatitis C (HCV) co-infection, and ongoing polysubstance abuse, who presented with one week of scleral icterus, pruritus, and abdominal pain[3]. She was not receiving ART and her CD4+ count was 7 cells/μL and VL 721 000 at presentation. CMV PCR in the blood was positive. Liver biopsy showed marked ductopenia and associated granulomatous inflammation. The authors imputed CMV as the culprit for VBDS, recognizing the potential contribution of HCV co-infection. The patient died one month after presentation

Table 1 Selected medications associated with chronic cholestasis and vanishing bile duct syndrome

Medication class	Medication
Antimicrobials	Penicillins
	Fluroquinolones
	Trimethoprim-sulfamethoxazole
	Azithromycin
	Tetracyclines
	Clindamycin
	Carbapenems
	Itraconazole
	Terbinafine
Antiretrovirals	Nevirapine
Anticonvulsants	Carbamazepine
	Valproic acid
NSAIDS	Ibuprofen
Sex steroids	Testosterone
Antidepressants	Amitriptyline
Antipsychotics	Risperidone
Immunomodulating	Interleukin-2
Anti-lipid Agents	Atorvastatin
	Fenofibrate

NSAIDS: Nonsteroidal antiinflammatory drugs.

Case 5 was a 25-year-old African male with advanced AIDS (CD4+ 108 cells/μL, VL 97 100 copies/mL) who presented with confusion, vomiting and jaundice two weeks after starting zidovudine, lamivudine and efavirenz[2]. ART was discontinued and he received ciprofloxacin and clarithromycin for pneumonia. Three weeks later, ART was reinitiated with tenofovir, lamivudine and nevirapine. One week later, he was readmitted with fever and worsening jaundice. Liver biopsy showed bile duct damage associated with minimal inflammation and absence of ducts in 40% of the visualized portal tracts. His clinical presentation, thought to be consistent with VBDS, was attributed to medications, especially nevirapine, since his clinical deterioration occurred after starting ART. He was referred for liver transplantation evaluation but died approximately one month after referral.

Case 6, available only in abstract form, was a 41-year-old male with HIV, previously well-controlled on a regimen of zidovudine, lamivudine, abacavir and tenofovir[5]. After his HIV RNA increased from < 50 to 454 copies/mL, his ART regimen was changed to abacavir, lamivudine and nevirapine. Liver enzymes prior to ART change were normal. Four weeks later, he developed jaundice and abdominal pain. Liver biopsy showed lack of bile ducts involving all portal tracts with lymphocytic infiltration without eosinophils. Given the temporal association with initiation of nevirapine, nevirapine-induced VBDS was imputed. In subsequent follow-up, the patient was diagnosed with lymphoma and died within a year of VBDS diagnosis (personal communication, Dr. Judith Berger, May 2, 2011).

DISCUSSION

VBDS is a rare cause of progressive cholestasis, most commonly described with drugs but also seen with infection, in cancer and cancer therapy, and in liver diseases such as allograft rejection, graft-versus-host disease, primary biliary cirrhosis and primary sclerosing cholangitis. Diagnosis is made by liver biopsy when interlobular and septal bile ducts cannot be identified in at least 50% of the portal tracts in a biopsy that contains at least 10 portal tracts for assessment[7].

Clinical presentation, similar in HIV-infected and non-HIV-infected patients, varies, with some cases presenting shortly after drug exposure with acute jaundice[4] and others appearing with cholestatic cirrhosis and complications of portal hypertension[7]. Evaluation should include a detailed medication history to identify possible drug causes of cholestasis, laboratory testing to rule out viral and autoimmune hepatitis and primary biliary cirrhosis, and imaging to rule out extrahepatic biliary obstruction. Liver biopsy is required for diagnosis.

The 6 cases in HIV-infected patients described to date share many risk factors for VBDS reported previously in non-HIV patients, including immunocompromise, multiple medication exposures, viral co-infection, and Hodgkin's lymphoma. Given the frequency of risk factors for VBDS in HIV-infected populations, it is perhaps surprising that the diagnosis is so rarely reported.

Remarkable features for the HIV-infected patients include near-total bile duct loss at the time of liver biopsy in 5 of the 6 patients described. The majority experienced irreversible progression with 5 of the 6 patients dying within months of diagnosis. Because diagnosis occurred over a range of CD4+ T-cell counts and levels of HIV viremia, it is difficult to conclude that advanced HIV disease is a predisposition. However, ART and associated immune reconstitution may play an underlying role in some cases, with 3 patients appearing shortly after antiretroviral regimen initiation or change.

Drugs

Many drugs with hepatic metabolism are known to cause hepatotoxicity and cholestasis and have been associated with VBDS[8]. The growing list of drugs associated with VBDS includes most antimicrobial classes, anticonvulsants, antipsychotics, antidepressants, antihistamines, NSAIDS, and sex steroids (Table 1). VBDS has also been described as a component of severe drug reactions, such as Stevens-Johnson syndrome (SJS) and toxic epidermal necrolysis[9,10].

The prognosis of drug-induced VBDS appears to be better than with other causes of VBDS with gradual improvement in liver test abnormalities and symptoms described in the majority of reports[11,12]. However, in a portion of patients, the disease is irreversible[10], as seen in 5 of the 6 HIV-positive patients described here.

Drug-induced ductopenia is thought to occur from direct damage caused by toxins or from an immunoallergic reaction where the drug or its metabolites act as haptens that induce an immune response against the biliary epithelium, destroying the intrahepatic bile ducts

and resulting in cholestasis[13]. Histologic descriptions of drug-induced VBDS include marked inflammatory infiltrates, including mononuclear cells, eosinophils, and neutrophils, surrounding bile ducts and accompanied by lobular cholestasis. Sequential biopsies demonstrate decreasing inflammation, increasing cholestasis and progressive ductopenia[13]. Interestingly, there does not appear to be any qualitative histologic difference between cases that improve and those that progress, though reports of ductopenia in allograft rejection suggest that a greater degree of bile duct loss was seen in irreversible cases[14].

Three of the HIV patients were treated with medications described in association with VBDS. Case 1 received empiric treatment for urethritis with ceftriaxone and azithromycin, two medications that could have potentially worsened his underlying liver disease. Case 2 received anti-MAC therapy and an unknown antibiotic for a respiratory infection. His antiepileptic, divalproex, is a pro-drug of valproic acid, a medication reported in association with VBDS. Case 5 received clarithromycin, ciprofloxacin, and ibuprofen. The role of these medications in causing or perpetuating the underlying liver damage is not known.

Though nevirapine has been associated with SJS and hepatoxicity, the 3 reports of VBDS in the setting of nevirapine use did not have features of SJS. However, given the temporal association of nevirapine with VBDS in cases 3 and 6, it has been added to the list of potentially causative agents.

Infection

Bile duct loss has been described in association with viral infections, most frequently HCV[15,16] and CMV,[17,18] less frequently HBV[19] and EBV[20], with most published cases occurring in the setting of transplant or other immunocompromised states.

Cholestatic presentation of HCV is well-described in liver transplant recipients[21], however ductopenia is infrequently observed in patients with chronic HCV. Rarely, this bile duct damage is associated with features of chronic cholestasis or progressive bile duct loss[16]. The mechanism of bile duct damage with HCV is not known. Proposed mechanisms include a direct cytopathic effect of HCV (HCV has been identified in bile duct epithelium[22]) and immune-mediated destruction[16]. Thus, HCV co-infection may have played a role in case 4, though other etiologies including advanced untreated HIV infection, CMV infection, and ongoing alcohol and substance abuse may also have contributed.

CMV infection is the best characterized viral cause of bile duct injury, both in neonates and adults[23,24], and is the most common viral cause of VBDS in liver transplants recipients[25]. The exact mechanism of CMV-related duct injury is not known; infection of the biliary epithelium or immune-mediated mechanisms have been proposed. Bile duct injury with CMV viral inclusions in the biliary epithelium has been shown in neonatal hepatitis and with epithelioid granulomas in adults[26]. One series of VBDS in liver transplant identified ongoing CMV replication in hepatocytes resulting in cholangiocyte death[25].

Case 4, a patient with advanced HIV disease not receiving ART and chronic HCV, had a positive CMV PCR in blood; no CMV studies were reported on the liver biopsy, however granulomatous inflammation was seen. The authors proposed CMV infection as the probable cause of the patient's VBDS. Cases 1 and 2 had low-level positive CMV PCR in blood, however liver biopsy found no evidence of CMV cytopathic effect. Cases 1 and 2 also presented with elevations in lipase consistent with pancreatitis, the etiology of which was not determined. CMV is a recognized cause of pancreatitis in immunocompromised and immunocompetent hosts[27,28], so CMV infection may have played a role in the development of VBDS in these cases.

A role for anti-CMV therapy for the treatment of CMV-associated ductopenic liver disease has not been established. Antiviral treatment in neonatal cholestatic hepatitis remains controversial[29]. The use of antiviral prophylaxis in transplant settings is associated with a decrease in early CMV disease and allograft rejection[30], however the impact on vanishing bile duct syndrome has not been shown.

Lymphoma

Many VBDS cases are associated with cancer and cancer therapy, frequently in association with Hodgkin's lymphoma[31]. Postulated mechanisms for VBDS in lymphoma include lymphoma-induced toxic cytokine release, resulting in bile duct damage either directly or through recruitment of other effector cells, or occult infiltration[31]. Though remission and improvement in cholestasis have been described with lymphoma therapy, the prognosis for VBDS and lymphoma is poor, in part because of chemotherapy dose reduction required to minimize further hepatotoxicity[31,32].

Two of the cases reported here, cases 1 and 6, were diagnosed with lymphoma after diagnosis of VBDS, a pattern of presentation described in non-HIV infected patients[32]. Lymphoma may have played a role in the other fatal HIV-related cases; however, post-mortem examination was not available for cases 2, 4 and 5. Given the high rate of lymphoma in HIV-infected adults[33], suspicion for incident lymphoma in HIV patients with VBDS should be high.

EBV infection could potentially contribute to the development of both lymphoma and VBDS. One patient with Hodgkin's lymphoma (case 1) was EBV DNA PCR positive in blood and liver, though EBV was not identified by in situ hybridization in liver.

Prognosis and treatment

Resolution of VBDS has been reported, both spontaneously[14,34] and in association with immunosuppressants[35,36] and ursodeoxycholic acid[9,37]. In cases of

possible drug-induced cholestasis, withdrawal of the drug is recommended, however in HIV-infected patients receiving ART, the risk of viral rebound and immune decline must be weighed against potential benefit of ART discontinuation. The majority of cases progress despite these interventions, however. In progressive cases, liver transplantation is the only potentially curative option. Successful transplantation has been described in idiopathic biliary ductopenia[38], however, recurrence of disease is frequently reported in patients undergoing liver transplant for chronic cholestatic conditions and with transplant-related ductopenia[39]. For HIV-infected adults access to liver transplant remains restricted, though data suggests that HIV-infected patients with non-viral hepatitis associated liver disease have a better prognosis than those with viral hepatitis[40].

In summary, VBDS is a rare cholestatic liver disease that is usually fatal in HIV-positive patients as a result of progressive destruction and disappearance of intrahepatic bile ducts leading to liver failure. Investigations should be aimed at potential associations of VBDS in HIV-positive patients, including medications, co-infection and lymphoma. Given the association of VBDS with Hodgkin's lymphoma, and the increased incidence of lymphoma in HIV-positive patients, evaluation for lymphoma is reasonable. In HIV-positive patients presenting with an acute cholestatic clinical picture, a high index of suspicion for VBDS, early diagnosis *via* liver biopsy, and referral to a liver transplant center, may improve clinical outcomes.

ACKNOWLEDGMENTS

The authors thank Dr. Joseph A Kovacs, NIH Clinical Center, Bethesda, MD, for his helpful comments on an earlier version of the manuscript and assistance with optimizing the images included in Figure 1.

REFERENCES

1 **Reau NS**, Jensen DM. Vanishing bile duct syndrome. *Clin Liver Dis* 2008; **12**: 203-217 [PMID: 18242505]
2 **Aldeen T**, Davies S. Vanishing bile duct syndrome in a patient with advanced AIDS. HIV Med 2007; 8: 70-72. *HIV Med* 2007; **8**: 573-574 [PMID: 17944694]
3 **Hindupur S**, Yeung M, Shroff P, Fritz J, Kirmani N. Vanishing bile duct syndrome in a patient with advanced AIDS. *HIV Med* 2007; **8**: 70-72 [PMID: 17305935]
4 **Kochar R**, Nevah MI, Lukens FJ, Fallon MB, Machicao VI. Vanishing bile duct syndrome in human immunodeficiency virus: nevirapine hepatotoxicity revisited. *World J Gastroenterol* 2010; **16**: 3335-3338 [PMID: 20614492]
5 **Stein DF**, Stein B, Halton P, Tirelli R, Meyers J, Berger J, Hwang R, Kim Y, Momeni M, Culliford A. Nevirapine (NVR) Induced Vanishing Bile Duct Syndrome: A Case Report. *Am J Gastroenterol* 2006; **101:** S310-S311 [DOI:10.1111/j.1572-0241.2006.00001_4.x]
6 **Pau AK**, Penzak SR, Boyd SD, McLaughlin M, Morse CG. Impaired maraviroc and raltegravir clearance in a human immunodeficiency virus-infected patient with end-stage liver disease and renal impairment: a management dilemma. *Pharmacotherapy* 2012; **32**: e1-e6 [PMID: 22392831 DOI: 10.1002/PHAR.1003]
7 **Ludwig J**, Wiesner RH, LaRusso NF. Idiopathic adulthood ductopenia. A cause of chronic cholestatic liver disease and biliary cirrhosis. *J Hepatol* 1988; **7**: 193-199 [PMID: 3057064]
8 **Desmet VJ**. Vanishing bile duct syndrome in drug-induced liver disease. *J Hepatol* 1997; **26** Suppl 1: 31-35 [PMID: 9138126]
9 **Taghian M**, Tran TA, Bresson-Hadni S, Menget A, Felix S, Jacquemin E. Acute vanishing bile duct syndrome after ibuprofen therapy in a child. *J Pediatr* 2004; **145**: 273-276 [PMID: 15289784 DOI: 10.1016/j.jpeds.2004.05.027]
10 **Juricic D**, Hrstic I, Radic D, Skegro M, Coric M, Vucelic B, Francetic I. Vanishing bile duct syndrome associated with azithromycin in a 62-year-old man. *Basic Clin Pharmacol Toxicol* 2010; **106**: 62-65 [PMID: 19906050]
11 **Gökçe S**, Durmaz O, Celtik C, Aydogan A, Güllüoglu M, Sökücü S. Valproic acid-associated vanishing bile duct syndrome. *J Child Neurol* 2010; **25**: 909-911 [PMID: 20388938]
12 **Vuppalanchi R**, Chalasani N, Saxena R. Restoration of bile ducts in drug-induced vanishing bile duct syndrome due to zonisamide. *Am J Surg Pathol* 2006; **30**: 1619-1623 [PMID: 17122520 DOI: 10.1097/01.pas.0000213342.78475.48]
13 **Degott C**, Feldmann G, Larrey D, Durand-Schneider AM, Grange D, Machayekhi JP, Moreau A, Potet F, Benhamou JP. Drug-induced prolonged cholestasis in adults: a histological semiquantitative study demonstrating progressive ductopenia. *Hepatology* 1992; **15**: 244-251 [PMID: 1735527]
14 **Hubscher SG**, Buckels JA, Elias E, McMaster P, Neuberger JM. Reversible vanishing bile duct syndrome after liver transplantation: report of 6 cases. *Transplant Proc* 1991; **23**: 1415-1416 [PMID: 1989250]
15 **Hoffmann RM**, Günther C, Diepolder HM, Zachoval R, Eissner HJ, Forst H, Anthuber M, Paumgartner G, Pape GR. Hepatitis C virus infection as a possible risk factor for ductopenic rejection (vanishing bile duct syndrome) after liver transplantation. *Transpl Int* 1995; **8**: 353-359 [PMID: 7576016]
16 **Kumar KS**, Saboorian MH, Lee WM. Cholestatic presentation of chronic hepatitis C: a clinical and histological study with a review of the literature. *Dig Dis Sci* 2001; **46**: 2066-2073 [PMID: 11680577]
17 **O'Grady JG**, Alexander GJ, Sutherland S, Donaldson PT, Harvey F, Portmann B, Calne RY, Williams R. Cytomegalovirus infection and donor/recipient HLA antigens: interdependent co-factors in pathogenesis of vanishing bile-duct syndrome after liver transplantation. *Lancet* 1988; **2**: 302-305 [PMID: 2899720]
18 **Martelius T**, Krogerus L, Höckerstedt K, Bruggeman C, Lautenschlager I. Cytomegalovirus infection is associated with increased inflammation and severe bile duct damage in rat liver allografts. *Hepatology* 1998; **27**: 996-1002 [PMID: 9537439]
19 **Umit H**, Unsal G, Tezel A, Soylu AR, Pamuk GE, Turgut B, Demir M, Tucer D, Ermantas N, Cevikbas U. Vanishing bile duct syndrome in a patient with Hodgkin's lymphoma and asymptomatic hepatitis B virus infection. *Acta Gastroenterol Belg* 2009; **72**: 277-278 [PMID: 19637792]
20 **Kikuchi K**, Miyakawa H, Abe K, Fujikawa H, Horiuchi T, Nagai K, Kako M. Vanishing bile duct syndrome associated with chronic EBV infection. *Dig Dis Sci* 2000; **45**: 160-165 [PMID: 10695629]
21 **Narang TK**, Ahrens W, Russo MW. Post-liver transplant cholestatic hepatitis C: a systematic review of clinical and pathological findings and application of consensus criteria. *Liver Transpl* 2010; **16**: 1228-1235 [PMID: 21031537 DOI: 10.1002/lt.22175]
22 **Nouri-Aria KT**, Sallie R, Mizokami M, Portmann BC, Williams R. Intrahepatic expression of hepatitis C virus antigens in chronic liver disease. *J Pathol* 1995; **175**: 77-83 [PMID: 7891230 DOI: 10.1002/path.1711750112]
23 **Lurie M**, Elmalach I, Schuger L, Weintraub Z. Liver findings

in infantile cytomegalovirus infection: similarity to extrahepatic biliary obstruction. *Histopathology* 1987; **11**: 1171-1180 [PMID: 2826325]
24 **Ten Napel HH**, Houthoff HJ, The TH. Cytomegalovirus hepatitis in normal and immune compromised hosts. *Liver* 1984; **4**: 184-194 [PMID: 6087073]
25 **Arnold JC**, Portmann BC, O'Grady JG, Naoumov NV, Alexander GJ, Williams R. Cytomegalovirus infection persists in the liver graft in the vanishing bile duct syndrome. *Hepatology* 1992; **16**: 285-292 [PMID: 1322347]
26 **Nakanuma Y**, Tsuneyama K, Harada K. Pathology and pathogenesis of intrahepatic bile duct loss. *J Hepatobiliary Pancreat Surg* 2001; **8**: 303-315 [PMID: 11521175 DOI: 10.1007/s0053410080303]
27 **Joe L**, Ansher AF, Gordin FM. Severe pancreatitis in an AIDS patient in association with cytomegalovirus infection. *South Med J* 1989; **82**: 1444-1445 [PMID: 2554512]
28 **Oku T**, Maeda M, Waga E, Wada Y, Nagamachi Y, Fujita M, Suzuki Y, Nagashima K, Niitsu Y. Cytomegalovirus cholangitis and pancreatitis in an immunocompetent patient. *J Gastroenterol* 2005; **40**: 987-992 [PMID: 16261436 DOI: 10.1007/s00535-005-1683-z]
29 **Vancíková Z**, Kucerová T, Pelikán L, Zikmundová L, Priglová M. Perinatal cytomegalovirus hepatitis: to treat or not to treat with ganciclovir. *J Paediatr Child Health* 2004; **40**: 444-448 [PMID: 15265185 DOI: 10.1111/j.1440-1754.2004.00430.x]
30 **Rubin RH**, Kemmerly SA, Conti D, Doran M, Murray BM, Neylan JF, Pappas C, Pitts D, Avery R, Pavlakis M, Del Busto R, DeNofrio D, Blumberg EA, Schoenfeld DA, Donohue T, Fisher SA, Fishman JA. Prevention of primary cytomegalovirus disease in organ transplant recipients with oral ganciclovir or oral acyclovir prophylaxis. *Transpl Infect Dis* 2000; **2**: 112-117 [PMID: 11429021]
31 **Hubscher SG**, Lumley MA, Elias E. Vanishing bile duct syndrome: a possible mechanism for intrahepatic cholestasis in Hodgkin's lymphoma. *Hepatology* 1993; **17**: 70-77 [PMID: 7678577]
32 **Pass AK**, McLin VA, Rushton JR, Kearney DL, Hastings CA, Margolin JF. Vanishing bile duct syndrome and Hodgkin disease: a case series and review of the literature. *J Pediatr Hematol Oncol* 2008; **30**: 976-980 [PMID: 19131796 DOI: 10.1097/MPH.0b013e31818b37c4]
33 **Grulich AE**, van Leeuwen MT, Falster MO, Vajdic CM. Incidence of cancers in people with HIV/AIDS compared with immunosuppressed transplant recipients: a meta-analysis. *Lancet* 2007; **370**: 59-67 [PMID: 17617273]
34 **Ramos AM**, Gayotto LC, Clemente CM, Mello ES, Luz KG, Freitas ML. Reversible vanishing bile duct syndrome induced by carbamazepine. *Eur J Gastroenterol Hepatol* 2002; **14**: 1019-1022 [PMID: 12352223]
35 **Jakab SS**, West AB, Meighan DM, Brown RS, Hale WB. Mycophenolate mofetil for drug-induced vanishing bile duct syndrome. *World J Gastroenterol* 2007; **13**: 6087-6089 [PMID: 18023105]
36 **Tajiri H**, Etani Y, Mushiake S, Ozono K, Nakayama M. A favorable response to steroid therapy in a child with drug-associated acute vanishing bile duct syndrome and skin disorder. *J Paediatr Child Health* 2008; **44**: 234-236 [PMID: 18377375]
37 **O'Brien CB**, Shields DS, Saul SH, Reddy KR. Drug-induced vanishing bile duct syndrome: response to ursodiol. *Am J Gastroenterol* 1996; **91**: 1456-1457 [PMID: 8678017]
38 **Rios R**, Herrero JI, Quiroga J, Sangro B, Sola I, Pardo F, Cienfuegos JA, Herraiz M, Prieto J. Idiopathic adulthood ductopenia: long-term follow-up after liver transplantation. *Dig Dis Sci* 2001; **46**: 1420-1423 [PMID: 11478493]
39 **van Hoek B**, Wiesner RH, Ludwig J, Paya C. Recurrence of ductopenic rejection in liver allografts after retransplantation for vanishing bile duct syndrome. *Transplant Proc* 1991; **23**: 1442-1443 [PMID: 1703343]
40 **Mindikoglu AL**, Regev A, Magder LS. Impact of human immunodeficiency virus on survival after liver transplantation: analysis of United Network for Organ Sharing database. *Transplantation* 2008; **85**: 359-368 [PMID: 18301332 DOI: 10.1097/TP.0b013e3181605fda]

P- Reviewers Ohkohchi N, Hackert T **S- Editor** Gou SX
L- Editor A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i1.122

World J Gastroenterol 2013 January 7; 19(1): 122-124
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Atresia of the appendix vermiformis: A rare case of developmental abnormality

Faik Yaylak, Mustafa Cem Algin, Yesim Gungor, Mehmet Korkmaz

Faik Yaylak, Mustafa Cem Algin, Department of General Surgery, Dumlupinar University Faculty of Medicine, 43020 Kutahya, Turkey
Yesim Gungor, Department of General Surgery, Kastamonu Munif Islamoglu State Hospital, 37100 Kastamonu, Turkey
Mehmet Korkmaz, Department of Radiology, Dumlupinar University Faculty of Medicine, 43020 Kutahya, Turkey
Author contributions: Yaylak F and Gungor Y were responsible for the review of the literature and the initial preparation of the paper; Yaylak F, Korkmaz M, and Algin MC prepared the final version of the manuscript.
Correspondence to: Faik Yaylak, MD, Assistant Professor of General Surgery, Department of General Surgery, Dumlupinar University Faculty of Medicine, Merkez Kampus, 43020 Kutahya, Turkey. surg.faikyaylak@yahoo.com
Telephone: +90-274-2652286 Fax: +90-274-2652286
Received: July 12, 2012 Revised: October 15, 2012
Accepted: October 30, 2012
Published online: January 7, 2013

Abstract

Acute appendicitis, which requires immediate surgical intervention, is an important diagnosis in patients with acute abdomen. However, developmental abnormalities may interfere with the preoperative diagnosis and surgical treatment in some cases. Agenesis and atresia of the cecal vermiform appendix is an extremely rare clinical diagnosis. In addition, preoperative diagnosis may be difficult in some cases. Thus, diagnosis of the congenital absence of the vermiform appendix requires a thorough exploration of the retrocecal and ileocecal regions. A 59-year-old male was admitted from the emergency services with right lower abdominal pain. A celiotomy was performed with the suspicion of acute appendicitis. However, an atresia of the vermiform appendix was observed. The patient's appendix was thus removed. Pathological examination confirmed suppurative appendicitis. This case underlines the importance of the clinical entity for surgeons who may deal with a similar case.

© 2013 Baishideng. All rights reserved.

Key words: Appendix vermiformis; Developmental abnormality; Agenesis; Atresia; Acute abdomen

Yaylak F, Algin MC, Gungor Y, Korkmaz M. Atresia of the appendix vermiformis: A rare case of developmental abnormality. *World J Gastroenterol* 2013; 19(1): 122-124 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i1/122.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i1.122

INTRODUCTION

Acute appendicitis is an important diagnosis in patients with acute abdomen, and requires immediate surgical intervention. However, developmental abnormalities of appendix vermiformis may interfere with the preoperative diagnosis and surgical treatment in some cases. Although rare, duplication and agenesis of appendix vermiformis have been reported previously[1-3]. Clinical diagnosis of developmental abnormalities merits a thorough exploration of the abdominal cavity when clinical and surgical findings are atypical[2,4,5]. A definite diagnosis is essential, not only to control the ongoing inflammation due to an overlooked appendix, but also to exclude a cancer misdiagnosis. Here we present an atresia of the vermiform appendix in a patient with acute abdomen.

CASE REPORT

A 59-year-old male was admitted with acute abdomen. His past medical and surgical history was significant for coronary heart disease and a coronary by-pass surgery with two vessels replacement in 2005. His medication included oral anticoagulants and acetaminophen as needed for pain. He denied tobacco, alcohol, or intravenous drug use. He was afebrile and in mild distress at the time of presentation. His abdominal examina-

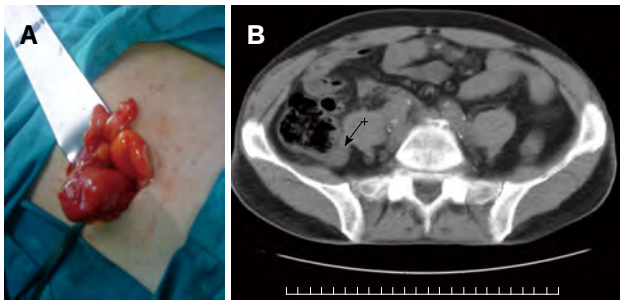


Figure 1 Base of cecum. A: Base of cecum; B: Computed tomography image.

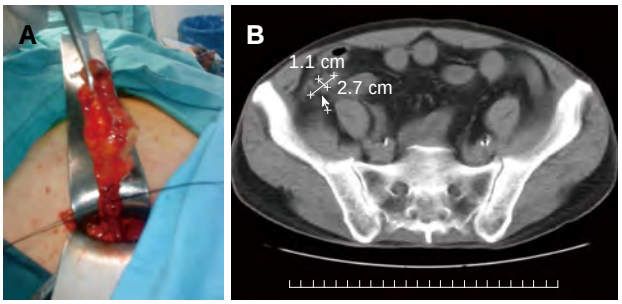


Figure 2 Appendix. A: Appendix; B: Computed tomography image.

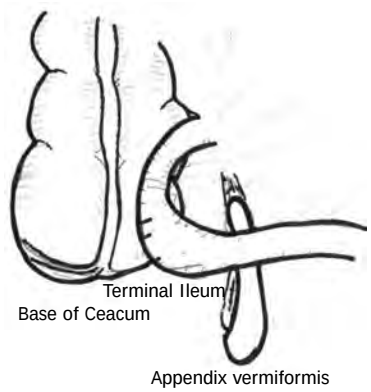


Figure 3 Illustration of the atresia of the appendix vermiformis.

tion was significant for tenderness at McBurney's point with voluntary guarding. Laboratory results revealed an elevated white blood cell count of 12.6 × 10^3/μL and a C-reactive protein level of 5.9 mg/L. Preoperative evaluation of the abdomen with computed tomography (CT) without contrast revealed mildly dilated loops of the small bowel, and questionable appendicitis with periappendiceal inflammation. The patient was thus scheduled for emergent diagnostic celiotomy. The preoperative diagnosis was suspicious for acute perforated appendicitis, and the intention was an appendectomy. Celiotomy was performed through a McBurney's incision. Purulent material in the pericecal and pelvic cavity was observed, and the surgical cavity was cleared with aspiration and irrigation. The cecum and retrocecal region were macroscopically normal in appearance without an appendix vermiformis (Figure 1A). A mass, with a free tip, was observed approximately 10 cm proximal of the ileocecal valve (Figure 2A). The tip of the mass was inflamed and the lumen was ending blindly without a connection to the cecum. The mass was removed after preparation of its mesoappendix-like pedicle (Figure 3). The vascular supply of the mass was the ileocecal artery. Meckel's diverticulum was excluded. After removal of the mass, the peritoneal and pelvic cavities were irrigated with saline, and a drain was placed in the pelvic cavity. Surgical incision was closed primarily. The drain was removed on the fifth postoperative day, and the surgical sutures were removed on the tenth day. Intravenous metronidazole (500 mg twice a day) was begun preoperatively and continued for five days. Analgesics were supplied as the patient needed. Pathological examination confirmed suppurative appendicitis. The initial surgical course was uneventful, but on the third day the patient was recognized to have a decrease in his speech ability and a loss of strength in his left leg. The end diagnosis was a transient ischemic attack from which the patient recovered after medical treatment with piracetam for five days and anticoagulation.

DISCUSSION

Developmental abnormalities of the appendix vermiformis, such as alterations in localization, duplication, or agenesis, may complicate the clinical and surgical presentation of patients with acute abdomen[2,3]. Thus, meticulous preoperative evaluation and surgical exploration of the abdominal cavity is essential in patients with atypical presentations[6-8]. Preoperative abdominal CT scanning may be helpful in some patients. In our case, an atresia of the appendix vermiformis was observed during surgical exploration. Preoperative abdominal CT images were reviewed with an experienced radiologist after surgery. Abdominal CT findings were found to be conclusive, with a focal increase in the thickness of the posteromedial wall of the cecum (Figure 1B). A tubular mass was noticed inferior to the cecum. There was no connection between this mass and the cecum (Figure 2B). In addition, the surrounding mesenteric fatty tissues were found to be dirty due to inflammation. Thus, this case also underlines the fact that a careful evaluation of abdominal CT images is the important part of the preoperative diagnosis. An experienced radiologist should be available to improve the preoperative diagnostic workup in the management of acute abdomen.

Previous reports with appendiceal developmental abnormalities have suggested an association with cancer[5]. In our experience, preoperative clinical presentation was suspicious for perforated appendicitis. However, a perforation was excluded with the presence of a healthy cecum and the observation of the inflamed mass in the ileocecal region. Cancer was excluded with histopathological examination of the resected mass. Routine histopathological examination should not be ignored in patients with appendicitis in order to exclude possible malign disorders[9].

In conclusion, this case is one of the first reports to demonstrate atresia of the appendix vermiformis. In such difficult cases, a surgeon should remember that a thorough exploration of the abdominal cavity is an essential part of the management of the patients with acute appendicitis. Preoperative diagnosis may be improved with the use of abdominal CT. Possible misdiagnoses may lead to inappropriate and inadequate treatment in patients with acute abdomen.

REFERENCES

1 **Yucel AF**, Pergel A, Kocakusak A, Aydin I, Bagci P, Sahin DA. Multiple caeco-appendiceal fistulas and diverticulosis: a newly defined congenital anomaly of the appendix-report of the first case. *J Gastrointest Surg* 2011; **15**: 2111-2113 [PMID: 21567291 DOI: 10.1007/s11605-011-1487-9]

2 **Sobhian B**, Mostegel M, Kunc C, Karner J. [Appendix vermiformis duplex--a rare surprise]. *Wien Klin Wochenschr* 2005; **117**: 492-494 [PMID: 16091877 DOI: 10.1007/s00508-005-0390-3]

3 **Woywodt A**, Verhaart S, Kiss A. Atresia of the appendix. *J Pediatr Surg* 1998; **33**: 1423-1425 [PMID: 9766372]

4 **Hsu KF**, Yu JC, Chan DC, Liu YC. Atypical acute appendicitis and its complications: a rare location of the appendix in the periduodenum. *Acta Gastroenterol Belg* 2011; **74**: 105 [PMID: 21563667]

5 **Freeman HJ**. Duplicated appendix complicated by appendiceal cancer. *World J Gastroenterol* 2011; **17**: 135-136 [PMID: 21218095 DOI: 10.3748/wjg.v17.i1.135]

6 **Chamisa I**, Nikolov S, Bam TQ. Duplex appendicitis. *S Afr Med J* 2007; **97**: 843 [PMID: 17985053]

7 **Samee A**, Alibhai M, Ameer N, Sparks M, Morgan A, Halliday M. Appendicitis in a duplex appendix mimicking intussusception. *BMJ Case Rep* 2010; **2010** [PMID: 22736757 DOI: 10.1136/bcr.12.2009.2516]

8 **Collins DC**. 71,000 Human appendix specimens. a final report, summarizing forty years' study. *Am J Proctol* 1963; **14**: 265-281 [PMID: 14098730]

9 **Chan W**, Fu KH. Value of routine histopathological examination of appendices in Hong Kong. *J Clin Pathol* 1987; **40**: 429-433 [PMID: 3584486]

P- Reviewers Amin AI, Leitman M **S- Editor** Gou SX
L- Editor Rutherford A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i1.125

World J Gastroenterol 2013 January 7; 19(1): 125-128
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Russell body duodenitis in a patient with retroperitoneal metastasis of ureteral cancer

Yoshihisa Takahashi, Seiichiro Shimizu, Koji Uraushihara, Toshio Fukusato

Yoshihisa Takahashi, Toshio Fukusato, Department of Pathology, Teikyo University School of Medicine, Tokyo 173-8605, Japan
Seiichiro Shimizu, Department of Pathology, Showa General Hospital, Tokyo 187-8510, Japan
Koji Uraushihara, Department of Gastrointestinal Medicine, Showa General Hospital, Tokyo 187-8510, Japan
Author contributions: Takahashi Y and Shimizu S made the pathological diagnosis; Uraushihara K provided the clinical data; Takahashi Y wrote the manuscript; Fukusato T checked and revised the manuscript.
Correspondence to: Yoshihisa Takahashi, MD, Department of Pathology, Teikyo University School of Medicine, 2-11-1 Kaga, Itabashi-ku, Tokyo 173-8605, Japan. ytakaha-tky@umin.ac.jp
Telephone: +81-3-39641211 Fax: +81-3-39649622
Received: May 1, 2012 Revised: September 26, 2012
Accepted: October 16, 2012
Published online: January 7, 2013

Abstract

Russell bodies are globular and eosinophilic inclusion bodies in the cytoplasm of mature plasma cells. Plasma cells whose cytoplasm is filled with Russell bodies are designated as Mott cells. Russell body duodenitis (RBD) is a unique form of chronic duodenitis that is characterized by infiltration of numerous Mott cells. RBD is very rare; only two cases have been reported to date. In this paper, we report a case of RBD in a patient with retroperitoneal metastasis of ureteral cancer. A 77-year-old man was admitted to our hospital complaining of appetite loss, vomiting, and upper abdominal distension. He had undergone left nephroureterectomy for ureteral cancer 4 years earlier. Upper digestive tract endoscopy revealed edema, stenosis, and punctate redness of the mucosa of the duodenum, and a biopsy was performed. Histological analysis showed that numerous Mott cells had infiltrated the lamina propria mucosae, and the condition was diagnosed as RBD. A mass lesion in the retroperitoneum adjacent to the duodenum was detected by abdominal computed tomography, and was diagnosed as metastatic urothelial carcinoma by biopsy. It is possible that chemokines produced by tumor cells caused RBD in this case.

© 2013 Baishideng. All rights reserved.

Key words: Russell body; Duodenitis; Ureteral cancer; Mott cells; Plasma cells

Takahashi Y, Shimizu S, Uraushihara K, Fukusato T. Russell body duodenitis in a patient with retroperitoneal metastasis of ureteral cancer. *World J Gastroenterol* 2013; 19(1): 125-128 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i1/125.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i1.125

INTRODUCTION

Russell bodies are globular and eosinophilic inclusion bodies in the cytoplasm of mature plasma cells, and were first described by Russell[1] in 1890. They correspond to markedly dilated rough endoplasmic reticulum (rER) filled with produced proteins. Plasma cells whose cytoplasm is filled with Russell bodies are designated as Mott cells. Although the appearance of small numbers of Mott cells in chronic inflammation lesions is not rare, the appearance of numerous Mott cells in these lesions is very rare.

In 1998, Tazawa *et al*[2] reported a case of chronic gastritis with the appearance of numerous Mott cells and proposed that such a lesion be designated as "Russell body gastritis (RBG)". Since then, there has been a gradual increase in reported cases of RBG[3-9], and association with *Helicobacter pylori* (*H. pylori*) infection[2,4,6,7], human immunodeficiency virus (HIV) infection[5], and alcohol abuse[2,3,5,8] has been suspected. However, Russell body duodenitis (RBD), which has a similar histological appearance to RBG, is very rare, and only two cases have been reported to date[10,11]. A previously reported case of

RBD occurred in an HIV-positive patient[10]; however, the pathogenesis and clinicopathological characteristics of RBD remain to be determined. In this paper, we report a case of RBD that occurred in a patient with retroperitoneal metastasis of ureteral cancer and discuss the pathogenesis of RBD.

CASE REPORT

A 77-year-old man was admitted to our hospital complaining of appetite loss, vomiting, and upper abdominal distension. He had undergone left nephroureterectomy for ureteral cancer 4 years earlier, and 2 years later, he had undergone chemotherapy (methotrexate + vinblastine + doxorubicin + cisplatin) and radiation therapy (39.6 Gy in total) for paraaortic lymph node metastasis. He did not abuse alcohol, and was HIV-negative. He had not undergone any treatment for *H. pylori*. Edema, stenosis, and punctate redness of the mucosa were observed in the second portion of the duodenum by upper digestive tract endoscopy (Figure 1), and a biopsy was performed. Endoscopic visualization revealed no tumorous lesions in the upper digestive tract mucosa. Computed tomography showed a mass lesion in the retroperitoneum adjacent to the duodenum (Figure 2), and the clinical symptoms were attributed to duodenal obstruction by the mass. Gastrojejunal bypass surgery was performed 2 wk after admission. Biopsy of the retroperitoneal mass was performed during surgery, and the lesion was diagnosed as a metastasis of ureteral cancer. The retroperitoneal metastatic mass subsequently increased in size, and the patient died 2 mo after the surgery. Autopsy was not permitted.

Three biopsy fragments were taken from the second portion of the duodenum. Numerous plasmacytoid cells, whose cytoplasm was filled with eosinophilic inclusion bodies and whose nuclei were eccentrically located, had infiltrated the lamina propria mucosae of one biopsy fragment (Figure 3A and B). Neither nuclear atypia nor mitosis was observed in these cells. These inclusion bodies were positive for periodic acid-Schiff (PAS) staining. Immunohistochemically, the cells were positive for CD138 (Figure 3C), CD38, and CD79a and negative for cytokeratin, confirming that they were plasma cells containing Russell bodies (Mott cells). Although scattered lymphocytes and plasma cells without Russell bodies were also present, these cells showed no nuclear atypia, and no lymphoepithelial lesions were observed. Immunohistochemical staining for κ and λ chains revealed that the infiltrating plasma cells were of polyclonal origin (Figure 3D and E), although Russell bodies showed no obvious positive staining for either antibody. No gastric metaplasia was observed in the epithelium, and no *H. pylori* infection was detected by immunohistochemical staining. The other two duodenal biopsy fragments showed only non-specific chronic inflammation. No cancer invasion was observed in the biopsy fragments. The histological appearance of the biopsy specimen from the retroperitoneal mass was compatible with that of metastatic urothelial carcinoma.

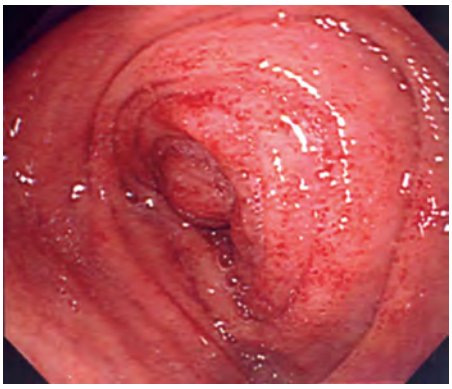


Figure 1 Endoscopic appearance of the duodenum. Edema, stenosis, and punctate redness of mucosa are observed.



Figure 2 Abdominal computed tomography image. A mass lesion is observed in the retroperitoneum adjacent to the duodenum (arrows).

DISCUSSION

The duodenal biopsy in this case helped us to reach a diagnosis of RBD. Differential diagnoses included B-cell lymphomas with plasmacytic differentiation, plasmacytoma, celiac disease, Whipple's disease, and lymphocytic gastroenteritis. Mott cells occasionally appear in B-cell lymphomas with plasmacytic differentiation (particularly mucosa-associated lymphoid tissue lymphoma) and plasmacytoma. However, RBD can be differentiated from B-cell lymphomas and plasmacytoma because no atypical lymphoid or plasmacytoid cells and lymphoepithelial lesions are present and the infiltrating plasma cells show a polyclonal pattern in immunohistochemical staining for κ and λ chains in RBD. In celiac disease, the endoscopy results indicate reduced or absent folds of the small intestinal mucosa, and the histological analysis indicates the accumulation of large fat globules in the surface epithelium. These features of celiac disease were not observed in the present case. In Whipple's disease, the inflammatory cells that aggregate in the lamina propria mucosae of the small intestine are not Mott cells, but large macrophages whose cytoplasm contains large amounts of a diastase-resistant, PAS-positive material. Lymphocytic gastroenteritis is characterized histologically by increased numbers of intraepithelial lymphocytes (mostly cytotoxic T-cells), which were not observed in the present case.

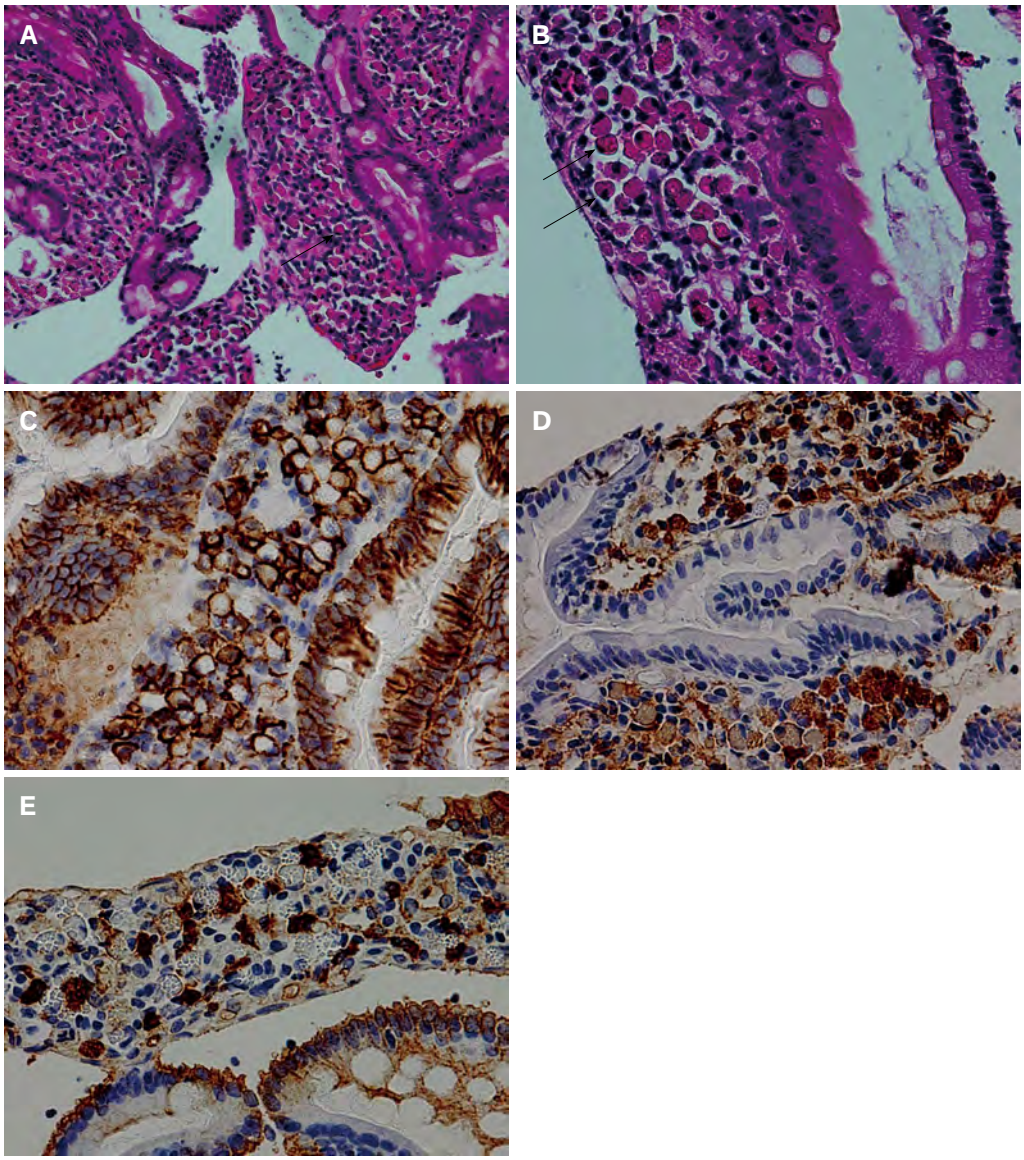


Figure 3 Histological appearance of the duodenal biopsy. A, B: Numerous Mott cells (arrows), whose cytoplasm is filled with eosinophilic inclusion bodies (Russell bodies), infiltrate the lamina propria mucosae (HE stain, A: × 200, B: × 400); C: Immunohistochemically, Mott cells are positive for CD138 (× 400); D, E: Infiltrating plasma cells show a polyclonal pattern upon the immunohistochemical staining for κ (D) and λ (E) chains (× 400).

Russell bodies are eosinophilic inclusions in the cytoplasm of plasma cells. They are situated within the cisternae of the rER and contain non-immunoglobulin molecules, by-products of immunoglobulin synthesis, or some altered form of immunoglobulins that can no longer be recognized by an anti-immunoglobulin antibody[12,13]. They may be formed by excessive production of, or failure in, the secretion of immunoglobulin molecules[12]. The appearance of numerous Mott cells in the lesion in cases of chronic inflammation is very rare, and the diagnosis of RBG/RBD is made when numerous Mott cells appear in a chronic gastritis/duodenitis lesion. RBG/RBD is thought to be a focal condition[2]; the fact that RBD was found in only one of three duodenal biopsy fragments in the present case is consistent with this hypothesis.

Although reported cases of RBG are gradually increasing in number[2-9], RBD is very rare, and only two cases have been reported to date[10,11]. The first reported case of RBD[10] was that of a 55-year-old HIV-positive man whose chief complaint was abdominal pain. The upper digestive tract endoscopy revealed non-specific gastritis and duodenitis. Histologically, gastric metaplasia was observed in the duodenal epithelium, although *H. pylori* infection was not detected. The second reported RBD case[11] was that of a 69-year-old woman. She complained of refractory dysphagia; however, this symptom was thought to be unrelated to the duodenal lesion. Using endoscopy, the RBD lesion was recognized as a nodule within the duodenal bulb. Histological analysis indicated gastric metaplasia in the duodenal epithelium, although neither *H. pylori* nor other discernible microorganisms were identified. The patient in our study was a 77-year-old HIV-negative man, and the clinical symptoms were appetite loss, vomiting, and upper abdominal distension; however, these symptoms were attributed to

obstruction of the duodenum by retroperitoneal metastasis of ureteral cancer. Endoscopic findings were nonspecific inflammation, and no *H. pylori* infection was detected. No gastric metaplasia was detected in the duodenal epithelium in the present case.

H. pylori infection was detected in more than half of the reported cases of RBG[2,4,6,7], and it is estimated that antigenic stimulation by *H. pylori* is an important factor in the etiology of RBG. Alcohol[3,5,8] or analgesic[3] abuse and/or HIV infection[5,10] were reported in the *H. pylori*-negative RBG/RBD cases, and stimulation or immunological abnormality caused by these factors may also be important in the pathogenesis of RBG/RBD. The present patient did not have any of these suggested etiological factors for RBG/RBD. It is noteworthy that metastatic urothelial carcinoma was present in the vicinity of the duodenum in the present case. Several cancer cases with prominent Mott cell proliferation have been reported[14,15], and it is speculated that chemokine production by tumor cells may be the cause of Mott cell accumulation in these cases[14]. Thus, chemokine production by tumor cells may be the cause of RBD in the present case, although cancer invasion was not present in the duodenal mucosa. It is possible that *H. pylori* infection is not important in the RBD pathogenesis, because no *H. pylori* infection has been detected in any of the reported RBD cases, including the present case. However, the pathogenesis of RBG/RBD should be further investigated using larger number of cases and molecular techniques.

In summary, this paper has described a case of RBD occurring in a patient with retroperitoneal metastasis of ureteral cancer. This is the third RBD case to be reported. It is possible that chemokines produced by tumor cells caused RBD in the present patient. It is important that pathologists maintain enough knowledge of the disease to avoid misdiagnosis of the condition as malignant diseases, such as B-cell lymphomas and plasmacytoma. The clinicopathological features of RBD should be further elucidated by continually accumulating and studying reported cases.

REFERENCES

1 **Russell W**. An Address on a Characteristic Organism of Cancer. *Br Med J* 1890; **2**: 1356-1360 [PMID: 20753194]

2 **Tazawa K**, Tsutsumi Y. Localized accumulation of Russell body-containing plasma cells in gastric mucosa with Helicobacter pylori infection: 'Russell body gastritis'. *Pathol Int* 1998; **48**: 242-244 [PMID: 9589496]

3 **Erbersdobler A**, Petri S, Lock G. Russell body gastritis: an unusual, tumor-like lesion of the gastric mucosa. *Arch Pathol Lab Med* 2004; **128**: 915-917 [PMID: 15270606]

4 **Ensari A**, Savas B, Okcu Heper A, Kuzu I, Idilman R. An unusual presentation of Helicobacter pylori infection: so-called "Russell body gastritis". *Virchows Arch* 2005; **446**: 463-466 [PMID: 15744498 DOI: 10.1007/s00428-005-1215-5]

5 **Drut R**, Olenchuk AB. Images in pathology. Russell body gastritis in an HIV-positive patient. *Int J Surg Pathol* 2006; **14**: 141-142 [PMID: 16703175 DOI: 10.1177/106689690601400206]

6 **Paik S**, Kim SH, Kim JH, Yang WI, Lee YC. Russell body gastritis associated with Helicobacter pylori infection: a case report. *J Clin Pathol* 2006; **59**: 1316-1319 [PMID: 17142575 DOI: 10.1136/jcp.2005.032185]

7 **Pizzolitto S**, Camilot D, DeMaglio G, Falconieri G. Russell body gastritis: expanding the spectrum of Helicobacter pylori - related diseases? *Pathol Res Pract* 2007; **203**: 457-460 [PMID: 17395398 DOI: 10.1016/j.prp.2007.01.009]

8 **Habib C**, Gang DL, Ghaoui R, Pantanowitz L. Russell body gastritis. *Am J Hematol* 2010; **85**: 951-952 [PMID: 21108327 DOI: 10.1002/ajh.21702]

9 **Del Gobbo A**, Elli L, Braidotti P, Di Nuovo F, Bosari S, Romagnoli S. Helicobacter pylori-negative Russell body gastritis: case report. *World J Gastroenterol* 2011; **17**: 1234-1236 [PMID: 21448431 DOI: 10.3748/wjg.v17.i9.1234]

10 **Savage NM**, Fortson T, Schubert M, Chamberlain S, Lee J, Ramalingam P. Isolated Russell body duodenitis. *Dig Dis Sci* 2011; **56**: 2202-2204 [PMID: 21234686 DOI: 10.1007/s10620-010-1559-9]

11 **Paniz Mondolfi A**, Samuel M, Kikhney J, Moter A, Feldman D, Slova D, Filatov A, Theise N. Russell body duodenitis: a histopathological and molecular approach to a rare clinical entity. *Pathol Res Pract* 2012; **208**: 415-419 [PMID: 22673188 DOI: 10.1016/j.prp.2012.05.007]

12 **Blom J**, Mansa B, Wilk A. A study of Russell bodies in human monoclonal plasma cells by means of immunofluorescence and electron microscopy. *Acta Pathol Microbiol Scand A* 1976; **84**: 335-349 [PMID: 785941]

13 **Hsu SM**, Hsu PL, McMillan PN, Fanger H. Russell bodies: a light and electron microscopic immunoperoxidase study. *Am J Clin Pathol* 1982; **77**: 26-31 [PMID: 6275697]

14 **Shinozaki A**, Ushiku T, Fukayama M. Prominent Mott cell proliferation in Epstein-Barr virus-associated gastric carcinoma. *Hum Pathol* 2010; **41**: 134-138 [PMID: 19665167 DOI: 10.1016/j.humpath.2009.07.004]

15 **Wolf EM**, Mrak K, Tschmelitsch J, Langner C. Signet ring cell cancer in a patient with Russell body gastritis--a possible diagnostic pitfall. *Histopathology* 2011; **58**: 1178-1180 [PMID: 21707721 DOI: 10.1111/j.1365-2559.2011.03876.x]

P- Reviewers Rodriguez DC, Gupta C **S- Editor** Gou SX
L- Editor Stewart GJ **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i1.129

World J Gastroenterol 2013 January 7; 19(1): 129-132
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Small hepatocellular carcinoma with ring calcification: A case report and literature review

Takashi Murakami, Daisuke Morioka, Hideki Takakura, Yasuhiko Miura, Shinji Togo

Takashi Murakami, Daisuke Morioka, Hideki Takakura, Yasuhiko Miura, Shinji Togo, Department of Surgery, Yokohama Ekisaikai Hospital, Yokohama, Kanagawa 231-0036, Japan

Author contributions: Murakami T, Morioka D and Togo S performed the surgery; Murakami T, Morioka D and Takakura H treated the patient early postoperatively; Miura Y and Togo S were responsible for this patient in the outpatient clinic; Murakami T and Morioka D wrote the manuscript; Takakura H, Miura Y and Togo S revised the manuscript for important intellectual content.

Correspondence to: Daisuke Morioka, MD, PhD, Department of Surgery, Yokohama Ekisaikai Hospital, 1-2 Yamadacho, Naka-ku, Yokohama, Kanagawa 231-0036, Japan. dmorioka@hotmail.com

Telephone: +81-45-2618191 Fax: +81-45-2618149
Received: August 10, 2012 Revised: September 26, 2012
Accepted: September 29, 2012
Published online: January 7, 2013

Abstract

Ring calcification in untreated hepatocellular carcinoma (HCC) is extremely rare, with only 3 previously reported cases in the English-language literature. A case of HCC with ring calcification was reported in this paper. Additionally, 3 previously reported cases of HCC with ring calcification were reviewed. In 3 of these 4 cases (including our case), surgery was performed. Although the size of the ring-calcified lesion ranged from 3.0-3.7 cm in previously reported cases, the size was only 1 cm in ours. The differentiation of the tumor was moderate in the 2 previously reported cases in the histological findings and poor in ours. In spite of their poor differentiation for their sizes, these tumors showed no early enhancement in dynamic computed tomography. All calcified tumors showed a thick fibrous capsule and extensive necrosis histologically. Ring calcification was considered to result from a circulatory disturbance caused by the imbalance between the less abundant arterial blood flow and high inner pressure induced by either the thick fibrous capsule or vigorous proliferation due to the poor differentiation. Ring calcification in untreated HCC may suggest a lower differentiation of the tumor. Even if its size is small, hepatic resection should be performed for any tumor with ring calcification because poor differentiation is considered to be one of the risk factors for recurrence after local ablation therapy, including radio frequency ablation.

© 2013 Baishideng. All rights reserved.

Key words: Hepatocellular carcinoma; Ring calcification; Calcification in untreated neoplasms; Process of forming calcification; Histological differentiation

Murakami T, Morioka D, Takakura H, Miura Y, Togo S. Small hepatocellular carcinoma with ring calcification: A case report and literature review. *World J Gastroenterol* 2013; 19(1): 129-132 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i1/129.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i1.129

INTRODUCTION

Macroscopic calcification in untreated hepatocellular carcinoma (HCC) (the term "untreated HCC" is defined as HCC that was not treated with local ablation, local injection, or embolization prior to the development of calcification) is considered rare, and has reportedly been observed in only 1.9%-3.3% of cases[1-5]. Ring calcification in untreated HCC is extremely rare, with only 3 previously reported cases in the English-language literature[6,7]. We present a case of small HCC (1 cm in diameter) showing ring calcification. To elucidate the clinical and histological characteristics of this entity, we reviewed previously reported cases of HCC with ring calcification in the English-language literature.

CASE REPORT

A 68-year-old woman with a 19-year history of chronic hepatitis C was referred for surgery because of a hepatic

tumor in segment 4 (defined by Couinaud's nomenclature for liver segmentation) (Figure 1). Despite no elevation in either the serum alpha-fetoprotein (AFP) or des-γ-carboxy prothrombin (DCP) levels and no enhancement in the dynamic computed tomography (CT) study, the tumor was suspected to be HCC from the patient' s history of hepatitis C. The patient was offered surgery for the tumor but rejected the offer at that time. Six months after the first diagnosis of the tumor, the serum AFP level was elevated (128 ng/mL, but no elevation in DCP level) and the tumor showed ring calcification (Figure 1). Furthermore, an irregularly shaped tumor, which was broadly in contact with the calcified lesion and showed early enhancement in dynamic CT, was observed, whereas the calcified lesion showed no enhancement in any phase (Figure 1). After a detailed informed consent process explaining the necessity of surgery, the patient accepted the offer of surgery and underwent segment 4 segmentectomy 9 mo after the first diagnosis.

Macroscopically, a 10-mm tumor with ring calcification and an 18-mm tumor were identified in the same thick fibrous capsule (Figure 2A). Microscopic examination revealed findings of poorly differentiated HCC in the calcified lesion and moderately differentiated HCC in the non-calcified lesion (Figure 2B). Each lesion comprised only a single differentiated component and had a thick fibrous capsule (Figure 2A). With regard to the calcified tumor, extensive necrosis was diffusely observed in the tumor, suggesting that the necrosis was caused by a circulatory disturbance. The ring calcification formed inside the thick fibrous capsule (Figure 3).

The postoperative course was uneventful, and the patient is doing well without recurrence 30 mo after the surgery.

Review of the literature

A PubMed search on August 2012 for articles published since 1965 with the key words "hepatocellular carcinoma" and "calcification" yielded 62 articles published in English-language journals. These publications were all reviewed. There were only 2 articles describing 3 cases of untreated HCC with ring calcification[6,7], resulting in a total of 4 reported cases. The demographic data are shown in Table 1. The previously reported sizes of ring-calcified lesions ranged from 3.0 to 3.7, but ours was only 1 cm. Three of the four cases underwent hepatic resection. The remaining case did not receive any treatment for HCC because of markedly poor general condition, resulting in no histological confirmation for the diagnosis of HCC. Of the 3 cases for which histological findings were obtained, differentiation was moderate in the 2 previously reported cases and poor in our case. All 3 calcified lesions consisted of a single differentiated component. These 3 cases had a thick fibrous capsule, and the ring calcification formed inside the fibrous capsule. Additionally, in the previous 2 cases with histological findings, similar findings to our own case were observed; extensive necrosis was diffusely observed in the tumor[6,7].

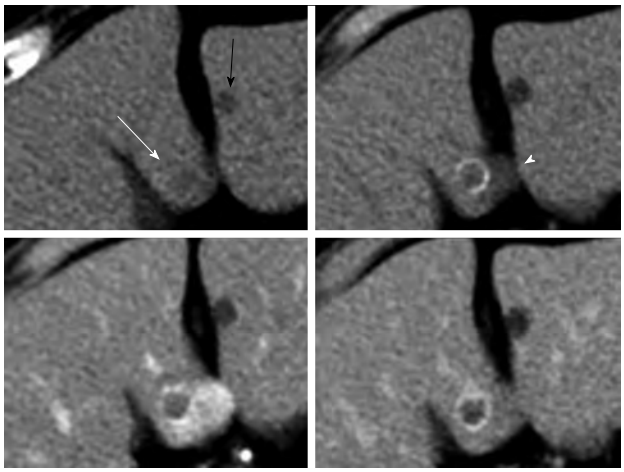


Figure 1 Findings of computed tomography. Upper left, plain abdominal computed tomography (CT) taken at the initial diagnosis revealed a 1-cm low-density lesion without calcification in liver segment 4 (white arrow). A small low-density area found in the left lateral section was a liver cyst (black arrow); upper right, plain abdominal CT taken 6 mo after the initial diagnosis revealed that the lesion had a ring calcification and an adjacent 1.8-cm lesion (white arrowhead); lower left and right, abdominal CT taken 6 mo after the initial diagnosis in the early phase (lower left) after a bolus administration of intravenous contrast material showed enhancement of the newly appeared lesion and the delayed phase (lower right) showed that it was hypoattenuated.

DISCUSSION

The cause of calcification in HCC is considered to be dystrophic calcification, in which deposition occurs locally in nonviable or dying tissues with normal serum levels of calcium and in the absence of a dysfunction in calcium metabolism[4-7]. Dystrophic calcification in untreated neoplasms is most commonly observed in meningioma, papillary thyroid carcinoma, and ovarian serous cystadenocarcinoma[8]. In such tumors, the mechanisms leading to the formation of calcifications in the tumors have been thoroughly analyzed[8]. Although the exact mechanism of calcification formation is not well understood, it has been suggested to be the following. In such tumors, spontaneous tumor necrosis occurs easily due to micro-infarction caused by their structural property, which predisposes microcirculatory disturbance. Matrix vesicles and matrix giant bodies derived from degenerated or necrotic cells sequester hydroxyapatite crystals to form seed crystals. Mineral deposition encrusts these crystals. Collagen and elastic fibers derived from degenerated basement membranes adjacent to necrotic cells are a prerequisite for the calcification to be identifiable because the expansion of hydroxyapatite deposition requires these connective tissues as nidi[8]. Namely, tumors with abundant stromal tissues, in which ischemic necrosis easily occurs, are likely to show identifiable dystrophic calcification. Therefore, the reason for why calcification in untreated HCC is rarely observed can be explained as the following. HCC is less abundant in stromal tissue and has a hypervascular structure, in which blood flow is primarily composed of arterial flow, which is less likely to cause spontaneous microcirculatory disturbance[9-11]. Converse-

Table 1 Summary of 4 cases of hepatocellular carcinoma with ring calcification

Case	No.	Age	Sex	Symptom	AFP (ng/mL)	Size of calcified lesion (cm)	Location	Early enhancement in dynamic CT	Viral infection	Background liver	Histology	Treatment
Fukuya *et al*[6]	1	72	F	No	178	3.0	S8	No	HCV	CH	Mod diff HCC	Partial hepatectomy
Fukuya *et al*[6]	2	77	F	No	9300	3.0	S8	Yes	HCV	LC	Not obtained	None
Kawada *et al*[7]	3	67	F	Abdominal pain Back pain	Not mentioned	3.7	S4	Yes	HCV	CH	Mod diff HCC	Left lobectomy
Our case	4	68	F	No	128	1.0	S4	No	HCV	CH	Poorly diff HCC	Left medial sectionectomy

AFP: Alpha-fetoprotein; CT: Computed tomography; F: Female; S8: Segment 8 (defined by the Couinaud's nomenclature for liver segmentation); S4: Segment 4; HCV: Hepatitis C virus; CH: Chronic hepatitis; LC: Liver cirrhosis; Mod diff: Moderately differentiated; Poorly diff: Poorly differentiated; HCC: Hepatocellular carcinoma.

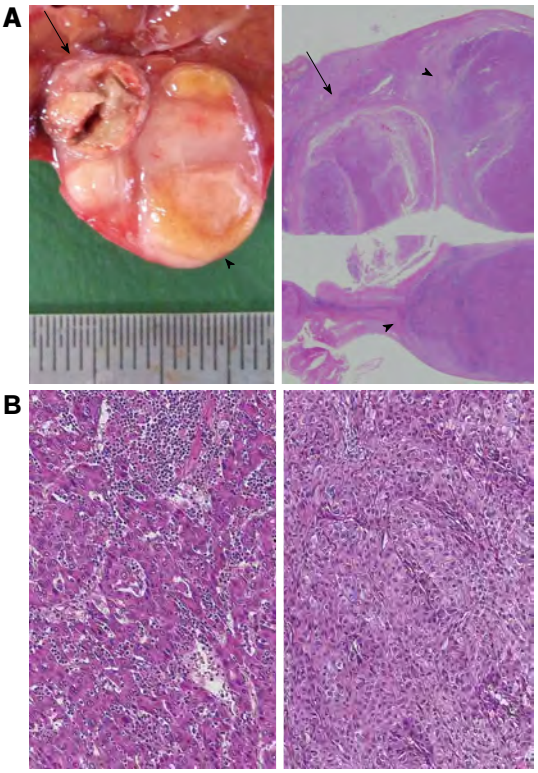


Figure 2 Macroscopic findings of the resected specimen, calcified and non-calcified lesions. A: Left, macroscopic examination showed a 10-mm tumor with ring calcification (arrow) and an 18-mm tumor (arrowhead) existing side-by-side and encapsulated within the same thick fibrous tissue; Right, a prepared specimen (loupe image, hematoxylin and eosin staining); B: Left, microscopic examination findings of the calcified lesion showed the poorly differentiated hepatocellular carcinoma (hematoxylin and eosin staining; original magnification, × 200); Right, microscopic examination findings of the non-calcified lesion showed moderately differentiated hepatocellular carcinoma (hematoxylin and eosin staining; original magnification, × 200).

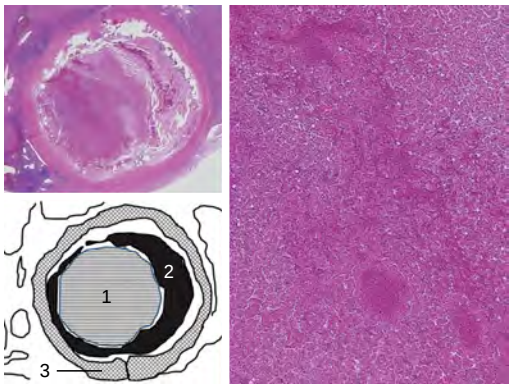


Figure 3 Schematic image of the structure of the calcified lesion. Upper left, loupe image of the calcified lesion (hematoxylin and eosin staining; original magnification, × 5); Right, microscopic findings of the calcified lesion showed that necrotic area was extensively observed in the tumor (hematoxylin and eosin staining; original magnification, × 40); Lower left, schematic of the structure of the calcified lesion. A poorly differentiated tumor, in which spotty necrosis and hemorrhagic necrosis were extensively observed, was surrounded by a fibrous capsule. Calcification was identified between the tumor and the fibrous capsule (1: Poorly differentiated tumor; 2: Calcification; 3: Fibrous capsule).

ly, calcification being observed often in HCC treated by trans-catheter arterial chemoembolization, which causes ischemic necrosis in tumors[4-7], suggests that extensive ischemic necrosis is essential for forming identifiable calcifications in neoplasms.

In the present study, tumors showing a ring calcification with histological findings revealed extensive necrosis histologically. A ring calcification formed in the marginal zone of relatively small tumors in contact with a thick fibrous capsule. Furthermore, these tumors consisted of a single relatively low-differentiation component. In fact, calcified lesions in the previously reported cases were composed of only a moderately differentiated component, whereas the calcified lesion in our case was only composed of a poorly differentiated component; all 3 tumors with histological findings showed poor differentiation for their sizes (1-3.7 cm)[6,7]. Furthermore, these tumors showed no early enhancement in dynamic CT study[6,7], suggesting that these tumors had less abundant arterial blood flow, unlike the usual cases of moderately or poorly differentiated HCC[9-11]. Furthermore, all 3 tumors had a thick fibrous capsule for their size. A fibrous capsule surrounding HCC is usually observed with relatively large and less histologically differentiated tumors[12-14]. It is not known exactly how the fibrous capsule is formed, but the formation is known to result from the interaction between the tumor and host liver and interferes with the growth and invasion of HCC[12-14]. The fibrous capsule is primarily composed of collagenous tissue produced principally

by stellate cells (the so-called fat-storing cell of Ito), which are present among the tumor cells in the vicinity of the boundary between the HCC and non-tumor liver[12-14]. Furthermore, it was reported that the fibrous capsule of HCC contains smooth muscle cells and has contractile ability, whereas the septa of multi-nodular HCC or hepatic cirrhosis does not have either[12]. Based on these findings, we hypothesize that ring calcification forms through the following process. Tumors with ring calcification in this series had less abundant arterial blood flow and had a high internal pressure induced by either the thick fibrous capsule or vigorous proliferation, as suggested by their low differentiation for their sizes. This imbalance between the blood flow and the internal pressure causes a circulatory disturbance in the tumor, resulting in extensive ischemic necrosis. Necrotic tumor cells cause mineral deposition that subsequently involves adjacent collagenous fibers produced by the stellate cell in the marginal zone and elastic fibers derived from the cytoskeleton of degenerated or necrotized tumor cells as the nidi to extend along the fibrous capsule, forming annular calcification. Identifiable calcification cannot be formed in the inner zone because of the absence of collagenous fiber as the nidus. In other words, the prerequisites for forming a ring calcification in untreated HCC are the following: small size, poor differentiation, a thick fibrous capsule, and less abundant arterial blood flow. These prerequisites are the reasons why a ring calcification in untreated HCC is extremely rare. On the other hand, tumors with ring calcification are considered to be likely to show poor differentiation for their sizes. Therefore, HCCs with ring calcification are likely to show a poorer prognosis than other tumors of their size when local ablation therapy is applied for treatment[15]. In treating these lesions, therefore, hepatic resection should be selected even if the size is small and ablation therapy seems feasible[15].

In conclusion, we present an extremely rare case of small HCC (1 cm in diameter) showing ring calcification and a review of the English-language literature including 3 other cases. We believe that ring calcification in untreated HCC is suggestive of the poorer differentiation of the tumors. Therefore, hepatic resection should be performed for these lesions because poor differentiation is considered to be one of the risk factors for recurrence after local ablation therapy.

REFERENCES

1 **Patton RB**, Horn RC. Primary liver carcinoma. Autopsy study of 60 cases. *Cancer* 1964; **17**: 757-768 [PMID: 14176989 DOI: 10.1002/1097-0142]

2 **Maeda M**, Kanayama M, Uchida T, Hasumura Y, Takeuchi J. A case of hepatocellular carcinoma associated with ossification. A case report. *Cancer* 1986; **57**: 134-137 [PMID: 3000570]

3 **Chin NW**, Chapman I, Coopersmith H, Jimenez FA. Parenchymal calcification in hepatocellular carcinoma. *N Y State J Med* 1986; **86**: 596-598 [PMID: 3025789]

4 **Moenandar IM**. Extensive calcification in the stroma of a primary hepatic carcinoma. *J Pathol* 1974; **114**: 53-54 [PMID: 4373560 DOI: 10.1002/path.1711140110]

5 **Teefey SA**, Stephens DH, Weiland LH. Calcification in hepatocellular carcinoma: not always an indication of fibrolamellar histology. *AJR Am J Roentgenol* 1987; **149**: 1173-1174 [PMID: 2825493]

6 **Fukuya T**, Yabuuchi H, Seo Y, Fukuda T, Koga M. Hepatocellular carcinoma with ring calcification: report of two cases. *Radiat Med* 1999; **17**: 379-382 [PMID: 10593291]

7 **Kawada N**, Murata M, Nakayama T, Yukawa M, Okajima K, Ohta Y, Kudo M, Inoue M. Unusual hepatocellular carcinoma with macroscopic biliary tumor thrombus accompanied by rim calcification and no arterial-phase enhancement. *Acta Med Kinki Univ* 2008; **33**: 59-63

8 **Das DK**. Psammoma body: a product of dystrophic calcification or of a biologically active process that aims at limiting the growth and spread of tumor? *Diagn Cytopathol* 2009; **37**: 534-541 [PMID: 19373908 DOI: 10.1002/dc.21081]

9 **Enzan H**, Himeno H, Iwamura S, Onishi S, Saibara T, Yamamoto Y, Hara H. Alpha-smooth muscle actin-positive perisinusoidal stromal cells in human hepatocellular carcinoma. *Hepatology* 1994; **19**: 895-903 [PMID: 8138263 DOI: 10.1002/hep.1840190415]

10 **Maeda T**, Adachi E, Kajiyama K, Takenaka K, Honda H, Sugimachi K, Tsuneyoshi M. CD34 expression in endothelial cells of small hepatocellular carcinoma: its correlation with tumour progression and angiographic findings. *J Gastroenterol Hepatol* 1995; **10**: 650-654 [PMID: 8580408 DOI: 10.1111/j.1440-1746.1995.tb01365.x]

11 **Kimura H**, Nakajima T, Kagawa K, Deguchi T, Kakusui M, Katagishi T, Okanoue T, Kashima K, Ashihara T. Angiogenesis in hepatocellular carcinoma as evaluated by CD34 immunohistochemistry. *Liver* 1998; **18**: 14-19 [PMID: 9548262 DOI: 10.1111/j.1600-0676.1998.tb00121.x]

12 **Kojima A**, Kaneda K, Ueda M, Maki A, Takabayashi A, Fukushima S, Sakurai M, Nagai R, Matsui-Yuasa I. Induction of smooth muscle cells in the fibrous capsule of human hepatocellular carcinoma but not in the septa of hepatic cirrhosis. *Virchows Arch* 1999; **434**: 413-422 [PMID: 10389624 DOI: 10.1007/s004280050360]

13 **Ishizaki M**, Ashida K, Higashi T, Nakatsukasa H, Kaneyoshi T, Fujiwara K, Nouso K, Kobayashi Y, Uemura M, Nakamura S, Tsuji T. The formation of capsule and septum in human hepatocellular carcinoma. *Virchows Arch* 2001; **438**: 574-580 [PMID: 11469689 DOI: 10.1007/s004280000391]

14 **Iguchi T**, Aishima S, Sanefuji K, Fujita N, Sugimachi K, Gion T, Taketomi A, Shirabe K, Maehara Y, Tsuneyoshi M. Both fibrous capsule formation and extracapsular penetration are powerful predictors of poor survival in human hepatocellular carcinoma: a histological assessment of 365 patients in Japan. *Ann Surg Oncol* 2009; **16**: 2539-2546 [PMID: 19533247 DOI: 10.1245/s10434-009-0453-1]

15 **Huang J**, Yan L, Cheng Z, Wu H, Du L, Wang J, Xu Y, Zeng Y. A randomized trial comparing radiofrequency ablation and surgical resection for HCC conforming to the Milan criteria. *Ann Surg* 2010; **252**: 903-912 [PMID: 21107100 DOI: 10.1097/SLA.0b013e3181efc656]

P-Reviewers Cucchetti A, Moesinger RC
S- Editor Gou SX **L- Editor** A **E- Editor** Li JY



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i1.133

World J Gastroenterol 2013 January 7; 19(1): 133-136
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Biliary phytobezoar resulting in intestinal obstruction

Yura Kim, Beom Jin Park, Min Ju Kim, Deuk Jae Sung, Dong-Sik Kim, Young-Dong Yu, Jeong Hyeon Lee

Yura Kim, Beom Jin Park, Min Ju Kim, Deuk Jae Sung, Department of Radiology, College of Medicine, Korea University, Anam Hospital, Seoul 136-705, South Korea
Dong-Sik Kim, Young-Dong Yu, Division of HBP Surgery and Liver Transplantation, Department of Surgery, College of Medicine, Korea University, Anam Hospital, Seoul 136-705, South Korea
Jeong Hyeon Lee, Department of Pathology, College of Medicine, Korea University, Anam Hospital, Seoul 136-705, South Korea
Author contributions: Kim Y and Park BJ interpreted the data and wrote the manuscript; Kim MJ and Sung DJ reviewed the literature and edited the manuscript; Kim DS and Yu YD contributed to revision of the surgical and scientific parts of the manuscript; Lee JH contributed to revisions of the pathology portions of the manuscript.
Correspondence to: Beom Jin Park, MD, PhD, Department of Radiology, College of Medicine, Korea University, Anam Hospital, 126-1, 5-Ka, Anam Dong, Sung buk-ku, Seoul 136-705, South Korea. rupture226@hanmail.net
Telephone: +82-2-9205578 Fax: +82-2-9203796
Received: September 9, 2012 Revised: September 20, 2012
Accepted: September 28, 2012
Published online: January 7, 2013

Abstract

Phytobezoar is the most common type of bezoar. It is composed of indigestible vegetable matter and is usually found in the stomach. Biliary phytobezoar is extremely rare and difficult to diagnose preoperatively. The pathogenesis is not clear, and there have been only a few reports of biliary bezoars associated with sphincteric impairmentat the ampulla of Vater. Here, we present a report of biliary bezoar that resulted in jejunal obstruction. We were unable to identifythe bezoar in the extrahepatic bile duct until it obstructed the small bowel lumen. To our knowledge, this is the first report of small bowel obstruction resulting frommigration of a biliary bezoar.

© 2013 Baishideng. All rights reserved.

Key words: Phytobezoar; Biliary; Intestinal obstruction; Choledochoduodenal; Fistula; Multidetector-row computed tomography

KimY, Park BJ, Kim MJ, Sung DJ, Kim DS, Yu YD, Lee JH. Biliary phytobezoar resulting in intestinal obstruction. *World J Gastroenterol* 2013; 19(1): 133-136 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i1/133.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i1.133

INTRODUCTION

A bezoar is an intragastric mass consisting of accumulated ingested material. Phytobezoars, the most common type of bezoar, are masses of indigested vegetable matter including skin, seeds, and fruit and vegetable fiber, and are most commonly reported in patients who have had previous gastric surgery[1]. Although normally found in the stomach, a phytobezoar may pass into the small bowel. Primary small bowel bezoarsare very rare, but can form in patients with underlying small bowel disease such as diverticulum, stricture, or tumor[2]. Phytobezoars can also develop secondarily if there are areas of sufficient stagnation within a dilated small bowel loop, as may occur in patients with strictures caused by previous surgery, Crohn's disease, or tuberculosis[3].

We encountered a case of small bowel obstruction by a 'biliary' phytobezoar, which was formed in the extrahepatic bile duct and migrated into the small bowel resulting complete obstruction. Intestinal obstruction due to bezoars is rare, reportedly constituting only 0.44% of all small intestinal obstructions[4,5], and there is an even greater paucity of reports regarding small bowel obstructions caused by the migration of biliary bezoar. The first case report of bezoar-induced obstructive jaundice caused by an intradiverticular bezoar was published by Seryrig *et al*[6] in 1989, and to date, fewer than 5 cases of biliary bezoar have been reported in the English language literature[6-9].

This report concerns a rare case of biliary bezoar resulting intestinal obstruction, including imaging and clinicopathologic details.

CASE REPORT

A 72-year-old woman visited the emergency department with a 3-d history of abdominal pain. She had undergone open cholecystectomy with common bile duct (CBD) exploration for acute calculus cholecystitis 12 years previously. Since then, she had experienced recurrent episodes of obstructive jaundice and been hospitalized 3 years ago for recurrent pyogenic cholangitis. Multi-detector computed tomography (MDCT) at that time had shown diffuse and marked bile duct dilatation with pneumobilia (Figure 1A). Although the extent of dilatation was remarkable, there was no evidence of an obstructive mass within the bile ducts. The patient underwent endoscopic retrograde cholangio-pancreatography (ERCP), which revealed a choledochoduodenal fistula (Figure 1B). She was treated with endoscopic nasobiliary drainage and antibiotics and subsequently discharged after a few days of hospital stay.

A routine MDCT checkup 6 mo after discharge showed neither interval changes of the extensive biliary dilatation nor pneumobilia. The choledochoduodenal fistula was seen, along with a newly visible large mass at the extrahepatic bile duct with multiple internal air densities and low attenuation areas resembling impacted food material that was associated with luminal dilatation (Figure 1C). These MDCT features were suggestive of CBD stone or refluxed food material from the second portion of the duodenum (Figure 1D).

When the patient presented to the emergency room, MDCT confirmed small-bowel obstruction and showed proximal luminal dilatation of the jejunum with focal wall thickening of the transition zone of the mid-jejunum. In the transition zone, well-defined, thin-walled, ovoid food remnants were observed, consistent with bezoars, and these were assumed to be the cause of the obstruction (Figure 2A and B).

The patient underwent exploratory laparotomy with enterotomy, and the obstructing mass at the proximal jejunum was removed. Inflammation-induced segmental wall thickening was observed in the involved small bowel however, there was no sign of bowel perforation or ischemia. The resected mass was pathologically confirmed as a biliary bezoar (Figure 2D), and the histological evaluation indicated that theremoved objects were revealed as fibrinoid materials with some cellular debris.

When we retrospectively reviewed the preoperative computed tomography (CT) images, we concluded that the previously noted CBD mass, which was thought to be refluxed food or a stone, had disappeared. This mass had apparently migrated along the gastrointestinal tract until it reached the jejunal loop of the small bowel, where it resulted in obstruction (Figure 2C). The patient was discharged 1 wk after surgery without any postoperative complications.

DISCUSSION

Phytobezoar is a type of bezoar that consists of remnants of poorly digested food materials. Phytobezoars are usually reported in patients who have poor mastication, a vegetarian diet, have ingested persimmons, or have undergone previous gastric surgery and vagotomy resulting in diminished motility[10-12].

Biliary phytobezoar is extremely rare, and is reported to develop in patients who have had previous cholecystectomy. In 1995, Lamotte *et al*[6] were the first to describe a biliary bezoar, which developed in a patient 15 years after cholecystogastrostomy[13]. In other reports, including the present case, biliary bezoar shave developed in patients who have undergone cholecystectomy and had subsequent formation of choledochoduodenal fistulas[9]. Thus, development of biliary bezoar seems to be related to loss of the sphincteric mechanism due to previous biliary-enteric anastomosis or sphincterotomy, which allows reflux of undigested vegetable fibers or food particles from the alimentary tract into the bile ducts[7]. However, biliary bezoarhas also developed in the patients without any sphincteric disruption or impairment. Thus, it is conceivable that some vegetable matter can enter the bile duct through an intact sphincter[7,8].

It is reported that MDCT is the most useful method for diagnosing of small-bowel bezoars because it is able to reveal precise details including location, degree, and features of bezoars[14]. When a patient has a well-defined round or ovoid mass in the intraluminal space with mottled gas pattern causing small-bowel obstruction, bezoar should be considered as the cause of obstruction[14]. A study from France on CT findings that could differentiate small-bowel bezoars from small-bowel feces suggested'floating fat density debris', as a CT feature of bezoars, which can be defined in images showing fat-density debris in a region proximal to the obstruction site with dilated bowel loops[15]. The definition proposes that this unusual density arises from the sum of the tissue density of the debris and the negative density of trapped air. However, in our case, no such low-density feature was noted.

MDCT findings for biliary bezoars causing intestinal obstruction have not been previously reported. A biliary bezoar entrapped in the small bowel can be confused with an intraluminal gallstone associated with gallstone ileus or with other types of bezoars formed within gastrointestinal tract. The Rigler's radiologic criteria (evidence of an ectopic stone in the intestine, air in the biliary tree and gall bladder, and small-bowel obstruction) and direct visualization of choledochoduodenal fistula by MDCT are well known pathognomonic factors for the diagnosis of the gall stone ileus[16,17]. Although the present case fulfilled most criteria for the diagnosis of the gall stone ileus, mottled air densities of intraluminal mass in transition zone of intestinal obstruction was different from Rigler's criteria and this finding provided diagnostic clue for the final diagnosis as the bezoar rather than gall stone.

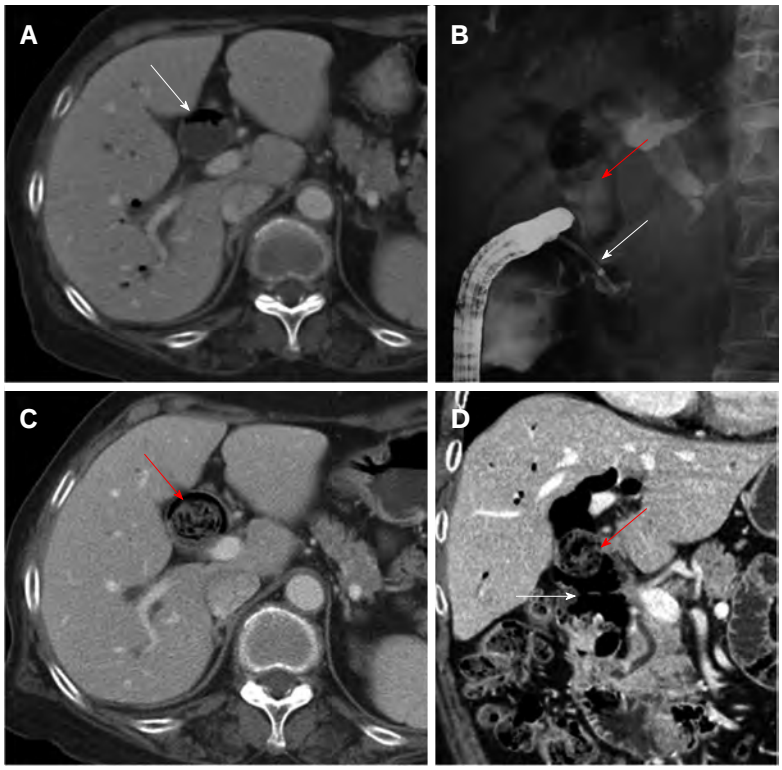


Figure 1 Development of a biliary phytobezoar in the extrahepatic bile duct. A: Axial portal venous-phase computed tomography (PVP CT) 3 years ago showing diffuse bile duct dilatation (white arrow) with pneumobilia; B: On endoscopic retrograde cholangiopancreatography, there was no abnormal filling defect except pneumobilia (red arrow) in the dilated extrahepatic bile duct (EHD). A choledochoduodenal fistula (white arrow) in the distal common bile duct which was cannulated by endoscopy; C and D: Axial PVP CT and its coronal reformatted image at 6-mo follow-up showed a dilated EHD and a newly developed intraductal ovoid mass with interstitial air (red arrows), as well as the choledochoduodenalfistula (white arrow).

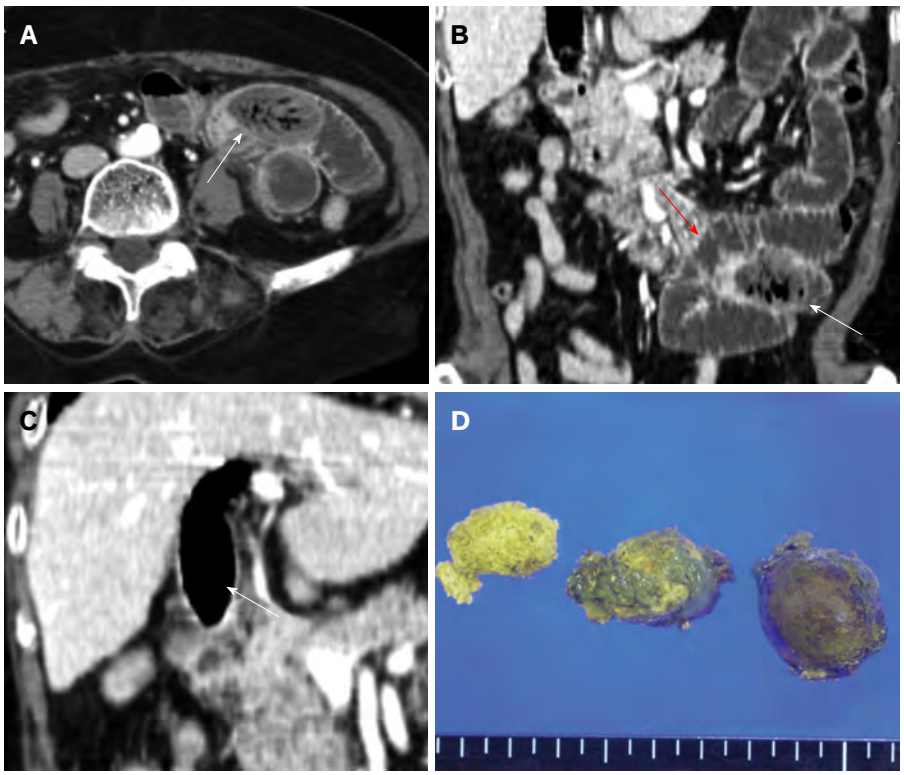


Figure 2 Migration of the biliary phytobezoar resulting in mechanical obstruction of the jejunum. A and B: Axial portal venous computed tomography and coronal reformatted image at admission showed the migration of the ovoid intraluminal mass (white arrows) with mottled gas pattern to the jejunal loop, as well as a dilated proximal loop (red arrow) containing air-fluid levels suggestive of obstruction; C: Empty extrahepatic bile duct (white arrow) with extensive residual pneumobilia after migration of the bezoar; D: Gross specimen and cut surface of the biliary bezoar.

It is not possible to classify biliary foreign bodies, including biliary bezoars, in patients who have not had previous surgery or who do not have a biliary enteric fistula, because the radiologic and macroscopic characteristics of biliary bezoarson MDCT and ERCP resemble those of bile duct stones[18]. Therefore, it is important to carefully check for the presence of biloenteric communication and predisposing factors related to bezoar formation ahead of a preoperative diagnosis of a biliary bezoar, because, as noted, most biliary bezoarsare associated with a previous history of biliary abnormality such as choledocho or cholecystoduodenal fistula or diverticula in the walls of the bile ducts and duodenum.

Surgical or laparoscopic excision is the treatment of choice for small bowel bezoars. The entire gastrointestinal tract must be explored to exclude synchronous bezoars and prevent recurrent bowel obstruction caused by residual bezoar[3]. Other non-surgical treatment options include sodium bicarbonate powder, a liquid diet, enzymatic digestion with various agents, endoscopic fragmentation, and oral intake (or endoscopic injection) of 'Coca-Cola'[19]. A study using combinations of the cola and the endoscopic treatment showed a relatively good response in terms of ability to dissolveor decrease the size of phytobezoars[20].

In conclusion, we have presented a case of small bowel obstruction caused by biliary phytobezoar. Although it is considered extremely rare, biliary bezoar can be one of the possible causes of the mechanical small bowel obstruction in patients with the previously mentioned predisposing factors. We have presented this case to promote further awareness of the interesting phenomenon of biliary bezoar formation associated with abilo digestive fistula, as well as the possibility of subsequent small bowel obstruction due tomigration of the biliary bezoar.

REFERENCES

1 **Angelelli G**, Magliocca M, Zaccheo N, Vinci R, Rotondo A. [Intestinal obstruction caused by phytobezoar: computerized tomography findings. Report of 3 cases]. *Radiol Med* 1997; **93**: 789-791 [PMID: 9411533]

2 **Teng H**, Nawawi O, Ng K, Yik Y. Phytobezoar: an unusual cause of intestinal obstruction. *Biomed Imaging Interv J* 2005; **1**: e4 [PMID: 21625276 DOI: 10.2349/biij.1.1.e4]

3 **Ezzat RF**, Rashid SA, Rashid AT, Abdullah KM, Ahmed SM. Small intestinal obstruction due to phytobezoar: a case report. *J Med Case Rep* 2009; **3**: 9312 [PMID: 20062741 DOI: 10.1186/1752-1947-3-9312]

4 **Quiroga S**, Alvarez-Castells A, Sebastià MC, Pallisa E, Barluenga E. Small bowel obstruction secondary to bezoar: CT diagnosis. *Abdom Imaging* 1997; **22**: 315-317 [PMID: 9107659 DOI: 10.1007/s002619900198]

5 **Goldstein SS**, Lewis JH, Rothstein R. Intestinal obstruction due to bezoars. *Am J Gastroenterol* 1984; **79**: 313-318 [PMID: 6711533]

6 **Lamotte M**, Kockx M, Hautekeete M, Holvoet J, Hubens H. Biliary phytobezoar: a medical curiosity. *Am J Gastroenterol* 1995; **90**: 1346-1348 [PMID: 7639247]

7 **Cetta F**, Lombardo F, Rossi S. Large foreign body as a nidus for a common duct stone in a patient without spontaneous biliary enteric fistula or previous abdominal surgery. *HPB Surg* 1993; **6**: 235-242 [PMID: 8489973]

8 **Procházka V**, Krausová D, Kod'ousek R, Zámecníková P. Foreign material as a cause of choledocholithiasis. *Endoscopy* 1999; **31**: 383-385 [PMID: 10433048 DOI: 10.1055/s-1999-36]

9 **Moghaddam JA**, Amini M, Adibnejad S. Development of bile duct bezoars following cholecystectomy caused by choledochoduodenal fistula formation: a case report. *BMC Gastroenterol* 2006; **6**: 1 [PMID: 16396681 DOI: 10.1186/1471-230X-6-1]

10 **Chisholm EM**, Leong HT, Chung SC, Li AK. Phytobezoar: an uncommon cause of small bowel obstruction. *Ann R Coll Surg Engl* 1992; **74**: 342-344 [PMID: 1416706]

11 **Rubin M**, Shimonov M, Grief F, Rotestein Z, Lelcuk S. Phytobezoar: a rare cause of intestinal obstruction. *Dig Surg* 1998; **15**: 52-54 [PMID: 9845563 DOI: 10.1159/000018586]

12 **Kement M**, Ozlem N, Colak E, Kesmer S, Gezen C, Vural S. Synergistic effect of multiple predisposing risk factors on the development of bezoars. *World J Gastroenterol* 2012; **18**: 960-964 [PMID: 22408356 DOI: 10.3748/wjg.v18.i9.960]

13 **Seyrig JA**, Chambon J, Fritsch J, Berger M, Liguory C, Chousterman M. [Cholestasis caused by an intradiverticular bezoar. Endoscopic treatment]. *Gastroenterol Clin Biol* 1989; **13**: 741-743 [PMID: 2509276]

14 **Delabrousse E**, Brunelle S, Saguet O, Destrumelle N, Landecy G, Kastler B. Small bowel obstruction secondary to phytobezoar CT findings. *Clin Imaging* 2001; **25**: 44-46 [PMID: 11435039 DOI: 10.1016/S0899-7071(01)00251-0]

15 **Delabrousse E**, Lubrano J, Sailley N, Aubry S, Mantion GA, Kastler BA. Small-bowel bezoar versus small-bowel feces: CT evaluation. *AJR Am J Roentgenol* 2008; **191**: 1465-1468 [PMID: 18941086 DOI: 10.2214/AJR.07.4004]

16 **Papavramidis TS**, Potsi S, Paramythiotis D, Michalopoulos A, Papadopoulos VN, Douros V, Pantoleon A, Foutzila-Kalogera A, Ekonomou I, Harlaftis N. Gallstone obstructive ileus 3 years post-cholecystectomy to a patient with an old ileoileal anastomosis. *J Korean Med Sci* 2009; **24**: 1216-1219 [PMID: 19949687 DOI: 10.3346/jkms.2009.24.6.1216]

17 **Lassandro F**, Romano S, Ragozzino A, Rossi G, Valente T, Ferrara I, Romano L, Grassi R. Role of helical CT in diagnosis of gallstone ileus and related conditions. *AJR Am J Roentgenol* 2005; **185**: 1159-1165 [PMID: 16247126 DOI: 10.2214/AJR.04.1371]

18 **Kim TO**, Lee SH, Kim GH, Heo J, Kang DH, Song GA, Lee JW, Cho M. Common bile duct stone caused by a phytobezoar. *Gastrointest Endosc* 2006; **63**: 324; discussion 324 [PMID: 16427945 DOI: 10.1016/j.gie.2005.09.037]

19 **Ha SS**, Lee HS, Jung MK, Jeon SW, Cho CM, Kim SK, Choi YH. Acute intestinal obstruction caused by a persimmon phytobezoar after dissolution therapy with Coca-Cola. *Korean J Intern Med* 2007; **22**: 300-303 [PMID: 18309693 DOI: 10.3904/kjim.2007.22.4.300]

20 **Lee BJ**, Park JJ, Chun HJ, Kim JH, Yeon JE, Jeen YT, Kim JS, Byun KS, Lee SW, Choi JH, Kim CD, Ryu HS, Bak YT. How good is cola for dissolution of gastric phytobezoars? *World J Gastroenterol* 2009; **15**: 2265-2269 [PMID: 19437568 DOI: 10.3748/wjg.15.2265]

P- Reviewers Papavramidis T, Vikram K, Manta R, Tsujikawa T, Cho YS **S- Editor** Gou SX **L- Editor** A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i1.137

World J Gastroenterol 2013 January 7; 19(1): 137-140
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Endoscopic resection of co-existing severe dysplasia and a small esophageal leiomyoma

Sun Young Ahn, Seong Woo Jeon

Sun Young Ahn, Seong Woo Jeon, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Kyungpook National University Hospital, Daegu 700-721, South Korea

Author contributions: Ahn SY and Jeon SW contributed equally to this work; Ahn SY and Jeon SW designed this article and discussed the clinical features; Ahn SY wrote the paper; Jeon SW contributed to revising this article critically for important intellectual content.

Correspondence to: Seong Woo Jeon, MD, PhD, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Kyungpook National University Hospital, Samduk 2-50, Junggu, Daegu 700-721, South Korea. swjeon@knu.ac.kr
Telephone: +82-53-2003517 Fax: +82-53-4268773
Received: October 8, 2012 Revised: October 30, 2012
Accepted: November 6, 2012
Published online: January 7, 2013

Abstract

Leiomyoma is the most common benign mesenchymal tumor of the esophagus. A small leiomyoma covered with endoscopically normal mucosa has a characteristic endoscopic ultrasonographic appearance, slow growth rate, and negligible risk of malignant transformation; therefore the histology does not need to be proven. Synchronous tumors such as an epithelial tumor and small subepithelial tumor in the upper gastrointestinal tract are uncommon. We describe a case with a co-existing small leiomyoma and severe dysplasia in the esophagus which were completely resected by endoscopic mucosal resection.

© 2013 Baishideng. All rights reserved.

Key words: Synchronous tumor; Leiomyoma; Dysplasia

Ahn SY, Jeon SW. Endoscopic resection of co-existing severe dysplasia and a small esophageal leiomyoma. *World J Gastroenterol* 2013; 19(1): 137-140 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i1/137.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i1.137

INTRODUCTION

Leiomyoma is the most common benign mesenchymal tumor in the esophagus[1,2]. A small and asymptomatic leiomyoma covered with endoscopically normal mucosa does not require surgical treatment such as resection because it has a characteristic endoscopic ultrasonographic appearance, slow growth rate, and negligible risk of malignant transformation[3]. A few cases involving co-existing tumors such as epithelial tumor and subepithelial tumor in the esophagus have been reported. However, in all of these reports the tumors were larger than 1 cm, were squamous cell carcinoma[4-8], and most were treated with surgery. A subepithelial lesion smaller than 1 cm combined with epithelial dysplasia is extremely unusual. We present a patient with an epithelial lesion of severe dysplasia overlying a small leiomyoma in the muscularis mucosa of the esophagus. Both lesions were completely removed simultaneously by endoscopic mucosal resection (EMR).

CASE REPORT

A 67-year-old male patient visited our hospital following the incidental finding of a subepithelial lesion at the mid-esophagus. He was a non-smoker, with no previous medical history and had good performance. Esophagogastroscopic examination showed a protruding mass, measuring 1 cm × 1 cm in dimension, without any ulcer or erosion, covered with normal mucosa (Figure 1A). We examined the lesion using narrow-band imaging (NBI) which showed scattered brown dots, and dilated and tortuous vessels on the top of the lesion (Figure 1B). We judged that the lesion might have dysplasia. Endoscopic ultrasonography was performed in order to determine the depth and nature of the tumor. Findings indicated

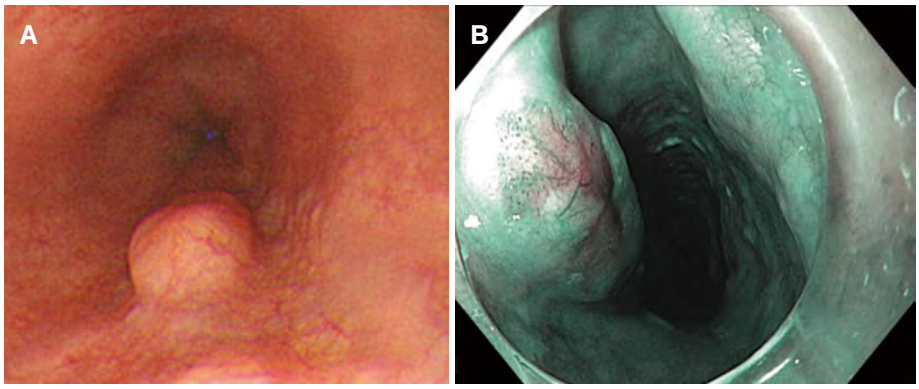


Figure 1 Endoscopic findings. A: Endoscopic findings demonstrate a subepithelial tumor with intact overlying mucosa; B: Chromoendoscopy with narrow band imaging showed scattered brown dots, and dilated and tortuous vessels on the top of the lesion.

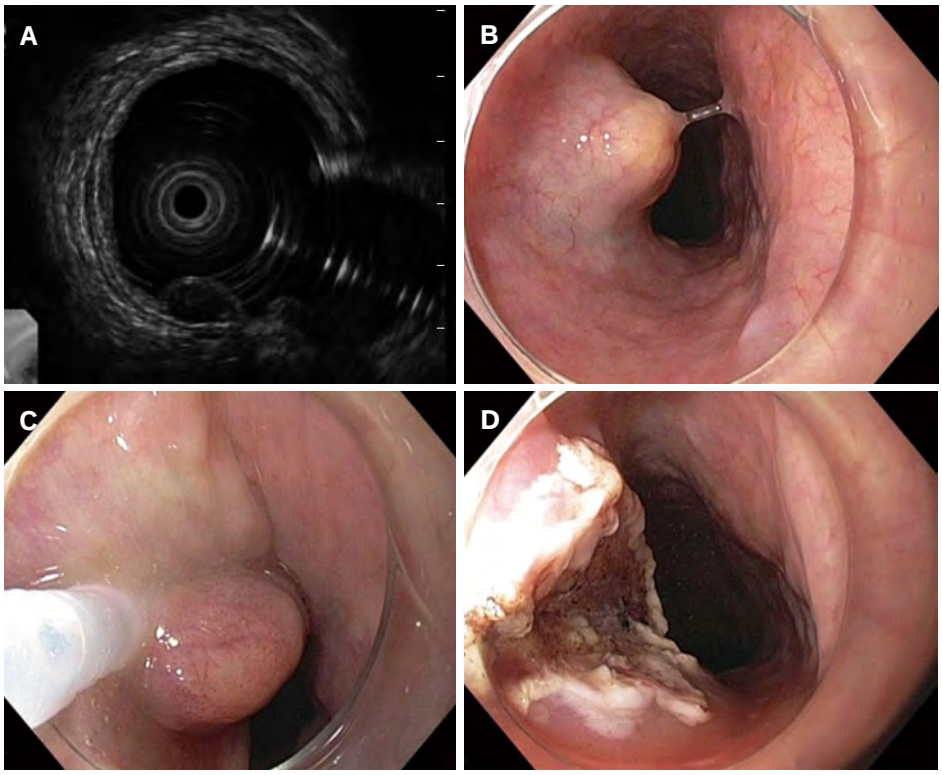


Figure 2 Endoscopic ultrasound findings and procedures for endoscopic mucosal resection. A: High frequency ultrasonic endoscopic findings showed a clear homogenous hypoechoic mass derived from the second layer of the esophagus; B: After making a submucosal bleb; C: The subepithelial lesion was captured using a snare; D: No immediate complications occurred after endoscopic mucosal resection.

that the lesion, which measured 5.0 mm × 4.0 mm in size, was a homogenous hypoechoic mass, confined to the second layer, the muscularis mucosa (Figure 2A). We performed a mucosal biopsy. Histological examination of the specimen indicated severe dysplasia. We diagnosed severe dysplasia overlying a small leiomyoma from the muscularis mucosa. Therefore, we planned *en bloc* resection by endoscopy for removal of the synchronous lesions. Endoscopic resection was performed using the EMR method. Following injection of hyaluronic acid into the submucosa, resection was performed using a snare (Figure 2B and C). No complications related to the procedure were observed (Figure 2D). Histopathological examination of the tumor revealed high-grade dysplasia with a clear resection margin and the results of immunohistochemical analyses revealed that the leiomyoma was negative for C-Kit and CD 34, and positive for smooth muscle actin (Figure 3).

DISCUSSION

The present case of severe epithelial dysplasia overlying a small subepithelial tumor was treated with EMR.

Due to improvements in diagnostic tools and prolonged lifespan rates, the diagnosis of esophageal subepithelial lesions is increasing. Leiomyoma is the most

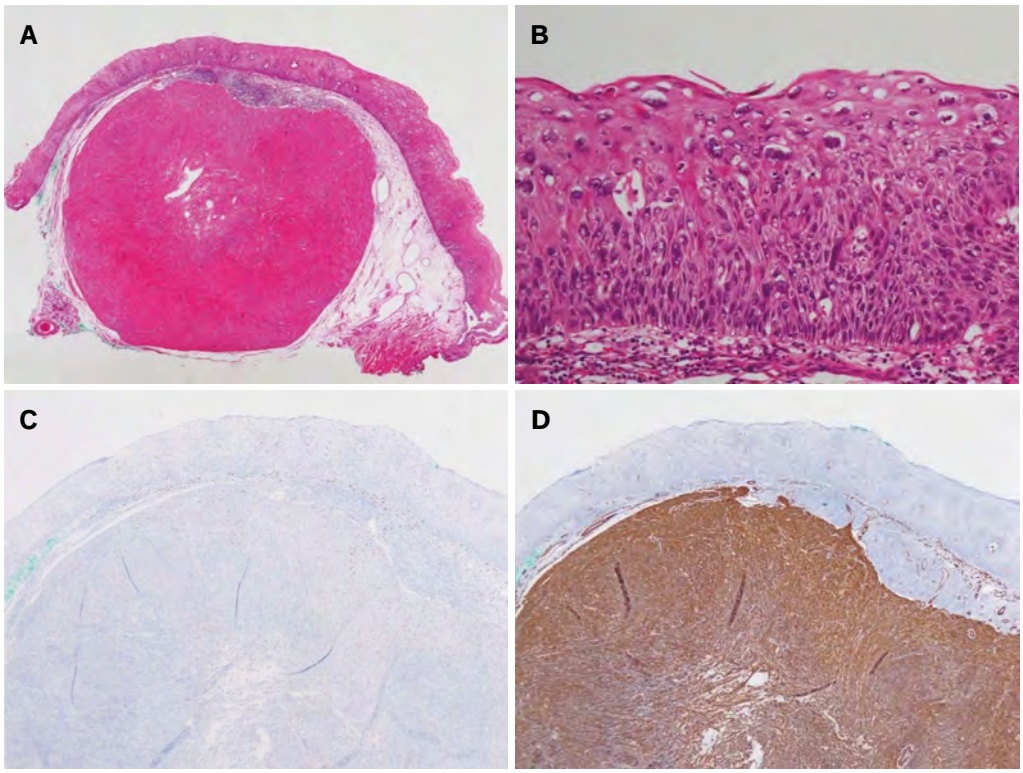


Figure 3 Histopathologic findings. A: Esophageal leiomyoma arising from the muscularis mucosa (HE, 20 ×); B: Atypical cells in all three areas of the epithelium, but not full thickness, showed severe dysplasia (HE, 200 ×); C: The esophageal leiomyoma was negative for CD117 (C-Kit); D: The esophageal leiomyoma showed strong and diffuse positive staining for smooth muscle actin (200 ×).

common subepithelial tumor of the esophagus. Esophageal leiomyomas account for approximately 12% of all gastrointestinal leiomyomas[9]. Esophageal leiomyomas may occur in the muscularis propria layer or the muscularis mucosa of the esophagus. With the development of the high frequency ultrasonic endoprobe, detection of the origin of leiomyomas, even in small lesions, appears to be easier[10]. On occasion, those arising from the muscularis mucosa can present as polypoid intraluminal tumors. In a review of 838 cases, only 1% were the polypoid intraluminal type[11]. The question of whether a leiomyoma can show malignant degeneration is controversial. If it were possible, the risk would be negligible. Thus, when a small leiomyoma covered with normal mucosa is encountered on endoscopy, a biopsy is not recommended[1].

The co-existence of an epithelial lesion and a subepithelial lesion is rare. Twelve patients in ten case reports with carcinoma located in the mucosa overlying a benign tumor have been reported[4-6,12]. All cases were squamous cell carcinoma and the subepithelial lesions were larger than 1 cm. Eight patients were treated surgically. However, this is the first case of epithelial dysplasia overlying a leiomyoma measuring less than 1 cm in the esophagus.

It is not clear whether both lesions occurred at the same time and whether there were any causative relationships. However, we suggest that there was synchronous development of the subepithelial and epithelial lesions. After development of a leiomyoma, external stimuli may change the mucosa. Epithelial dysplasia is the principal precursor lesion of esophageal squamous cell carcinoma. Studies have shown that esophageal squamous cell carcinoma develops through a progressive sequence from mild to severe dysplasia[13]. Predisposition to esophageal dysplasia may be related to certain carcinogenic stimuli, dietary factors, and individual genetic susceptibility[13]. The patient was a non-smoker and did not have any relevant medical history. He did not have any risk factors for esophageal cancer. We assume that focal mechanical stimuli might induce mucosa dysplasia. However, determination of a dysplastic lesion on conventional endoscopy is difficult, even when using an iodine stain. The prevalence of severe dysplasia derived from iodine-stained tissue is quite low (< 1%)[14]. The specificity of NBI for severe dysplasia performed by experienced endoscopists has been reported to be up to 100%[15]. Therefore, a subepithelial lesion presenting with an intraluminal polypoid mass should be examined closely using electronic chromoendoscopy with NBI and tissue biopsy. However, there is a risk of bleeding and scattering malignant cells with tissue biopsy. Furthermore, scarring after tissue biopsy can create problems in the complete removal of lesions. The examination of simultaneous lesions is important. If NBI findings suggest dysplastic change, it is better to resect the lesion without tissue biopsy. Severe dysplasia overlying a leiomyoma originating from the muscularis mucosa can be removed safely using an endoscopic technique[10].

In conclusion, a polypoid subepithelial tumor of the

esophagus is usually very small; however, it can occur with simultaneous epithelial dysplastic change. If the lesions are detected early using novel endoscopic imaging, they can be removed endoscopically.

REFERENCES

1 **Fountain SW**. Leiomyoma of the esophagus. *Thorac Cardiovasc Surg* 1986; **34**: 194-195 [PMID: 2426836 DOI: 10.1055/s-2007-1020408]

2 **Punpale A**, Rangole A, Bhambhani N, Karimundackal G, Desai N, de Souza A, Pramesh CS, Jambhekar N, Mistry RC. Leiomyoma of esophagus. *Ann Thorac Cardiovasc Surg* 2007; **13**: 78-81 [PMID: 17505413]

3 **Mutrie CJ**, Donahue DM, Wain JC, Wright CD, Gaissert HA, Grillo HC, Mathisen DJ, Allan JS. Esophageal leiomyoma: a 40-year experience. *Ann Thorac Surg* 2005; **79**: 1122-1125 [PMID: 15797036 DOI: 10.1016/j.athoracsur.2004.08.029]

4 **Kuwano H**, Sadanaga N, Watanabe M, Yasuda M, Nozoe T, Sugimachi K. Esophageal squamous cell carcinoma occurring in the surface epithelium over a benign tumor. *J Surg Oncol* 1995; **59**: 268-272 [PMID: 7630176]

5 **Watanabe M**, Baba T, Hotchi M. A case of leiomyoma of the lamina muscularis mucosae of the esophagus with a complication of carcinoma in situ of the overlying mucosa. *Acta Pathol Jpn* 1987; **37**: 1845-1851 [PMID: 3439459]

6 **Iizuka T**, Kato H, Watanabe H, Itabashi M, Hirota T. Superficial carcinoma of the esophagus coexisting with esophageal leiomyoma: a case report and review of the Japanese literature. *Jpn J Clin Oncol* 1984; **14**: 115-122 [PMID: 6708309]

7 **Iwaya T**, Maesawa C, Uesugi N, Kimura T, Ikeda K, Kimura Y, Mitomo S, Ishida K, Sato N, Wakabayashi G. Coexistence of esophageal superficial carcinoma and multiple leiomyomas: A case report. *World J Gastroenterol* 2006; **12**: 4588-4592 [PMID: 16874880]

8 **Mizobuchi S**, Kuge K, Matsumoto Y, Yokoyama Y, Ookawauchi K, Tamura S, Kurabayashi A, Sasaguri S. Co-existence of early esophageal carcinoma and leiomyoma: a case report. *Jpn J Clin Oncol* 2004; **34**: 751-754 [PMID: 15640507 DOI: 10.1093/jjco/hyh133]

9 **Choong CK**, Meyers BF. Benign esophageal tumors: introduction, incidence, classification, and clinical features. *Semin Thorac Cardiovasc Surg* 2003; **15**: 3-8 [PMID: 12813683]

10 **Lee SJ**, Paik YH, Lee DK, Lee KS, Lee SI. The diagnostic value of endoprobe for small esophageal leiomyomas derived from the muscularis mucosae. *Yonsei Med J* 2005; **46**: 61-65 [PMID: 15744806]

11 **Seremetis MG**, Lyons WS, deGuzman VC, Peabody JW. Leiomyomata of the esophagus. An analysis of 838 cases. *Cancer* 1976; **38**: 2166-2177 [PMID: 991129]

12 **Ishihara R**, Yamamoto S, Yamamoto S, Iishi H, Takeuchi Y, Kato M, Masuda E, Tatsumi K, Higashino K, Uedo N, Tatsuta M. Endoscopic resection of the esophageal squamous cell carcinoma overlying leiomyoma. *Gastrointest Endosc* 2008; **67**: 745-747 [PMID: 18206883 DOI: 10.1016/j.gie.2007.09.021]

13 **Mao WM**, Zheng WH, Ling ZQ. Epidemiologic risk factors for esophageal cancer development. *Asian Pac J Cancer Prev* 2011; **12**: 2461-2466 [PMID: 22320939]

14 **Fagundes RB**, de Barros SG, Pütten AC, Mello ES, Wagner M, Bassi LA, Bombassaro MA, Gobbi D, Souto EB. Occult dysplasia is disclosed by Lugol chromoendoscopy in alcoholics at high risk for squamous cell carcinoma of the esophagus. *Endoscopy* 1999; **31**: 281-285 [PMID: 10376452 DOI: 10.1055/s-1999-122]

15 **Ishihara R**, Takeuchi Y, Chatani R, Kidu T, Inoue T, Hanaoka N, Yamamoto S, Higashino K, Uedo N, Iishi H, Tatsuta M, Tomita Y, Ishiguro S. Prospective evaluation of narrow-band imaging endoscopy for screening of esophageal squamous mucosal high-grade neoplasia in experienced and less experienced endoscopists. *Dis Esophagus* 2010; **23**: 480-486 [PMID: 20095991 DOI: 10.1111/j.1442-2050.2009.01039.x]

P- Reviewer Stanojevic GZ **S- Editor** Song XX
L- Editor Webster JR **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i1.141

World J Gastroenterol 2013 January 7; 19(1): 141-143
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

Somatic second-hit mutations leads to polycystic liver diseases

Jesús M Banales, Patricia Munoz-Garrido, Luis Bujanda

Jesús M Banales, Patricia Munoz-Garrido, Luis Bujanda, Division of Hepatology and Gastroenterology, Donostia Universitary Hospital, Biodonostia Research Institute, E-20014 San Sebastián, Spain
Jesús M Banales, Luis Bujanda, Department of Liver and Gastrointestinal Diseases, National Institute for the Study of Liver and Gastrointestinal Diseases (CIBERehd, funded by the Spanish Carlos III Institute), 08036 Barcelona, Spain
Jesús M Banales, Ikerbasque, Basque Foundation for Science, 48011 Bilbao, Spain
Jesús M Banales, Spanish Association Against Cancer (AECC), 28040 Madrid, Spain
Author contributions: Banales JM, Munoz-Garrido P and Bujanda L performed the manuscript concept and design, drafting of the text and critical revision.
Correspondence to: Jesús M Banales, PhD, Division of Hepatology, Biodonostia Research Institute, Donostia Universitary Hospital, Paseo Dr. Begiristain s/n, E-20014 San Sebastián, Spain. jesus.banales@biodonostia.org
Telephone: +34-94-3006067 Fax: +34-94-3006250
Received: August 7, 2012 Revised: August 10, 2012
Accepted: August 26, 2012
Published online: January 7, 2013

Abstract

Polycystic liver diseases (PCLDs) are a heterogeneous group of genetic disorders characterized by the development of multiple fluid-filled cysts in the liver, which derive from cholangiocytes, the epithelial cells lining the bile ducts. When these cysts grow, symptoms such as abdominal distension, nausea, and abdominal pain may occur. PCLDs may exist isolated (i.e., autosomal dominant polycystic liver disease, ADPLD) or in combination with renal cystogenesis (i.e., autosomal dominant polycystic kidney disease and autosomal recessive polycystic liver disease). The exact prevalence of PCLDs is unknown, but is estimated to occur in approximately 1:1000 persons. Although the pathogenesis of each form of PCLD appears to be different, increasing evidences indicate that hepatic cystogenesis is a phenomenon that may involve somatic loss of heterozygosity (LOH) in those pathological conditions inherited in a dominant form. A recent report, using highly sophisticated methodology, demonstrated that ADPLD patients with a germline mutation in the protein kinase C substrate 80K-H (*PRKCSH*) gene mostly develop hepatic cystogenesis through a second somatic mutation. While hepatocystin, the PRKCSH-encoding protein, was absent in the hepatic cysts with LOH, it was still expressed in the heterozygous cysts. On the other hand, no additional trans-heterozygous mutations on the *SEC63 homolog* (*S. cerevisiae*/*SEC63*) gene (also involved in the development of PCLDs) were observed. These data indicate that PCLD is recessive at the cellular level, and point out the important role of hepatocystin loss in cystogenesis. In this commentary, we discuss the knowledge regarding the role of somatic second-hit mutations in the development of PCLDs, and the most relevant findings have been highlighted.

© 2013 Baishideng. All rights reserved.

Key words: Polycystic liver diseases; Cholangiocyte; Cystogenesis; Loss of heterozygosity; Protein kinase C substrate 80K-H; SEC63

Banales JM, Munoz-Garrido P, Bujanda L. Somatic second-hit mutations leads to polycystic liver diseases. *World J Gastroenterol* 2013; 19(1): 141-143 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i1/141.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i1.141

TO THE EDITOR

We have read with great interest the recently publication by Janssen *et al*[1] in *Gastroenterology*. The manuscript nicely demonstrate that cystogenesis in polycystic liver diseases is mostly associated with a loss of heterozygosity (LOH) in the biliary epithelial cells, termed cholangiocytes[2]. In this study, the group of professor Drenth employed blood and liver cystic tissue from 8 female ADPLD

patients, who had a heterozygous germline mutation in the protein kinase C substrate 80K-H (*PRKCSH*) gene (5 patients with a c.1341-2A>G mutation and 3 with a c.292+1G>C mutation) resulting in premature stop codons. Moreover, no additional germline mutations in *SEC63* were detected in any of these patients. By using laser microdissection of hepatic cysts, they found that 76% of the liver cysts presented a secondary somatic mutation in the normal *PRKCSH* allele (i.e., LOH). In contrast, the surrounding liver tissue and blood of the patients showed heterozygosity for this gene (Figure 1). Additionally, no detectable somatic changes in the *SEC63 homolog*-(*S. cerevisiae*/*SEC63*) gene sequence were found in cystic epithelium compared to non-epithelial cells or blood DNA, excluding trans-heterozygous *SEC63* somatic mutations as a cause of cyst formation in cysts without somatic *PRKCSH* mutations.

The authors also indicated that hepatic cysts presented different somatic mutations extended through out the 70-kilo-base region in the majority (97%) of the cysts. These data support the concept that cyst formation results from a specific genetic event in a subset of cells. Although all known mutations in ADPLD are heterozygous, the fact that ADPLD bile ducts express hepatocystin and ADPLD cystic cholangiocytes do not express this protein *via* a 2-hit somatic genomic mutation support the hypothesis that this disease in recessive on a cellular level. Interestingly, sequencing of full *PRKCSH* gene in those 12 cysts without LOH showed that 2 of them, despite expressing hepatocystin, developed a somatic missense mutation. Therefore, the other cysts without LOH in the *PRKCSH* gene may represent all the other mutations in other genes that may lead to cyst formation.

We agree with the authors that it is important to remark that although somatic 2-hit mutations in *PRKCSH* may develop anywhere in the body, cyst formation is only seen in the liver. This observation indicates that the liver-specific phenotype of ADPLD patients is a consequence of a more sensitive response of cholangiocytes to the lack of hepatocystin. Because liver cysts display cholangiocyte features and no malignant transformation, the possibility that a somatic 2-hit mutation originating from a liver progenitor cell would explain the cyst development and the hyperproliferative properties of the cysts. There have been reports on the presence of 2-hit mechanisms in other non-malignant diseases including autosomal dominant polycystic kidney disease (ADPKD), where a somatic LOH was found in a proportion of both kidney and liver cysts[3,4].

Polycystic liver diseases (PCLDs) are genetic disorders characterized by the development of fluid-filled cysts in the liver derived from cholangiocytes[5-7]. The exact prevalence of PCLDs is unknown, but is estimated to occur in approximately 1:1000 persons. The most common symptoms are caused by enlargement of the liver, and include abdominal distension, dyspepsia, abdominal pain, and early satiety. Although not found

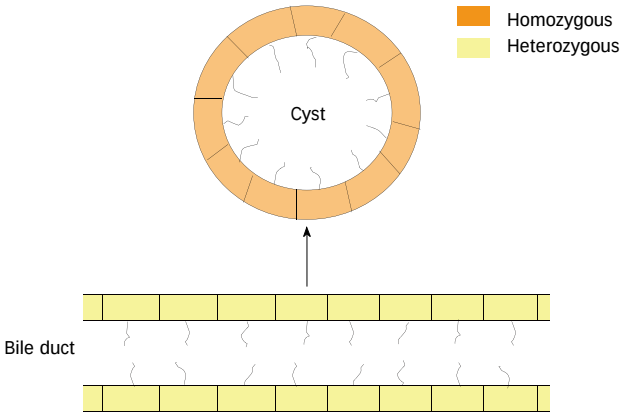


Figure 1 Chart representing the second somatic mutations occurring in the majority of cysts from autosomal dominant polycystic liver disease patients. Most of autosomal dominant polycystic liver disease cysts with a protein kinase C substrate 80K-H germline mutation develop over time a second somatic mutations in the same gene that result in the loss of heterozygosity and cyst formation.

frequently, serious complications such as portal hypertension and haemorrhage, or infections of cysts, may also occur[5-7]. The liver phenotype may exist isolated (i.e., ADPLD) or in combination with renal cystogenesis (i.e., autosomal dominant polycystic liver disease ADPLD, and autosomal recessive polycystic liver disease, ARPKD)[5-7]. ADPLD is a rare disease that can be caused by mutations in *PRKCSH* or *SEC63* genes, which encode for the endoplasmic reticulum (ER)-associated proteins hepatocystin and Sec63, involved in glycosylation and transport of glycoproteins into and out of the ER, respectively[5]. On the other hand, the genes responsible for the development of ADPKD (1:1000 prevalence) are *PKD1* and *PKD2*, which encode for polycystin-1 and polycystin-2, respectively[6,7]. Polycystin-1 is a mechanoreceptor involved in calcium signalling, and polycystin-2 a non-selective calcium channel. Finally, ARPKD (1:20.000 prevalence) is caused by mutations in the *PKHD1* gene, which encodes for fibrocystin, a protein with unknown function[6,7]. The pathogenesis of each form of PCLD appears to be different. However, alterations in different mechanisms such as proliferation, fluid secretion, and cell-matrix interactions seem to play an important role in all of them. Moreover, these altered events seem to be also associated with changes in the microRNA expression pattern, with increased levels of cyclic adenosine monophosphate levels, and with downregulation of the intracellular calcium levels[8,9]. Currently, there is no standard treatment for PCLDs. Pharmacological approaches include somatostatin analogues and mammalian target of rapamycin-inhibitors, but most clinical trials have only shown a small reduction of liver volume. On the other hand, the most frequently used surgical procedures are aspiration and sclerotherapy, fenestration, segmental hepatic resection, and liver transplantation, which have a better short term effect, but high recurrence and complication rate preclude wide spread use[5].

In summary, this article represents a milestone in the understanding of the molecular mechanisms involved in liver cystogenesis and leads to several questions such as the unknown mechanisms causing LOH in PCLDs, as well as the role of 2-hit somatic mutations in all forms of PCLDs, including ADPLD patients with a germline mutation in SEC63.

REFERENCES

1 **Janssen MJ**, Waanders E, Te Morsche RH, Xing R, Dijkman HB, Woudenberg J, Drenth JP. Secondary, somatic mutations might promote cyst formation in patients with autosomal dominant polycystic liver disease. *Gastroenterology* 2011; **141**: 2056-2063.e2 [PMID: 21856269 DOI: 10.1053/j.gastro.2011.08.]

2 **Banales JM**, Prieto J, Medina JF. Cholangiocyte anion exchange and biliary bicarbonate excretion. *World J Gastroenterol* 2006; **12**: 3496-3511 [PMID: 16773707]

3 **Qian F**, Germino GG. "Mistakes happen": somatic mutation and disease. *Am J Hum Genet* 1997; **61**: 1000-1005 [PMID: 9345111 DOI: 10.1086/301618]

4 **Qian F**, Watnick TJ, Onuchic LF, Germino GG. The molecular basis of focal cyst formation in human autosomal dominant polycystic kidney disease type I. *Cell* 1996; **87**: 979-987 [PMID: 8978603 DOI: 10.1016/S0092-8674(00)]

5 **Drenth JP**, Chrispijn M, Nagorney DM, Kamath PS, Torres VE. Medical and surgical treatment options for polycystic liver disease. *Hepatology* 2010; **52**: 2223-2230 [PMID: 21105111 DOI: 10.1002/hep.24036]

6 **Torres VE**, Harris PC, Pirson Y. Autosomal dominant polycystic kidney disease. *Lancet* 2007; **369**: 1287-1301 [PMID: 17434405 DOI: 10.1016/S0140-6736(07)60601-1]

7 **Strazzabosco M**, Somlo S. Polycystic liver diseases: congenital disorders of cholangiocyte signaling. *Gastroenterology* 2011; **140**: 1855-189, 1859.e1 [PMID: 21515270]

8 **Banales JM**, Masyuk TV, Gradilone SA, Masyuk AI, Medina JF, LaRusso NF. The cAMP effectors Epac and protein kinase a (PKA) are involved in the hepatic cystogenesis of an animal model of autosomal recessive polycystic kidney disease (ARPKD). *Hepatology* 2009; **49**: 160-174 [PMID: 19065671 DOI: 10.1002/hep.22636]

9 **Gradilone SA**, Masyuk TV, Huang BQ, Banales JM, Lehmann GL, Radtke BN, Stroope A, Masyuk AI, Splinter PL, LaRusso NF. Activation of Trpv4 reduces the hyperproliferative phenotype of cystic cholangiocytes from an animal model of ARPKD. *Gastroenterology* 2010; **139**: 304-14.e2 [PMID: 20399209]

P- Reviewer Levesque H **S- Editor** Jia F
L- Editor A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i1.144

World J Gastroenterol 2013 January 7; 19(1): 144-146
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

Is exon mutation analysis needed for adjuvant treatment of gastrointestinal stromal tumor?

Mehmet Ali Nahit Şendur, Nuriye Yıldırım Özdemir, Muhammed Bülent Akıncı, Dogan Uncu, Nurullah Zengin, Sercan Aksoy

Mehmet Ali Nahit Şendur, Nuriye Yıldırım Özdemir, Muhammed Bülent Akıncı, Dogan Uncu, Nurullah Zengin, Department of Medical Oncology, Ankara Numune Education and Research Hospital, Ankara 06100, Turkey
Sercan Aksoy, Department of Medical Oncology, Hacettepe University Institute of Oncology, Ankara 06100, Turkey
Author contributions: Şendur MAN, Aksoy S and Özdemir NY wrote the paper; Akıncı MB, Uncu D and Zengin N designed the paper.
Correspondence to: Mehmet Ali Nahit Şendur, MD, Department of Medical Oncology, Ankara Numune Education and Research Hospital, Sihhiye, Ankara 06100, Turkey. masendur@yahoo.com.tr
Telephone: +90-312-5084600 Fax: +90-312-3112778
Received: June 22, 2012 Revised: September 25, 2012
Accepted: November 11, 2012
Published online: January 7, 2013

Abstract

Gastrointestinal stromal tumors (GISTs) are the most common soft tissue sarcoma of the gastrointestinal tract, resulting from an activating mutation of stem cell factor receptor (KIT), and an activating mutation of the homologous platelet-derived growth factor receptor alpha (PDGFRA) kinase. Most GISTs (90%-95%) are KIT-positive. About 5% of GISTs are truly negative for KIT expression. GISTs have been documented to resistant conventional chemotherapeutics. Due to the KIT activation that occurs in the majority of the cases, KIT inhibition is the primary treatment approach in the adjuvant treatment of metastatic GISTs. Imatinib mesylate is an oral agent that is a selective protein tyrosine kinase inhibitor of the KIT protein tyrosine kinase, and it has demonstrated clinical benefit and objective tumor responses in most GIST patients in phase Ⅱ and Ⅲ trials. The presence and the type of KIT or PDGFRA mutation are predictive of response to imatinib therapy in patients with advanced and metastatic disease. Molecular analysis in phase Ⅰ-Ⅱ trials revealed significant differences in objective response, progression-free survival, and overall survival between GISTs with different kinase mutations. The aim of this letter is to touch on the need for exon mutation analysis for adjuvant treatment with imatinib in GIST patients.

© 2013 Baishideng. All rights reserved.

Key words: Imatinib; Gastrointestinal stromal tumor; Activating mutation; Stem cell factor receptor; Platelet-derived growth factor receptor alpha; Mutation analysis

Şendur MAN, Özdemir NY, Akıncı MB, Uncu D, Zengin N, Aksoy S. Is exon mutation analysis needed for adjuvant treatment of gastrointestinal stromal tumor? *World J Gastroenterol* 2013; 19(1): 144-146 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i1/144.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i1.144

TO THE EDITOR

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal neoplasms of the gastrointestinal tract. In general, 75% to 85% of GISTs have an activating mutation of stem cell factor receptor (KIT), 5% to 7% have an activating mutation of the homologous platelet-derived growth factor receptor alpha (PDGFRA) kinase, and approximately 12% to 15% of GISTs do not have a detectable mutation[1-3]. Imatinib mesylate is an oral agent that is a selective tyrosine kinase inhibitor of the KIT, PDGFRA, Abelson (ABL), and ABL activated by fusion with the *BCR* gene tyrosine kinases. Phase Ⅱ and Ⅲ trials have demonstrated that imatinib is effective against metastatic GIST in more than 50% of patients[4-7]. The presence and the type of KIT or PDGFRA

mutation are predictive of response to imatinib therapy in patients with advanced and metastatic disease. Molecular analysis in phase Ⅰ to Ⅱ trials revealed significant differences in objective response, progression-free survival (PFS), and overall survival (OS) between GISTs with different kinase mutations[8]. Previously published studies have shown that the outcomes of patients with KIT exon 11-mutant GIST were better than for patients with KIT exon 9-mutant GIST and tumors without a detectable KIT mutation[1,9]. In a recent trial, Joensuu *et al*[10] reported that in the adjuvant treatment of high risk of recurrence GIST patients, 36 mo of imatinib treatment improved recurrence-free survival (RFS) and OS compared with 12 mo of imatinib treatment. In this study, the KIT exon 11 mutation was found in nearly 65% of both groups. In exon 11 mutation-positive patients, RFS was statistically improved in the 36-mo imatinib treatment group compared with the 12-mo treatment group. However, RFS was not statistically significantly changed in all remaining patients who had a positive mutation other than exon 11, and who had extension of the treatment to 36 mo compared to 12 mo. The presence and the type of KIT or PDGFRA mutation are predictive of response to imatinib treatment. Heinrich *et al*[8] reported that the presence of the KIT exon 11 mutation in advanced GIST patients who were treated with imatinib correlated with improved objective response, time to tumor progression and OS, when compared with the presence of the KIT exon 9 mutation and wild-type genotypes. In the Finnish B2222 trial, assessing imatinib treatment in metastatic GIST patients, the partial response rate was 83.5% for those patients with positive exon 11 mutation, whereas it was 47.8% in exon 9 mutation-positive patients[1].

A randomized European Organisation for Research and Treatment of Cancer phase Ⅲ trial reported that treatment with imatinib 800 mg/d had a significantly superior PFS compared to imatinib 400 mg/d in advanced GIST patients with an exon 9 mutation[11]. Also, Heinrich *et al*[8] reported that imatinib 800 mg/d had improved response rates compared to imatinib 400 mg/d in advanced GIST patients with positive exon 9 mutation. DeMatteo *et al*[12] reported that adjuvant imatinib therapy compared to placebo treatment appears to prolong RFS following the resection of primary GIST, but the effect of mutation on RFS was not reported. On these grounds, the optimal duration of treatment and the optimal dose are not known for operable GIST with a high risk of recurrence, other than for exon 11 mutation patients.

In conclusion, analysis can disclose the type of exon mutation which can be predictive of response to adjuvant imatinib treatment in GIST patients.

REFERENCES

1 **Heinrich MC**, Corless CL, Demetri GD, Blanke CD, von Mehren M, Joensuu H, McGreevey LS, Chen CJ, Van den Abbeele AD, Druker BJ, Kiese B, Eisenberg B, Roberts PJ, Singer S, Fletcher CD, Silberman S, Dimitrijevic S, Fletcher JA. Kinase mutations and imatinib response in patients with metastatic gastrointestinal stromal tumor. *J Clin Oncol* 2003; **21**: 4342-4349 [PMID: 14645423 DOI: 10.1200/JCO.2003.04.190]

2 **Corless CL**, Fletcher JA, Heinrich MC. Biology of gastrointestinal stromal tumors. *J Clin Oncol* 2004; **22**: 3813-3825 [PMID: 15365079 DOI: 10.1200/JCO.2004.05.140]

3 **Heinrich MC**, Corless CL, Duensing A, McGreevey L, Chen CJ, Joseph N, Singer S, Griffith DJ, Haley A, Town A, Demetri GD, Fletcher CD, Fletcher JA. PDGFRA activating mutations in gastrointestinal stromal tumors. *Science* 2003; **299**: 708-710 [PMID: 12522257 DOI: 10.1126/science.1079666]

4 **Demetri GD**, von Mehren M, Blanke CD, Van den Abbeele AD, Eisenberg B, Roberts PJ, Heinrich MC, Tuveson DA, Singer S, Janicek M, Fletcher JA, Silverman SG, Silberman SL, Capdeville R, Kiese B, Peng B, Dimitrijevic S, Druker BJ, Corless C, Fletcher CD, Joensuu H. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. *N Engl J Med* 2002; **347**: 472-480 [PMID: 12181401 DOI: 10.1056/NEJMoa020461]

5 **Blanke CD**, Demetri GD, von Mehren M, Heinrich MC, Eisenberg B, Fletcher JA, Corless CL, Fletcher CD, Roberts PJ, Heinz D, Wehre E, Nikolova Z, Joensuu H. Long-term results from a randomized phase II trial of standard- versus higher-dose imatinib mesylate for patients with unresectable or metastatic gastrointestinal stromal tumors expressing KIT. *J Clin Oncol* 2008; **26**: 620-625 [PMID: 18235121 DOI: 10.1200/JCO.2007.13.4403]

6 **Blanke CD**, Rankin C, Demetri GD, Ryan CW, von Mehren M, Benjamin RS, Raymond AK, Bramwell VH, Baker LH, Maki RG, Tanaka M, Hecht JR, Heinrich MC, Fletcher CD, Crowley JJ, Borden EC. Phase III randomized, intergroup trial assessing imatinib mesylate at two dose levels in patients with unresectable or metastatic gastrointestinal stromal tumors expressing the kit receptor tyrosine kinase: S0033. *J Clin Oncol* 2008; **26**: 626-632 [PMID: 18235122 DOI: 10.1200/JCO.2007.13.4452]

7 **Li J**, Gong JF, Li J, Gao J, Sun NP, Shen L. Efficacy of imatinib dose escalation in Chinese gastrointestinal stromal tumor patients. *World J Gastroenterol* 2012; **18**: 698-703 [PMID: 22363143 DOI: 10.3748/wjg.v18.I7.698]

8 **Heinrich MC**, Owzar K, Corless CL, Hollis D, Borden EC, Fletcher CD, Ryan CW, von Mehren M, Blanke CD, Rankin C, Benjamin RS, Bramwell VH, Demetri GD, Bertagnolli MM, Fletcher JA. Correlation of kinase genotype and clinical outcome in the North American Intergroup Phase III Trial of imatinib mesylate for treatment of advanced gastrointestinal stromal tumor: CALGB 150105 Study by Cancer and Leukemia Group B and Southwest Oncology Group. *J Clin Oncol* 2008; **26**: 5360-5367 [PMID: 18955451 DOI: 10.1200/JCO.2008.17.4284]

9 **Debiec-Rychter M**, Dumez H, Judson I, Wasag B, Verweij J, Brown M, Dimitrijevic S, Sciot R, Stul M, Vranck H, Scurr M, Hagemeijer A, van Glabbeke M, van Oosterom AT. Use of c-KIT/PDGFRA mutational analysis to predict the clinical response to imatinib in patients with advanced gastrointestinal stromal tumours entered on phase I and II studies of the EORTC Soft Tissue and Bone Sarcoma Group. *Eur J Cancer* 2004; **40**: 689-695 [PMID: 15010069 DOI: 10.1016/j.ejca.2003.11.025]

10 **Joensuu H**, Eriksson M, Sundby Hall K, Hartmann JT, Pink D, Schütte J, Ramadori G, Hohenberger P, Duyster J, Al-Batran SE, Schlemmer M, Bauer S, Wardelmann E, Sarlomo-Rikala M, Nilsson B, Sihto H, Monge OR, Bono P, Kallio R, Vehtari A, Leinonen M, Alvegård T, Reichardt P. One vs three years of adjuvant imatinib for operable gastrointes-

tinal stromal tumor: a randomized trial. *JAMA* 2012; **307**: 1265-1272 [PMID: 22453568 DOI: 10.1001/jama.2012.347]

11 **Debiec-Rychter M**, Sciot R, Le Cesne A, Schlemmer M, Hohenberger P, van Oosterom AT, Blay JY, Leyvraz S, Stul M, Casali PG, Zalcberg J, Verweij J, Van Glabbeke M, Hagemeijer A, Judson I. KIT mutations and dose selection for imatinib in patients with advanced gastrointestinal stromal tumours. *Eur J Cancer* 2006; **42**: 1093-1103 [PMID: 16624552]

12 **Dematteo RP**, Ballman KV, Antonescu CR, Maki RG, Pisters PW, Demetri GD, Blackstein ME, Blanke CD, von Mehren M, Brennan MF, Patel S, McCarter MD, Polikoff JA, Tan BR, Owzar K. Adjuvant imatinib mesylate after resection of localised, primary gastrointestinal stromal tumour: a randomised, double-blind, placebo-controlled trial. *Lancet* 2009; **373**: 1097-1104 [PMID: 19303137]

P- Reviewers Peparini N, KhanI **S- Editor** Gou SX
L- Editor Logan S **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
www.wjgnet.com

World J Gastroenterol 2013 January 7; 19(1): I-VI
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

GENERAL INFORMATION

World Journal of Gastroenterology (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access (OA) journal. *WJG* was established on October 1, 1995. It is published weekly on the 7th, 14th, 21st, and 28th each month. The *WJG* Editorial Board consists of 1352 experts in gastroenterology and hepatology from 64 countries.

Aims and scope

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

WJG is published by Baishideng Publishing Group (BPG) in both electronic and online forms. All *WJG* articles are published in *WJG* website and PubMed Central. The major advantages of OA journals are faster release and delivery, no page or graph restrictions, and increased visibility, usage and impact. Full-text PDF articles and electronic/online versions are freely available to global readers. After the paper is published, the author(s) can obtain high-quality PDF files, which contain the journal cover, a list of editorial board members, table of contents, text, and back cover of the journal. BPG has a strong professional editorial team composed of editorial board members, editors-in-chief, science editors, language editors, and electronic editors. BPG currently publishes 42 OA clinical medical journals, including 41 in English, has a total of 15 471 editorial board members or peer reviewers, and is a world first-class publisher.

Columns

The columns in the issues of *WJG* will include: (1) Editorial: The editorial board members are invited to make comments on an important topic in their field in terms of its current research status and future directions to lead the development of this discipline; (2) Frontier: The editorial board members are invited to select a highly cited cutting-edge original paper of his/her own to summarize major findings, the problems that have been resolved and remain to be resolved, and future research directions to help readers understand his/her important academic point of view and future research directions in the field; (3) Diagnostic Advances: The editorial board members are invited to write high-quality diagnostic advances in their field to improve the diagnostic skills of readers. The topic covers general clinical diagnosis, differential diagnosis, pathological diagnosis, laboratory diagnosis, imaging diagnosis, endoscopic diagnosis, biotechnological diagnosis, functional diagnosis, and physical diagnosis; (4) Therapeutics Advances: The editorial board members are invited to write high-quality therapeutic advances in their field to help improve the therapeutic skills of readers. The topic covers medication therapy, psychotherapy, physical therapy, replacement therapy, interventional therapy, minimally invasive therapy, endoscopic therapy, transplantation therapy, and surgical therapy; (5) Field of Vision: The editorial board members are invited to write commentaries on classic articles, hot topic articles, or latest articles to keep readers at the forefront of research and increase their levels of clinical research. Classic articles refer to papers that are included in Web of Knowledge and have received a large number of citations (ranking in the top 1%) after being published for more than years, reflecting the quality and impact of papers. Hot topic articles refer to papers that are included in Web of Knowledge and have received a large number of citations after being published for no more than 2 years, reflecting cutting-edge trends in scientific research. Latest articles refer to the latest published high-quality papers that are included in PubMed, reflecting the latest research trends. These commentary articles should focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions. Basic information about the article to be commented (including authors, article title, journal name, year, volume, and inclusive page numbers; (6) Minireviews: The editorial board members are invited to write short reviews on recent advances and trends in research of molecular biology, genomics, and related cutting-edge technologies to provide readers with the latest knowledge and help improve their diagnostic and therapeutic skills; (7) Review: To make a systematic review to focus on the status quo of research, the most important research topics, the problems that have now been resolved and remain to be resolved, and future research directions; (8) Topic Highlight: The editorial board members are invited to write a series of articles (7-10 articles) to comment and discuss a hot topic to help improve the diagnostic and therapeutic skills of readers; (9) Medical Ethics: The editorial board members are invited to write articles about medical ethics to increase readers' knowledge of medical ethics. The topic covers international ethics guidelines, animal studies, clinical trials, organ transplantation, *etc.*; (10) Clinical Case Conference or Clinicopathological Conference: The editorial board members are invited to contribute high-quality clinical case conference; (11) Original Articles: To report innovative and original findings in gastroenterology and hepatology; (12) Brief

Articles: To briefly report the novel and innovative findings in gastroenterology and hepatology; (13) Meta-Analysis: To evaluate the clinical effectiveness in gastroenterology and hepatology by using data from two or more randomised control trials; (14) Case Report: To report a rare or typical case; (15) Letters to the Editor: To discuss and make reply to the contributions published in *WJG*, or to introduce and comment on a controversial issue of general interest; (16) Book Reviews: To introduce and comment on quality monographs of gastroenterology and hepatology; and (17) Autobiography: The editorial board members are invited to write their autobiography to provide readers with stories of success or failure in their scientific research career. The topic covers their basic personal information and information about when they started doing research work, where and how they did research work, what they have achieved, and their lessons from success or failure.

Name of journal
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

Launch date
October 1, 1995

Frequency
Weekly

Editor-in-chief
Ferruccio Bonino, MD, PhD, Professor of Gastroenterology, Director of Liver and Digestive Disease Division, Department of Internal Medicine, University of Pisa, Director of General Medicine 2 Unit University Hospital of Pisa, Via Roma 67, 56124 Pisa, Italy

Myung-Hwan Kim, MD, PhD, Professor, Head, Department of Gastroenterology, Director, Center for Biliary Diseases, University of Ulsan College of Medicine, Asan Medical Center, 388-1 Pungnap-2dong, Songpa-gu, Seoul 138-736, South Korea

Kjell Öberg, MD, PhD, Professor, Department of Endocrine Oncology, Uppsala University Hospital, SE-751 85 Uppsala, Sweden

Matt D Rutter, MBBS, MD, FRCP, Consultant Gastroenterologist, Senior Lecturer, Director, Tees Bowel Cancer Screening Centre, University Hospital of North Tees, Durham University, Stockton-on-Tees, Cleveland TS19 8PE, United Kingdom

Andrzej S Tarnawski, MD, PhD, DSc (Med), Professor of Medicine, Chief Gastroenterology, VA Long Beach Health Care System, University of California, Irvine, CA, 5901 E. Seventh Str., Long Beach, CA 90822, United States

Editorial office
Jian-Xia Cheng, Director
Jin-Lei Wang, Vice Director
World Journal of Gastroenterology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com
http://www.wjgnet.com

Publisher
Baishideng Publishing Group Co., Limited
Room 1701, 17/F, Henan Building,
No. 90 Jaffe Road, Wanchai,
Hong Kong, China
Fax: +852-3115-8812
Telephone: +852-5804-2046
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

Production center
Beijing Baishideng BioMed Scientific Co., Limited
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-85381892
Fax: +86-10-85381893

Representative office
USA Office
8226 Regency Drive,
Pleasanton, CA 94588-3144, United States
Telephone: +1-925-2238242
Fax: +1-925-2238243

Instructions to authors
Full instructions are available online at http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm

Indexed and abstracted in
Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, Digital Object Identifier, and Directory of Open Access Journals. ISI, Thomson Reuters, 2011 Impact Factor: 2.471 (32/74 Gastroenterology and Hepatology).

SPECIAL STATEMENT

All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

Biostatistical editing
Statistical review is performed after peer review. We invite an expert in Biomedical Statistics to evaluate the statistical method used in the paper, including *t*-test (group or paired comparisons), chi-squared test, ridit, probit, logit, regression (linear, curvilinear, or stepwise), correlation, analysis of variance, analysis of covariance, *etc*. The reviewing points include: (1) Statistical methods should be described when they are used to verify the results; (2) Whether the statistical techniques are suitable or correct; (3) Only homogeneous data can be averaged. Standard deviations are preferred to standard errors. Give the number of observations and subjects (*n*). Losses in observations, such as drop-outs from the study should be reported; (4) Values such as ED50, LD50, IC50 should have their 95% confidence limits calculated and compared by weighted probit analysis (Bliss and Finney); and (5) The word 'significantly' should be replaced by its synonyms (if it indicates extent) or the *P* value (if it indicates statistical significance).

Conflict-of-interest statement
In the interests of transparency and to help reviewers assess any potential bias, *WJG* requires authors of all papers to declare any competing commercial, personal, political, intellectual, or religious interests in relation to the submitted work. Referees are also asked

to indicate any potential conflict they might have reviewing a particular paper. Before submitting, authors are suggested to read "Uniform Requirements for Manuscripts Submitted to Biomedical Journals: Ethical Considerations in the Conduct and Reporting of Research: Conflicts of Interest" from International Committee of Medical Journal Editors (ICMJE), which is available at: http://www.icmje.org/ethical_4conflicts.html.

Statement of informed consent

Manuscripts should contain a statement to the effect that all human studies have been reviewed by the appropriate ethics committee or it should be stated clearly in the text that all persons gave their informed consent prior to their inclusion in the study. Details that might disclose the identity of the subjects under study should be omitted. Authors should also draw attention to the Code of Ethics of the World Medical Association (Declaration of Helsinki, 1964, as revised in 2004).

Sample wording: [Name of individual] has received fees for serving as a speaker, a consultant and an advisory board member for [names of organizations], and has received research funding from [names of organization]. [Name of individual] is an employee of [name of organization]. [Name of individual] owns stocks and shares in [name of organization]. [Name of individual] owns patent [patent identification and brief description].

Statement of human and animal rights

When reporting the results from experiments, authors should follow the highest standards and the trial should conform to Good Clinical Practice (for example, US Food and Drug Administration Good Clinical Practice in FDA-Regulated Clinical Trials; UK Medicines Research Council Guidelines for Good Clinical Practice in Clinical Trials) and/or the World Medical Association Declaration of Helsinki. Generally, we suggest authors follow the lead investigator's national standard. If doubt exists whether the research was conducted in accordance with the above standards, the authors must explain the rationale for their approach and demonstrate that the institutional review body explicitly approved the doubtful aspects of the study.

Before submitting, authors should make their study approved by the relevant research ethics committee or institutional review board. If human participants were involved, manuscripts must be accompanied by a statement that the experiments were undertaken with the understanding and appropriate informed consent of each. Any personal item or information will not be published without explicit consents from the involved patients. If experimental animals were used, the materials and methods (experimental procedures) section must clearly indicate that appropriate measures were taken to minimize pain or discomfort, and details of animal care should be provided.

SUBMISSION OF MANUSCRIPTS

Manuscripts should be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Number all pages consecutively, and start each of the following sections on a new page: Title Page, Abstract, Introduction, Materials and Methods, Results, Discussion, Acknowledgements, References, Tables, Figures, and Figure Legends. Neither the editors nor the publisher are responsible for the opinions expressed by contributors. Manuscripts formally accepted for publication become the permanent property of Baishideng Publishing Group Co., Limited, and may not be reproduced by any means, in whole or in part, without the written permission of both the authors and the publisher. We reserve the right to copy-edit and put onto our website accepted manuscripts. Authors should follow the relevant guidelines for the care and use of laboratory animals of their institution or national animal welfare committee. For the sake of transparency in regard to the performance and reporting of clinical trials, we endorse the policy of the ICMJE to refuse to publish papers on clinical trial results if the trial was not recorded in a publicly-accessible registry at its outset. The only register now available, to our knowledge, is http://www.clinicaltrials.gov sponsored by the United States National Library of Medicine and we encourage all potential contributors to register with it. However, in the case that other registers become available you will be duly notified. A letter of recommendation from each author's organization should be provided with the contributed article to ensure the privacy and secrecy of research is protected.

Authors should retain one copy of the text, tables, photographs and illustrations because rejected manuscripts will not be returned to the author(s) and the editors will not be responsible for loss or damage to photographs and illustrations sustained during mailing.

Online submissions

Manuscripts should be submitted through the Online Submission System at: http://www.wjgnet.com/esps/. Authors are highly recommended to consult the ONLINE INSTRUCTIONS TO AUTHORS (http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm) before attempting to submit online. For assistance, authors encountering problems with the Online Submission System may send an email describing the problem to wjg@wjgnet.com, or by telephone: +86-10-5908-0039. If you submit your manuscript online, do not make a postal contribution. Repeated online submission for the same manuscript is strictly prohibited.

MANUSCRIPT PREPARATION

All contributions should be written in English. All articles must be submitted using word-processing software. All submissions must be typed in 1.5 line spacing and 12 pt. Book Antiqua with ample margins. Style should conform to our house format. Required information for each of the manuscript sections is as follows:

Title page

Title: Title should be less than 12 words.

Running title: A short running title of less than 6 words should be provided.

Authorship: Authorship credit should be in accordance with the standard proposed by ICMJE, based on (1) substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; (2) drafting the article or revising it critically for important intellectual content; and (3) final approval of the version to be published. Authors should meet conditions 1, 2, and 3.

Institution: Author names should be given first, then the complete name of institution, city, province and postcode. For example, Xu-Chen Zhang, Li-Xin Mei, Department of Pathology, Chengde Medical College, Chengde 067000, Hebei Province, China. One author may be represented from two institutions, for example, George Sgourakis, Department of General, Visceral, and Transplantation Surgery, Essen 45122, Germany; George Sgourakis, 2nd Surgical Department, Korgialenio-Benakio Red Cross Hospital, Athens 15451, Greece.

Author contributions: The format of this section should be: Author contributions: Wang CL and Liang L contributed equally to this work; Wang CL, Liang L, Fu JF, Zou CC, Hong F and Wu XM designed the research; Wang CL, Zou CC, Hong F and Wu XM performed the research; Xue JZ and Lu JR contributed new reagents/analytic tools; Wang CL, Liang L and Fu JF analyzed the data; and Wang CL, Liang L and Fu JF wrote the paper.

Supportive foundations: The complete name and number of supportive foundations should be provided, e.g. Supported by National Natural Science Foundation of China, No. 30224801

Correspondence to: Only one corresponding address should be provided. Author names should be given first, then author title, affiliation, the complete name of institution, city, postcode, province, country, and email. All the letters in the email should be in lower case. A space interval should be inserted between country name and email address. For example, Montgomery Bissell, MD, Professor of Medicine, Chief, Liver Center, Gastroenterology Division, University of California, Box 0538, San Francisco, CA 94143, United States. montgomery.bissell@ucsf.edu

Telephone and fax: Telephone and fax should consist of +, country number, district number and telephone or fax number, e.g. Telephone: +86-10-59080039 Fax: +86-10-85381893

Peer reviewers: All articles received are subject to peer review. Normally, three experts are invited for each article. Decision on acceptance is made only when at least two experts recommend publication of an article. All peer-reviewers are acknowledged on Express Submission and Peer-review System website.

Abstract

There are unstructured abstracts (no less than 200 words) and structured abstracts. The specific requirements for structured abstracts are as follows:

An informative, structured abstract should accompany each manuscript. Abstracts of original contributions should be structured into the following sections: AIM (no more than 20 words; Only the purpose of the study should be included. Please write the Aim in the form of "To investigate/study/…"), METHODS (no less than 140 words for Original Articles; and no less than 80 words for Brief Articles), RESULTS (no less than 150 words for Original Articles and no less than 120 words for Brief Articles; You should present *P* values where appropriate and must provide relevant data to illustrate how they were obtained, e.g. 6.92 ± 3.86 *vs* 3.61 ± 1.67, $P < 0.001$), and CONCLUSION (no more than 26 words).

Key words

Please list 5-10 key words, selected mainly from *Index Medicus*, which reflect the content of the study.

Core tip

Please write a summary of less than 100 words to outline the most innovative and important arguments and core contents in your paper to attract readers.

Text

For articles of these sections, original articles and brief articles, the main text should be structured into the following sections: INTRODUCTION, MATERIALS AND METHODS, RESULTS and DISCUSSION, and should include appropriate Figures and Tables. Data should be presented in the main text or in Figures and Tables, but not in both.

Illustrations

Figures should be numbered as 1, 2, 3, *etc*., and mentioned clearly in the main text. Provide a brief title for each figure on a separate page. Detailed legends should not be provided under the figures. This part should be added into the text where the figures are applicable. Keeping all elements compiled is necessary in line-art image. Scale bars should be used rather than magnification factors, with the length of the bar defined in the legend rather than on the bar itself. File names should identify the figure and panel. Avoid layering type directly over shaded or textured areas. Please use uniform legends for the same subjects. For example: Figure 1 Pathological changes in atrophic gastritis after treatment. A:...; B:...; C:...; D:...; E:...; F:...; G: …*etc*. It is our principle to publish high resolution-figures for the E-versions.

Tables

Three-line tables should be numbered 1, 2, 3, *etc*., and mentioned clearly in the main text. Provide a brief title for each table. Detailed legends should not be included under tables, but rather added into the text where applicable. The information should complement, but not duplicate the text. Use one horizontal line under the title, a second under column heads, and a third below the Table, above any footnotes. Vertical and italic lines should be omitted.

Notes in tables and illustrations

Data that are not statistically significant should not be noted. $^{a}P < 0.05$, $^{b}P < 0.01$ should be noted ($P > 0.05$ should not be noted). If there are other series of *P* values, $^{c}P < 0.05$ and $^{d}P < 0.01$ are used. A third series of *P* values can be expressed as $^{e}P < 0.05$ and $^{f}P < 0.01$. Other notes in tables or under illustrations should be expressed as ^{1}F, ^{2}F, ^{3}F; or sometimes as other symbols with a superscript (Arabic numerals) in the upper left corner. In a multi-curve illustration, each curve should be labeled with ●, ○, ■, □, ▲, △, *etc*., in a certain sequence.

Acknowledgments

Brief acknowledgments of persons who have made genuine contributions to the manuscript and who endorse the data and conclusions should be included. Authors are responsible for obtaining written permission to use any copyrighted text and/or illustrations.

REFERENCES

Coding system

The author should number the references in Arabic numerals according to the citation order in the text. Put reference numbers in square brackets in superscript at the end of citation content or after the cited author's name. For citation content which is part of the narration, the coding number and square brackets should be typeset normally. For example, "Crohn's disease (CD) is associated with increased intestinal permeability[1,2]". If references are cited directly in the text, they should be put together within the text, for example, "From references[19,22-24], we know that...".

When the authors write the references, please ensure that the order in text is the same as in the references section, and also ensure the spelling accuracy of the first author's name. Do not list the same citation twice.

PMID and DOI

Pleased provide PubMed citation numbers to the reference list,

e.g. PMID and DOI, which can be found at http://www.ncbi.nlm.nihgov/sites/entrez?db=pubmed and http://www.crossref.org/SimpleTextQuery/, respectively. The numbers will be used in E-version of this journal.

Style for journal references

Authors: the name of the first author should be typed in bold-faced letters. The family name of all authors should be typed with the initial letter capitalized, followed by their abbreviated first and middle initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR). The title of the cited article and italicized journal title (journal title should be in its abbreviated form as shown in PubMed), publication date, volume number (in black), start page, and end page [PMID: 11819634 DOI: 10.3748/wjg.13.5396].

Style for book references

Authors: the name of the first author should be typed in bold-faced letters. The surname of all authors should be typed with the initial letter capitalized, followed by their abbreviated middle and first initials. (For example, Lian-Sheng Ma is abbreviated as Ma LS, Bo-Rong Pan as Pan BR) Book title. Publication number. Publication place: Publication press, Year: start page and end page.

Format

Journals

English journal article (list all authors and include the PMID where applicable)

1 **Jung EM**, Clevert DA, Schreyer AG, Schmitt S, Rennert J, Kubale R, Feuerbach S, Jung F. Evaluation of quantitative contrast harmonic imaging to assess malignancy of liver tumors: A prospective controlled two-center study. *World J Gastroenterol* 2007; **13**: 6356-6364 [PMID: 18081224 DOI: 10.3748/wjg.13.6356]

Chinese journal article (list all authors and include the PMID where applicable)

2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.00000 35706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

8 **Banit DM**, Kaufer H, Hartford JM. Intraoperative frozen section analysis in revision total joint arthroplasty. *Clin Orthop Relat Res* 2002; (**401**): 230-238 [PMID: 12151900 DOI:10.1097/00003086-200208000-00026]

No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001 Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

Statistical data

Write as mean ± SD or mean ± SE.

Statistical expression

Express *t* test as *t* (in italics), *F* test as *F* (in italics), chi square test as χ^2 (in Greek), related coefficient as *r* (in italics), degree of freedom as υ (in Greek), sample number as *n* (in italics), and probability as *P* (in italics).

Units

Use SI units. For example: body mass, m (B) = 78 kg; blood pressure, p (B) = 16.2/12.3 kPa; incubation time, t (incubation) = 96 h, blood glucose concentration, c (glucose) 6.4 ± 2.1 mmol/L; blood CEA mass concentration, p (CEA) = 8.6 24.5 μg/L; CO_2 volume fraction, 50 mL/L CO_2, not 5% CO_2; likewise for 40 g/L formaldehyde, not 10% formalin; and mass fraction, 8 ng/g, *etc.* Arabic numerals such as 23, 243, 641 should be read 23 243 641.

The format for how to accurately write common units and quantums can be found at: http://www.wjgnet.com/1007-9327/g_info_20100315223018.htm.

Abbreviations

Standard abbreviations should be defined in the abstract and on first mention in the text. In general, terms should not be abbreviated unless they are used repeatedly and the abbreviation is helpful to the reader. Permissible abbreviations are listed in Units, Symbols and Abbreviations: A Guide for Biological and Medical Editors and Authors (Ed. Baron DN, 1988) published by The Royal Society of Medicine, London. Certain commonly used abbreviations, such as DNA, RNA, HIV, LD50, PCR, HBV, ECG, WBC, RBC, CT, ESR, CSF, IgG, ELISA, PBS, ATP, EDTA, mAb, can be used directly without further explanation.

Italics

Quantities: *t* time or temperature, *c* concentration, *A* area, *l* length, *m* mass, *V* volume.
Genotypes: *gyrA*, *arg* 1, *c myc*, *c fos*, *etc.*
Restriction enzymes: *Eco*RI, *Hin*dI, *Bam*HI, *Kbo* I, *Kpn* I, *etc.*
Biology: *H. pylori*, *E coli*, *etc.*

Examples for paper writing

All types of articles' writing style and requirement will be found in the link: http://www.wjgnet.com/esps/NavigationInfo.aspx?id=15.

RESUBMISSION OF THE REVISED MANUSCRIPTS

Authors must revise their manuscript carefully according to the revision policies of Baishideng Publishing Group Co., Limited. The revised version, along with the signed copyright transfer agreement, responses to the reviewers, and English language Grade B certificate (for non-native speakers of English), should be submitted to the online system *via* the link contained in the e-mail sent by the editor. If you have any questions about the revision, please send e-mail to esps@wjgnet.com.

Language evaluation

The language of a manuscript will be graded before it is sent for revision. (1) Grade A: priority publishing; (2) Grade B: minor language polishing; (3) Grade C: a great deal of language polishing needed; and (4) Grade D: rejected. Revised articles should reach Grade A or B.

Copyright assignment form

Please download a Copyright assignment form from http://www.wjgnet.com/1007-9327/g_info_20100315222818.htm.

Responses to reviewers

Please revise your article according to the comments/suggestions provided by the reviewers. The format for responses to the reviewers' comments can be found at: http://www.wjgnet.com/1007-9327/g_info_20100315222607.htm

Proof of financial support

For papers supported by a foundation, authors should provide a copy of the approval document and serial number of the foundation.

Links to documents related to the manuscript

WJG will be initiating a platform to promote dynamic interactions between the editors, peer reviewers, readers and authors. After a manuscript is published online, links to the PDF version of the submitted manuscript, the peer-reviewers' report and the revised manuscript will be put on-line. Readers can make comments on the peer reviewer's report, authors' responses to peer reviewers, and the revised manuscript. We hope that authors will benefit from this feedback and be able to revise the manuscript accordingly in a timely manner.

Publication fee

WJG is an international, peer-reviewed, open access, online journal. Articles published by this journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license. Authors of accepted articles must pay a publication fee. Publication fee: 1365 USD per article. All invited articles are published free of charge.

ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

World J Gastroenterol 2013 January 14; 19(2): 147-320



Baishideng Publishing Group
www.wjgnet.com

World Journal of Gastroenterology

Contents

Weekly Volume 19 Number 2 January 14, 2013

EDITORIAL

147 History, ethics, advantages and limitations of experimental models for hepatic ablation
Ong SL, Gravante G, Metcalfe MS, Dennison AR

FIELD OF VISION

155 Bowel preparation prior to colonoscopy: A continual search for excellence
Bechtold ML, Choudhary A

158 Recurrent hepatitis C virus after transplant and the importance of plasma cells on biopsy
Kallwitz ER

161 Internal frontier: The pathophysiology of the small intestine
Sugimura H, Osawa S

TOPIC HIGHLIGHT

165 Endoscopic management of difficult common bile duct stones
Trikudanathan G, Navaneethan U, Parsi MA

GUIDELINES FOR BASIC SCIENCE

174 Prognostic value of innate and adaptive immunity in colorectal cancer
Grizzi F, Bianchi P, Malesci A, Laghi L

REVIEW

185 Gastrointestinal radiation injury: Symptoms, risk factors and mechanisms
Shadad AK, Sullivan FJ, Martin JD, Egan LJ

199 Gastrointestinal radiation injury: Prevention and treatment
Shadad AK, Sullivan FJ, Martin JD, Egan LJ

ORIGINAL ARTICLE

209 Intestinal injury can be reduced by intra-arterial postischemic perfusion with hypertonic saline
Kornyushin O, Galagudza M, Kotslova A, Nutfullina G, Shved N, Nevorotin A, Sedov V, Vlasov T

219 Characterization of focal liver masses using acoustic radiation force impulse elastography
Park H, Park JY, Kim DY, Ahn SH, Chon CY, Han KH, Kim SU

227 Inositol-requiring enzyme 1α is required for gut development in *Xenopus lavies* embryos
Guo J, Li XX, Feng JJ, Yin CY, Wang XJ, Wang N, Yuan L

BRIEF ARTICLE

235 Partially hydrolyzed guar gum in pediatric functional abdominal pain
Romano C, Comito D, Famiani A, Calamarà S, Loddo I

241 Atorvastatin and rosuvastatin do not prevent thioacetamide induced liver cirrhosis in rats
Shirin H, Sharvit E, Aeed H, Gavish D, Bruck R

249 Hyperglycemia is a significant prognostic factor of hepatocellular carcinoma after curative therapy
Hosokawa T, Kurosaki M, Tsuchiya K, Matsuda S, Muraoka M, Suzuki Y, Tamaki N, Yasui Y, Nakata T, Nishimura T, Suzuki S, Ueda K, Nakanishi H, Itakura J, Takahashi Y, Izumi N

258 Gastrointestinal tuberculosis is not associated with proton pump inhibitors: A retrospective cohort study
Hong KS, Kang SJ, Choi JK, Kim JH, Seo H, Lee S, Jung JW, Kang HR, Cho SH, Kim JS

265 Clinical outcomes and predictive factors in oral corticosteroid-refractory active ulcerative colitis
Jeon HH, Lee HJ, Jang HW, Yoon JY, Jung YS, Park SJ, Hong SP, Kim TI, Kim WH, Cheon JH

274 Dual probiotic strains suppress high fructose-induced metabolic syndrome
Park DY, Ahn YT, Huh CS, McGregor RA, Choi MS

284 Clinical significance and risk factors of postembolization fever in patients with hepatocellular carcinoma
Jun CH, Ki HS, Lee HK, Park KJ, Park SY, Cho SB, Park CH, Joo YE, Kim HS, Choi SK, Rew JS

290 IL28B polymorphism and cytomegalovirus predict response to treatment in Egyptian HCV type 4 patients
EI Awady MK, Bader El Din NG, Tabll A, EI Hosary Y, Abdel Aziz AO, El Khayat H, Salama M, Abdelhafez TH

CASE REPORT

299 Lubiprostone induced ischemic colitis
Sherid M, Sifuentes H, Samo S, Deepak P, Sridhar S

304 Lesson from an intriguing case of cryoglobulinemia
Barone M, Licinio R, Amoruso A, Viggiani MT, Larocca AMV, Di Leo A

307 Recurrent cervical esophageal stenosis after colon conduit failure: Use of myocutaneous flap
Sa YJ, Kim YD, Kim CK, Park JK, Moon SW

311 A case of ascending colon variceal bleeding treated with venous coil embolization
Ko BS, Kim WT, Chang SS, Kim EH, Lee SW, Park WS, Kim YS, Nam SW, Lee DS, Kim JC, Kang SB

316 Atypical presentation of pseudomembranous colitis localized in adenomatous polyps
Hernández-Rocha C, Barra-Carrasco J, Guzmán AM, Paredes-Sabja D, Lezcano G, Zoroquiaín P, Álvarez-Lobos M

LETTERS TO THE EDITOR **319** Anesthetic management of the SRS^{TM} endoscopic stapling system for gastro-esophageal reflux disease
Topuz U, Umutoglu T, Bakan M, Ozturk E

World Journal of Gastroenterology
Volume 19 Number 2 January 14, 2013

Contents

APPENDIX	I-VI	Instructions to authors
ABOUT COVER		Editorial Board Member of *World Journal of Gastroenterology*, Dr. Mukaddes Esrefoglu, Professor, Department of Histology and Embryology, Inonu University, 44280 Malatya, Turkey

AIMS AND SCOPE

World Journal of Gastroenterology (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. *WJG* was established on October 1, 1995. It is published weekly on the 7th, 14th, 21st, and 28th each month. The *WJG* Editorial Board consists of 1352 experts in gastroenterology and hepatology from 64 countries.

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

INDEXING/ABSTRACTING

World Journal of Gastroenterology is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, Digital Object Identifier, and Directory of Open Access Journals. ISI, Journal Citation Reports®, Gastroenterology and Hepatology, 2011 Impact Factor: 2.471 (32/74); Total Cites: 16951 (7/74); Current Articles: 677 (1/74); and Eigenfactor® Score: 0.06035 (5/74).

FLYLEAF	I-IX	Editorial Board

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Shuai Ma*
Responsible Science Editor: *Xin-Zhen Huang*
Responsible Electronic Editor: *Dan-Ni Zhang*
Proofing Editorial Office Director: *Jin-Lei Wang*
Proofing Editor-in-Chief: *Lian-Sheng Ma*

NAME OF JOURNAL
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

EDITOR-IN-CHIEF
Ferruccio Bonino, MD, PhD, Professor of Gastroenterology, Director of Liver and Digestive Disease Division, Department of Internal Medicine, University of Pisa, Director of General Medicine 2 Unit University Hospital of Pisa, Via Roma 67, 56124 Pisa, Italy

Myung-Hwan Kim, MD, PhD, Professor, Head, Department of Gastroenterology, Director, Center for Biliary Diseases, University of Ulsan College of Medicine, Asan Medical Center, 388-1 Pungnap-2dong, Songpa-gu, Seoul 138-736, South Korea

Kjell Öberg, MD, PhD, Professor, Department of Endocrine Oncology, Uppsala University Hospital, SE-751 85 Uppsala, Sweden

Matt D Rutter, MBBS, MD, FRCP, Consultant Gastroenterologist, Senior Lecturer, Director, Tees Bowel Cancer Screening Centre, University Hospital of North Tees, Durham University, Stockton-on-Tees, Cleveland TS19 8PE, United Kingdom

Andrzej S Tarnawski, MD, PhD, DSc (Med), Professor of Medicine, Chief Gastroenterology, VA Long Beach Health Care System, University of California, Irvine, CA, 5901 E. Seventh Str., Long Beach, CA 90822, United States

EDITORIAL OFFICE
Jian-Xia Cheng, Director
Jin-Lei Wang, Vice Director
World Journal of Gastroenterology
Room 903, Building D, Ocean International Center, No. 62 Dongsihuan Zhonglu, Chaoyang District, Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Co., Limited
Room 1701, 17/F, Henan Building,
No.90 Jaffe Road, Wanchai, Hong Kong, China
Fax: +852-31158812
Telephone: +852-58042046
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

PUBLICATION DATE
January 14, 2013

COPYRIGHT
© 2013 Baishideng. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT
All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i2.147

World J Gastroenterol 2013 January 14; 19(2): 147-154
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

EDITORIAL

History, ethics, advantages and limitations of experimental models for hepatic ablation

Seok Ling Ong, Gianpiero Gravante, Matthew S Metcalfe, Ashley R Dennison

Seok Ling Ong, Gianpiero Gravante, Matthew S Metcalfe, Ashley R Dennison, Department of Hepatobiliary and Pancreatic Surgery, Leicester General Hospital, Leicester LE5 4PW, United Kingdom

Author contributions: Ong SL, Gravante G, Metcalfe MS, Dennison AR were responsible for the acquisition of data, analysis and interpretation, drafting the article or revising it critically for important intellectual content and final approval of the version to be published; Ong SL, Dennison AR were responsible for substantial contributions to conception and design.

Correspondence to: Dr. Gianpiero Gravante, Department of Hepatobiliary and Pancreatic Surgery, Leicester General Hospital, Gwendolen Road, Leicester LE5 4PW, United Kingdom. ggravante@hotmail.com
Telephone: +44-116-2588244 Fax: +44-116-2584708

Received: March 19, 2012 Revised: September 10, 2012
Accepted: September 19, 2012
Published online: January 14, 2013

Abstract

Numerous techniques developed in medicine require careful evaluation to determine their indications, limitations and potential side effects prior to their clinical use. At present this generally involves the use of animal models which is undesirable from an ethical standpoint, requires complex and time-consuming authorization, and is very expensive. This process is exemplified in the development of hepatic ablation techniques, starting experiments on explanted livers and progressing to safety and efficacy studies in living animals prior to clinical studies. The two main approaches used are *ex vivo* isolated non-perfused liver models and *in vivo* animal models. *Ex vivo* non perfused models are less expensive, easier to obtain but not suitable to study the heat sink effect or experiments requiring several hours. *In vivo* animal models closely resemble clinical subjects but often are expensive and have small sample sizes due to ethical guidelines. Isolated perfused *ex vivo* liver models have been used to study drug toxicity, liver failure, organ transplantation and hepatic ablation and combine advantages of both previous models.

© 2013 Baishideng. All rights reserved.

Key words: Liver; Ablation; Experiment; *Ex vivo*; *In vivo*; Perfusion

Ong SL, Gravante G, Metcalfe MS, Dennison AR. History, ethics, advantages and limitations of experimental models for hepatic ablation. *World J Gastroenterol* 2013; 19(2): 147-154 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i2/147.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i2.147

INTRODUCTION

Progress in science and medicine depends on new ideas being developed, tested and introduced into mainstream practice. Animal experimentation is used not only in medicine and surgery but also in science, the pharmaceutical industry, the military and educational establishments. In medicine it is essential to establish beyond doubt the safety of a drug, instrument or procedure before it even begins phase 1 of clinical trials. To establish the safety of this heterogeneous range of treatments the medical profession has frequently had no option but to the use of live animals. Although this practice is highly undesirable and many people are morally opposed to these experiments, unless and until viable alternatives are available legislation prevents the introduction of these new drugs and procedures without this prior testing. Indeed virtually all drugs and surgical treatments that are in use today have been tested on animals.

Although the best treatment for liver tumour remains surgical resection[1] the majority of patients are still affected by unresectable lesions. To these patients hepatic ablation provides an opportunity to increase survival[2]. Experimental research over the last twenty years has seen the development of a number of ablative modalities and

several of these have been applied clinically[3,4]. Ablation experiments are now focused to increase the diameter of the ablation zone to achieve better tumor margins[5-12], or to conduct safe ablations on lesions positioned close to major vessels[13-16]. Numerous experimental models are available, each with different advantages, disadvantages and ethical implications. It is therefore imperative that the researcher that wants to explore the field of experimental hepatic ablation is aware of their characteristics.

HISTORICAL BACKGROUND OF ANIMAL EXPERIMENTATION

The Greeks almost 2500 years ago described the earliest recorded animal experiments. Aristotle (384-322 BC), and Erasistratus (304-258 BC)[17,18] both performed studies on live animals and in 2nd century Rome Galen who is known as the father of vivisection dissected live goats and pigs[19]. These studies continued into the Roman era and were then passed on to medical schools in Arabia. The practice died out after this and was absent completely in the Dark Ages only being revived in Italy in the 16th century. Subsequently live animals have been used throughout history to study a wide range of problems and to assess new treatments particularly drugs and vaccines. In the 17th century, many pivotal discoveries came to light because of these studies including the understanding of lung function and the circulation of the blood. In the 1840's general anesthesia emerged (initially ether and chloroform) and it became possible to study unconscious animals. In 1881, 250 experiments were carried out in Britain, the first year that records were kept of the procedures carried out on animals. The following year (1822) the first animal protection law was passed in Britain and in 1876 the Cruelty to Animals Act came into being and was the first law specifically designed to regulate animal testing.

Many advances in our understanding did result from these animal studies including those in basic science and also medicine. These included Antoine Lavoisier demonstrating that respiration was a form of combustion using guinea pigs in calorimeters[20], Stephen Hales measuring blood pressure in the horses, and in the 1880's Louis Pasteur demonstrating the concept of "germs" by giving anthrax to sheep[21]. Also in the 1880's and 1890's Emil von Behring was able to isolate the diphtheria toxin and not only demonstrate its effects in guinea pigs but also, by 1898 produce immunity by the injection of a mixture of toxin and antitoxin, for which he was awarded the Nobel Prize in Physiology and Medicine in 1901. In 1921 Banting ligated the pancreatic duct of dogs and demonstrated that pancreatic isolates could be used to keep these diabetic animals alive, and by 1922 working with John Macloed he isolated insulin from bovine sources and famously treated Leonard Thompson, a 14-year-old diabetic boy with diabetes[22].

OBJECTIONS TO LIVE ANIMAL EXPERIMENTS

Despite these advances and those that took place subsequently many people remained understandably opposed to any form of live animal experimentation. Claude Bernard was known as the "prince of vivisectors" and the father of physiology[23] but ironically his wife Marie Francoise Martin founded the first anti-vivisection society in France in 1883 and famously wrote in 1865 that "the science of life is a superb and dazzlingly lighted hall which may be reached only by passing through a long and ghastly kitchen[23,24]. Opposition to animal experimentation continued and many eminent scientists voiced their disapproval. Charles Darwin in 1871 wrote to Ray Lancaster in reply and stated "You ask about my opinion on vivisection. I quite agree that it is justifiable for real investigations on physiology; but not for mere damnable and detestable curiosity. It is a subject which makes me sick with horror, so I will not say another word about it, else I shall not sleep tonight"[25].

Objections to live animal experiments emerged in the United States in the 1860's and Henry Bergh founded the American Society for the Prevention of Cruelty to Animals. The first movement which specifically opposed vivisection was the American Antivivisection Society, which was founded in 1883. The continued opposition to the use of animals together with the dramatic increase in the number of procedure that were being performed (in 2002 in the United Kingdom 2.7 million live animal experiments were authorized and in the United States, it is estimated that between 19 and 29 million experiments were performed although exact estimates were difficult because 90% of the animals were rats, mice and other species recently exempt from legislation) encouraged many researchers in science and medicine to seek other methods to test drugs and procedures and much of this ethos has been incorporated into legislation or guidelines. Although British law requires that any new drug be tested on at least two different species of animals (one must be a large non-rodent), United Kingdom regulations in respect of animal experimentation are very strict and the Animals Act of 1986 requires that no animal experiments be conducted if there is a realistic alternative. It also exhorts investigators to examine their research carefully to determine the smallest number of animals that may be used to answer the questions posed by the research.

There remains however a dilemma with both ethical and legislational dimensions. All scientists agree that animal experimentation is unacceptable if there is a viable alternative but legislation will not allow the introduction of new products without rigorous testing. In 1988, the American Medical Association published a white paper defending biomedical research in animals[26], and stated that "In fact, virtually every advance in medical science

in the 20th century, from antibiotics and vaccines to antidepressant drugs and organ transplants, has been achieved either directly or indirectly through the use of animals in laboratory experiments". In Europe European Centre for the Validation of Alternative Methods was established in October 1991 by a communication from the Commission to the Council and the Parliament. The aim was to try and organize research and exchange information to limit the need for animal experimentation. As defined in the communication it was felt that this could be achieved in four ways: (1) to coordinate the validation of alternative test methods at the European Union level; (2) to act as a focal point for the exchange of information on the development of alternative test methods; (3) to set up, maintain and manage a database on alternative procedures; and (4) to promote dialogue between legislators, industries, biomedical scientists, consumer organizations and animal welfare groups, with a view to the development, validation and international recognition of alternative test methods.

Although there has been considerable progress in these areas, notably with the use of fresh and cryo-preserved cells (particularly hepatocytes) problems remain when it is necessary to investigate a treatment prior to its use inpatients. The problem is particularly acute in the field of biotechnology in which many of the devices that are proposed and developed are potentially harmful and effects in living tissue are essentially unknown. Today it is certainly possible to computer model some of the effects (widely used in the pharmaceutical industry to predict the usefulness and safety of new drugs) but many results remain unpredictable. Fresh tissue can be used and will provide answers if the procedure takes a short time and its effects and results do not rely on changes (particularly at the cellular level) that require interaction with living cells or evolve over a significant time period (several hours or longer). Unfortunately many devices do produce changes due to interaction with normally vascularized tissue and frequently take prolonged periods to produce effects. With these the only way to date that the results could be examined in detail was in living animals. In addition although prototypes could be studied in small animals instruments that have been developed for human use because of their size can often only be studied in large animals.

LIVER ABLATION EXPERIMENTAL MODELS

The number of patients with liver lesions, which are potentially suitable for treatment, continues to rise with advances in critical care, anesthesia, surgery and oncology. At present, the gold standard remains surgical resection that produces long-term survivors and cure in a significant number of patients. The advent of laparoscopic resection has further widened the indications but by far the most attractive concept remains ablation of the liver (for primary or secondary tumors) containing the tumour. The ablation techniques currently in place for the treatment of liver tumors include radiofrequency ablation (RFA), microwave ablation (MWA), cryotherapy, percutaneous ethanol injection, high intensity focused ultrasound, interstitial laser photocoagulation, electrolysis and bimodal electric tissue ablation[4]. The additional availability of methods for real-time monitoring to ensure that a sufficient volume is ablated (to engulf the tumor and reduce the likelihood of local recurrence) has seen an upsurge in the number of procedures performed and the consequent emergence of survival data that are very encouraging. In addition there is evidence that ablative techniques produce immunologically active components that may confer some extra survival advantage but also systemic effects with potentially harmful consequences.

The development of any new hepatic ablative therapy is a long process that requires a detailed understanding of the basic physical and chemical properties of the ablative modality and their application in clinical settings. It includes designing equipment suitable for clinical application, identifying methods of assessing tissue response to ablation, optimizing treatment monitoring, exploring local and systemic effect of the ablation, and finally, demonstrating the safety and efficacy in humans. Understanding of the tissue temperature changes and radiological evolution of lesion generated by each ablative modality is crucial before it can be used in a clinical setting. Further problems occur with some of the techniques that rely on temperature changes for their effectiveness. Particularly with RFA and MWA the effect can be significantly attenuated in the vicinity of large blood vessels due to the "heat sink effect" where the large flow in the vessels conducts heat away sufficiently rapidly to interfere with the treatment and potentially leave viable tumor cells around these vessels. Flows within the hepatic vessels have been shown to reduce the ablative energy via heat sink effects resulting in a greater energy requirement when ablating in close proximity to larger vessels with an associated higher risk of vascular and bile duct complications[15,27]. For the same reasons, thermal ablation techniques particularly are influenced by the effects of the separate occlusion of the hepatic artery, portal vein or both (Pringle maneuver). This delicate balance between safety and efficacy of ablation has been the focus of many experimental studies[15,28,29].

One further attraction of ablative techniques is the fact that they can often be performed percutaneously or laparoscopically and considerable effort has been expended in the development of suitable probes. These probes can be complex and are generally modeled initially on computers to predict the likely outcome after the delivery of a set amount of energy. Unfortunately the alterations in tissue resistance and electrical and thermal conductivity consequent upon the use of the probes, together with the complex designs (and frequent use of multiple probes or probes that assume unpredictable

shapes after deployment) means that to date it has been essential to test the devices in animal models. These will also be necessary to "calibrate" all new devices and produce standard dose-response curves, so that in addition to real-time monitoring a prediction can be made about the amount of energy that needs to be delivered to ablate a predetermined volume.

The increasing popularity and deployment of these techniques means that to accomplish these aims would require the use of a large number of experimental animals, and because of this it is valuable to consider whether there is an alternative approach that can produce reliable data of sufficient quality to facilitate the incorporation of these methods into clinical practice without the need for subsequent large animal studies. *Ex vivo* perfused liver models have been developed recently for the study of the hepatic toxicity of new pharmacological agents[30-32], and in an attempt to improve the outcome from organ transplantation. Organ transplantation models, which use pulsatile perfusion after harvesting, would seem to offer an excellent alternative that could closely mimic the *in vivo* situation and allow data to be collected for up to 24 h. In a sufficiently well developed perfusion model in which parameters can be accurately controlled it seems likely that data generated would be genuinely useful and achieve the aim of avoiding the use of live animal models.

EX VIVO NON-PERFUSED MODELS

The most commonly used model for the study of hepatic ablation is liver explanted from animals. Bovine and porcine livers are most frequently used because of their similarity to human livers in terms of size and density, and because they are farm animals that are readily available and inexpensive. In addition long-term use has resulted in a familiarity with the animals' anatomy that is essential in studies on the liver where the lobulation and blood supply are an important determinants of outcome. Although the organ is not perfused the position of the ablation in respect of vessels and bile ducts remains important. Their size also allows a number of ablations to be carried out on each individual model, which minimizes the number of animals required.

These models can be used to examine the relationship between ablative energy and lesion size, to assess the effect of different antenna configurations on lesion size and to study the dielectric properties of liver tissues[33-36]. Crocetti *et al*[37] used *ex vivo* calf livers to assess the feasibility and validity of fusion imaging that combines ultrasound and computed tomography in the monitoring of RFA. Unfortunately the results of this study were not directly transferable to the clinical setting because of the absence of respiratory excursion and subject motions of this model. The absence of perfusion within *ex vivo* models also limits their ability to assess the effect of blood flow on the size of the lesion created by ablation with a set amount of energy. Furthermore detailed studies of the cellular changes in the ablated area cannot be conducted due to the ischemia produced by explantation and the rapid cessation of metabolic and physiochemical processes.

IN VIVO MODELS

In view of the limitation of *ex vivo* models particularly with respect to the absence of blood flow, *in vivo* animal models have been widely used for the study of hepatic ablation. Data generated from these studies are very reliable and the close similarity to results in humans allows the experience to be directly transferred to clinical practice. Small laboratory animal models such as murine models are easier to handle but have significant differences in organ size, function and anatomy compared to the human liver. The relatively small organ size also limits the number of ablations that can be carried out per liver. In contrast, *in vivo* porcine liver closely resembles human livers in size, vascularity and metabolic function and although the shape is different the organ is also lobulated in a similar way to the human liver. This allows comparisons to be drawn with respect of the positioning of probes, real dosage levels and consequences of treatment. The result is that the porcine model is by far the most commonly used at present for the final evaluation of all ablative techniques.

The two major approaches to date for the study of hepatic ablation in animals, *ex vivo* non-perfused liver models and *in vivo* animal studies, and their respective advantages and limitations are compared in Table 1. A number of studies have been carried out simultaneously on *in vivo* and *ex vivo* non-perfused models to try and assess the magnitude of the changes produced and also to evaluate the specific differences. The volume of the ablated regions is always significantly smaller in the *in vivo* models when similar energy of ablation is applied[5,38,39]. These findings can probably be attributed to the presence of vascular flow, which removes some of the energy (heat) during the treatment in the *in vivo* models and hence these models are used to examine the influence of vascular occlusion on the size of ablated area. *In vivo* models also allow histological study of different zones, and the evolution of these zones in lesions generated by thermal ablations.

Results generated from *in vivo* models are directly transferable to clinical practice and are at present the gold standard for pre-clinical experimental studies of hepatic ablation. However the use of living animals requires specific laboratory facilities and research teams with expertise in anesthetizing and handling different species. The size and temperament of larger animal models such as porcine and bovine models poses practical problems when animals need to be anesthetized, moved or examined post-procedure (particularly if blood sampling is required).

Table 1 Advantages and limitations of *ex vivo* and *in vivo* models for experimental study of hepatic ablation

Models	Applications	Advantages	Limitations
Ex vivo (non perfused) models	Compare the efficacy of different antenna configurations Trial of different energy setting	Allow histological examination of whole lesion to study zones of ablation Cheap Larger study sample size Easy to manipulate during experiment Does not require ethical approval/animal license	Non-physiological Homogenous parenchyma Absence of respiratory excursion and subject motions Lack of cooling effect secondary to tissue perfusion Unable to study heat sink effect
In vivo models	Study of lesion evolution over time Histological examination of lesion Study of heat sink effect and the effect of bile duct cooling Study of systemic responses to ablation Study of the effect of large volume ablation (in larger animals)	Small animals Easier to handle Cheaper Ability to have larger sample size	Small animals Small volume of liver Limit number of ablation on each liver Not suitable for the study of large volume ablation
		Large animals Closer resemblance to human liver in terms of size and physiology More ablations can be carried out in each liver	Large animals Size and temperament poses challenges during anesthesia Difficult vascular access in porcine models Also limited by strict ethic regulation Small study sample size
			Common limitations Expensive Expertise in animal handling and anesthesia is required Duration of study is limited to the lifespan of the model Absence of interacting organ systems which may have a role in generating systemic response Unable to assess the impact of ablation on end organs
Isolated perfused *ex vivo* liver models	Study of lesion evolution over time Study of heat sink effect and the effect of bile duct cooling Study of early inflammatory response	Cheaper than *in vivo* experiments Sophisticated and accurate manipulation of hepatic inflow (e.g., Pringle manoeuvre) Does not require ethical approval Greater control of perfusion characteristics (e.g., portal vein and hepatic arterial flows and pressures)	Perfusion circuit itself may activate some degree of systemic response

EX VIVO PERFUSED MODELS

Ex vivo non-perfused and *in vivo* models have significant advantages with respect to specific scientific questions but an alternative model, the isolated perfused *ex vivo* model, theoretically would have most of the advantages of both. A normothermic liver perfusion system using autologous blood maintains physiological and metabolic functions and the hemodynamic changes resemble those of *in vivo* models with good preservation of liver architecture[40-42]. The organ would be perfused over a number of hours, and within this period valuable data could be produced within a relatively inexpensive model with no ethical problems and no requirement for licensing (Table 1)[43-45]. This should permit the histological study of lesions generated by hepatic thermal ablation, real-time monitoring of the evolution of the lesions and experiments designed to study the "heat sink" effect with different modalities. In addition, with a sufficiently sophisticated and stable model it should also be possible (by venous sampling) to study the early inflammatory responses following hepatic ablation[44,46].

Liver perfusion studies date back as far as the 19th century when they were first performed for research on physiological function of the liver[47]. A number of studies have been conducted subsequently to establish valid models of liver perfusion using asanguineous perfusates or autologous blood[41,48-50]. These models have been used for physiological research, treatment of liver failure, studies of metabolism and toxicity of new pharmacological compounds and in transplant units for the recovery and preservation of organs, which are to be implanted[51-53]. Hildebrand *et al*[54] have advocated the use of a perfused *ex vivo* liver model for the training in laparoscopic RFA.

For the *ex vivo* perfused model the main limitation is the limited lifespan and the determination of intervals during this lifespan when valid data can be extracted. Nevertheless perfusion models, and particularly those using autologous blood and pulsatile perfusions, have become very sophisticated and physiological parameters can be very accurately maintained for many hours. The interaction of blood with non-biological surfaces of the perfusion circuit does activate a number of biological pathways producing some degree of systemic response and interpretation of responses to ablation will have to take these into account. However the response will be the same for each experiment and should not interfere with comparisons of different dose responses or different modalities[55]. The absence of other interacting

organs may also affect the extent of systemic responses following hepatic ablation but this model still potentially represents an exciting new method of studying these new devices while avoiding the need to use live animals. The true potential and weakness of an isolated perfused liver model for the study of hepatic ablation remain to be seen (and will be determined by future research) but recent advances have certainly developed the model to the stage that merits this research[56].

FUTURE DIRECTIONS AND ADVANCES

To overcome the limitations of disconnecting the liver from the remaining organs, Chung *et al*[56] recently created the first multiorgan *ex vivo* perfused model in which the liver was serially connected with the kidney. The viability of both organs and the inflammatory reaction elicited have been investigated in two separate studies[56,57]. The addition of the kidney to the circuit improved the biochemical milieu of the circuit and consented a more physiological environment for those experiments requiring strict conditions[57]. At the same time, the combination of two organs in the circuit did not increase the cytokine production compared to the classic "liver-alone" circuit[56] and therefore could be used to test the inflammatory reaction produced by ablative techniques[44] without significant interferences by the newly added organ.

CONCLUSION

The key advantages of isolated perfused liver models are the avoidance of live animal experiments, the ability to control accurately physiological parameters and to analyze morphological changes following hepatic ablation. In addition detailed study of the "heat sink" effect and changes consequent upon bile duct perfusion can be assessed. Other organs and tissues from the same animals from which the livers are procured can also be used in other research leading to an overall reduction in the number of animals required. Validation of the role of isolated perfused liver models for experimental studies of hepatic ablation is essential to establish their position in this field of research.

REFERENCES

1 **Wong SL**, Mangu PB, Choti MA, Crocenzi TS, Dodd GD, Dorfman GS, Eng C, Fong Y, Giusti AF, Lu D, Marsland TA, Michelson R, Poston GJ, Schrag D, Seidenfeld J, Benson AB. American Society of Clinical Oncology 2009 clinical evidence review on radiofrequency ablation of hepatic metastases from colorectal cancer. *J Clin Oncol* 2010; **28**: 493-508 [PMID: 19841322 DOI: 10.1200/JCO.2009.23.4450]

2 **Cho YK**, Kim JK, Kim MY, Rhim H, Han JK. Systematic review of randomized trials for hepatocellular carcinoma treated with percutaneous ablation therapies. *Hepatology* 2009; **49**: 453-459 [PMID: 19065676 DOI: 10.1002/hep.22648]

3 **Bhardwaj N**, Strickland AD, Ahmad F, Dennison AR, Lloyd DM. Liver ablation techniques: a review. *Surg Endosc* 2010; **24**: 254-265 [PMID: 19554370 DOI: 10.1007/s00464-009-0590-4]

4 **Gannon CJ**, Curley SA. The role of focal liver ablation in the treatment of unresectable primary and secondary malignant liver tumors. *Semin Radiat Oncol* 2005; **15**: 265-272 [PMID: 16183480 DOI: 10.1016/j.semradonc.2005.04.004]

5 **Hines-Peralta AU**, Pirani N, Clegg P, Cronin N, Ryan TP, Liu Z, Goldberg SN. Microwave ablation: results with a 2.45-GHz applicator in ex vivo bovine and in vivo porcine liver. *Radiology* 2006; **239**: 94-102 [PMID: 16484351 DOI: 10.1148/radiol.2383050262]

6 **Awad MM**, Devgan L, Kamel IR, Torbensen M, Choti MA. Microwave ablation in a hepatic porcine model: correlation of CT and histopathologic findings. *HPB (Oxford)* 2007; **9**: 357-362 [PMID: 18345319 DOI: 10.1080/13651820701646222]

7 **Simon CJ**, Dupuy DE, Iannitti DA, Lu DS, Yu NC, Aswad BI, Busuttil RW, Lassman C. Intraoperative triple antenna hepatic microwave ablation. *AJR Am J Roentgenol* 2006; **187**: W333-W340 [PMID: 16985103 DOI: 10.2214/AJR.05.0804]

8 **Goldberg SN**, Solbiati L, Hahn PF, Cosman E, Conrad JE, Fogle R, Gazelle GS. Large-volume tissue ablation with radio frequency by using a clustered, internally cooled electrode technique: laboratory and clinical experience in liver metastases. *Radiology* 1998; **209**: 371-379 [PMID: 9807561]

9 **Mulier S**, Miao Y, Mulier P, Dupas B, Pereira P, de Baere T, Lencioni R, Leveillee R, Marchal G, Michel L, Ni Y. Electrodes and multiple electrode systems for radiofrequency ablation: a proposal for updated terminology. *Eur Radiol* 2005; **15**: 798-808 [PMID: 15711846 DOI: 10.1007/s00330-004-2584-x]

10 **Mulier S**, Ni Y, Frich L, Burdio F, Denys AL, De Wispelaere JF, Dupas B, Habib N, Hoey M, Jansen MC, Lacrosse M, Leveillee R, Miao Y, Mulier P, Mutter D, Ng KK, Santambrogio R, Stippel D, Tamaki K, van Gulik TM, Marchal G, Michel L. Experimental and clinical radiofrequency ablation: proposal for standardized description of coagulation size and geometry. *Ann Surg Oncol* 2007; **14**: 1381-1396 [PMID: 17242989 DOI: 10.1245/s10434-006-9033-9]

11 **Yu NC**, Lu DS, Raman SS, Dupuy DE, Simon CJ, Lassman C, Aswad BI, Ianniti D, Busuttil RW. Hepatocellular carcinoma: microwave ablation with multiple straight and loop antenna clusters--pilot comparison with pathologic findings. *Radiology* 2006; **239**: 269-275 [PMID: 16493013 DOI: 10.1148/radiol.2383041592]

12 **Shen P**, Geisinger KR, Zagoria R, Levine EA. Pathologic correlation study of microwave coagulation therapy for hepatic malignancies using a three-ring probe. *J Gastrointest Surg* 2007; **11**: 603-611 [PMID: 17393259 DOI: 10.1007/s11605-006-0046-2]

13 **Wemyss-Holden SA**, Dennison AR, Finch GJ, Hall Pd Pde L, Maddern GJ. Electrolytic ablation as an adjunct to liver resection: experimental studies of predictability and safety. *Br J Surg* 2002; **89**: 579-585 [PMID: 11972547 DOI: 10.1046/j.1365-2168.2002.02064.x]

14 **Wemyss-Holden SA**, de la M Hall P, Robertson GS, Dennison AR, Vanderzon PS, Maddern GJ. The safety of electrolytically induced hepatic necrosis in a pig model. *Aust N Z J Surg* 2000; **70**: 607-612 [PMID: 10945557 DOI: 10.1046/j.1440-1622.2000.01907.x]

15 **Metcalfe MS**, Mullin EJ, Texler M, Berry DP, Dennison AR, Maddern GJ. The safety and efficacy of radiofrequency and electrolytic ablation created adjacent to large hepatic veins in a porcine model. *Eur J Surg Oncol* 2007; **33**: 662-667 [PMID: 17412548 DOI: 10.1016/j.ejso.2007.02.011]

16 **Wemyss-Holden SA**, Dennison AR, Berry DP, Maddern GJ. Local ablation for unresectable liver tumors: is thermal best? *J Hepatobiliary Pancreat Surg* 2004; **11**: 97-106 [PMID: 15127271 DOI: 10.1007/s00534-002-0715-9]

17 **Singer CJ**. Greek biology and Greek medicine. London: Oxford University Press, 1922: 6-14

18 **Wilson LG**. Erasistratus, Galen, and the pneuma. *Bull Hist Med* 1959; **33**: 293-314 [PMID: 13845095]
19 **Prendergast JS**. The Background of Galen's Life and Activities, and its Influence on his Achievements. *Proc R Soc Med* 1930; **23**: 1131-1148 [PMID: 19987627]
20 **Buchholz AC**, Schoeller DA. Is a calorie a calorie? *Am J Clin Nutr* 2004; **79**: 899S-906S [PMID: 15113737]
21 **Mock M**, Fouet A. Anthrax. *Annu Rev Microbiol* 2001; **55**: 647-671 [PMID: 11544370 DOI: 10.1146/annurev.micro.55.1.647]
22 **Gorden P**. Non-insulin dependent diabetes--the past, present and future. *Ann Acad Med Singapore* 1997; **26**: 326-330 [PMID: 9285027]
23 **Croce P**. Vivisection or science? An Investigation into Testing Drugs and Safeguarding Health. London and New York: Zed Books, 1999: 11-30
24 **Rudacille D**. The scalpel and the butterfly: the conflict. New York: Farrar, Straus and Giroux, 2000: 19-20
25 **Bowlby J**. Charles Darwin: a new life. London and New York: W.W.Norton, 1992: 420-421
26 **Hendee WR**. Use of animals in biomedical research: the challenge and response. AMA white paper. Chicago: American Medical Association, 1989
27 **Teratani T**, Yoshida H, Shiina S, Obi S, Sato S, Tateishi R, Mine N, Kondo Y, Kawabe T, Omata M. Radiofrequency ablation for hepatocellular carcinoma in so-called high-risk locations. *Hepatology* 2006; **43**: 1101-1108 [PMID: 16628706 DOI: 10.1002/hep.21164]
28 **Welp C**, Siebers S, Ermert H, Werner J. Investigation of the influence of blood flow rate on large vessel cooling in hepatic radiofrequency ablation. *Biomed Tech (Berl)* 2006; **51**: 337-346 [PMID: 17155870 DOI: 10.1515/BMT.2006.067]
29 **Thanos L**, Mylona S, Galani P, Pomoni M, Pomoni A, Koskinas I. Overcoming the heat-sink phenomenon: successful radiofrequency thermal ablation of liver tumors in contact with blood vessels. *Diagn Interv Radiol* 2008; **14**: 51-56 [PMID: 18306146]
30 **Vuppugalla R**, Mehvar R. Selective effects of nitric oxide on the disposition of chlorzoxazone and dextromethorphan in isolated perfused rat livers. *Drug Metab Dispos* 2006; **34**: 1160-1166 [PMID: 16621933 DOI: 10.1124/dmd.105.009050]
31 **Strubelt O**, Kremer J, Tilse A, Keogh J, Pentz R, Younes M. Comparative studies on the toxicity of mercury, cadmium, and copper toward the isolated perfused rat liver. *J Toxicol Environ Health* 1996; **47**: 267-283 [PMID: 8604150 DOI: 10.1080/009841096161780]
32 **Hung DY**, Siebert GA, Chang P, Anissimov YG, Roberts MS. Disposition kinetics of propranolol isomers in the perfused rat liver. *J Pharmacol Exp Ther* 2004; **311**: 822-829 [PMID: 15192084 DOI: 10.1124/jpet.104.070011]
33 **Brace CL**, Laeseke PF, van der Weide DW, Lee FT. Microwave Ablation With a Triaxial Antenna: Results in ex vivo Bovine Liver. *IEEE Trans Microw Theory Tech* 2005; **53**: 215-220 [PMID: 18079981 DOI: 10.1109/TMTT.2004.839308]
34 **Lee JM**, Han JK, Kim HC, Choi YH, Kim SH, Choi JY, Choi BI. Switching monopolar radiofrequency ablation technique using multiple, internally cooled electrodes and a multichannel generator: ex vivo and in vivo pilot study. *Invest Radiol* 2007; **42**: 163-171 [PMID: 17287646]
35 **Navarro AC**, Burdío F, Berjano EJ, Güemes A, Burdío JM, Sousa R, Lozano R, Tejero E, de Gregorio MA. Small ablation zones created previous to saline infusion result in enlargement of the coagulated area during perfusion RF ablation: an ex vivo experimental study. *Physiol Meas* 2007; **28**: N29-N37 [PMID: 17664615 DOI: 10.1088/0967-3334/28/6/N02]
36 **O'Rourke AP**, Lazebnik M, Bertram JM, Converse MC, Hagness SC, Webster JG, Mahvi DM. Dielectric properties of human normal, malignant and cirrhotic liver tissue: in vivo and ex vivo measurements from 0.5 to 20 GHz using a precision open-ended coaxial probe. *Phys Med Biol* 2007; **52**: 4707-4719 [PMID: 17634659 DOI: 10.1088/0031-9155/52/15/022]
37 **Crocetti L**, Lencioni R, Debeni S, See TC, Pina CD, Bartolozzi C. Targeting liver lesions for radiofrequency ablation: an experimental feasibility study using a CT-US fusion imaging system. *Invest Radiol* 2008; **43**: 33-39 [PMID: 18097275]
38 **Solazzo SA**, Ahmed M, Liu Z, Hines-Peralta AU, Goldberg SN. High-power generator for radiofrequency ablation: larger electrodes and pulsing algorithms in bovine ex vivo and porcine in vivo settings. *Radiology* 2007; **242**: 743-750 [PMID: 17244719 DOI: 10.1148/radiol.2423052039]
39 **Wang Y**, Sun Y, Feng L, Gao Y, Ni X, Liang P. Internally cooled antenna for microwave ablation: results in ex vivo and in vivo porcine livers. *Eur J Radiol* 2008; **67**: 357-361 [PMID: 17768024 DOI: 10.1016/j.ejrad.2007.07.015]
40 **Imber CJ**, St Peter SD, de Cenarruzabeitia IL, Lemonde H, Rees M, Butler A, Clayton PT, Friend PJ. Optimisation of bile production during normothermic preservation of porcine livers. *Am J Transplant* 2002; **2**: 593-599 [PMID: 12201359 DOI: 10.1034/j.1600-6143.2002.20703.x]
41 **Imber CJ**, St Peter SD, Lopez de Cenarruzabeitia I, Pigott D, James T, Taylor R, McGuire J, Hughes D, Butler A, Rees M, Friend PJ. Advantages of normothermic perfusion over cold storage in liver preservation. *Transplantation* 2002; **73**: 701-709 [PMID: 11907414 DOI: 10.1097/00007890-200203150-00008]
42 **Butler AJ**, Rees MA, Wight DG, Casey ND, Alexander G, White DJ, Friend PJ. Successful extracorporeal porcine liver perfusion for 72 hr. *Transplantation* 2002; **73**: 1212-1218 [PMID: 11981411]
43 **Gravante G**, Ong SL, Metcalfe MS, Sorge R, Fox AJ, Lloyd DM, Maddern GJ, Dennison AR. Changes in acid-base balance during electrolytic ablation in an ex vivo perfused liver model. *Am J Surg* 2012; **204**: 666-670 [PMID: 20451173 DOI: 10.1016/j.amjsurg.2009.12.019]
44 **Gravante G**, Ong SL, Metcalfe MS, Sorge R, Overton J, Lloyd DM, Maddern GJ, Dennison AR. Cytokine response of electrolytic ablation in an ex vivo perfused liver model. *ANZ J Surg* 2010; **80**: 537-541 [PMID: 20795969 DOI: 10.1111/j.1445-2197.2010.05380.x]
45 **Gravante G**, Ong SL, West K, McGregor A, Maddern GJ, Metcalfe MS, Lloyd DM, Dennison AR. Patterns of histological changes following hepatic electrolytic ablation in an ex-vivo perfused model. *Pathol Oncol Res* 2012; **18**: 1085-1089 [PMID: 22706978]
46 **Gravante G**, Ong SL, Metcalfe MS, Sorge R, Sconocchia G, Orlando G, Lloyd DM, Dennison AR. Cytokine response to ischemia/reperfusion injury in an ex vivo perfused porcine liver model. *Transplant Proc* 2009; **41**: 1107-1112 [PMID: 19460492 DOI: 10.1016/j.transproceed.2009.02.054]
47 **Bernard C**. Sur le mécanisme de la formation du sucre dans le foie. *C R hebd Acad Sci* 1855; **41**: 461-469
48 **Hobbs KE**, Hunt AC, Palmer DB, Badric FE, Morris AM, Mitra SK, Peacock JH, Immelman EJ, Riddell AG. Hypothermic perfusion as a method of shor-term porcine liver storage. *Br J Surg* 1968; **55**: 862 [PMID: 5686999]
49 **Hobbs KE**, Hunt AC, Palmer DB, Badrick FE, Morris AM, Mitra SK, Peacock JH, Immelman EJ, Riddell AG. Hypothermic low flow liver perfusion as a means of porcine hepatic storage for six hours. *Br J Surg* 1968; **55**: 696-703 [PMID: 5676704 DOI: 10.1002/bjs.1800550913]
50 **Drapanas T**, Zemel R, Vang JO. Hemodynamics of the isolated perfused pig liver: metabolism according to routes of perfusion and rates of flow. *Ann Surg* 1966; **164**: 522-537 [PMID: 5927633]
51 **Abouna GM**, Ashcroft T, Hull C, Hodson A, Kirkley J,

Walder DN. The assessment of function of the isolated perfused porcine liver. *Br J Surg* 1969; **56**: 289-295 [PMID: 4952478 DOI: 10.1002/bjs.1800560413]

52 **Abouna GM**, Fisher LM, Still WJ, Hume DM. Acute hepatic coma successfully treated by extracorporeal baboon liver perfusions. *Br Med J* 1972; **1**: 23-25 [PMID: 5061783 DOI: 10.1136/bmj.1.5791.23]

53 **Powell GM**, Hughes HM, Curtis CG. Isolated perfused liver technology for studying metabolic and toxicological problems. *Drug Metabol Drug Interact* 1989; **7**: 53-86 [PMID: 2699284 DOI: 10.1515/DMDI.1989.7.1.53]

54 **Hildebrand P**, Kleemann M, Roblick U, Mirow L, Bruch HP, Bürk C. Development of a perfused ex vivo tumor-mimic model for the training of laparoscopic radiofrequency ablation. *Surg Endosc* 2007; **21**: 1745-1749 [PMID: 17332954 DOI: 10.1007/s00464-007-9216-x]

55 **Lappegård KT**, Fung M, Bergseth G, Riesenfeld J, Mollnes TE. Artificial surface-induced cytokine synthesis: effect of heparin coating and complement inhibition. *Ann Thorac Surg* 2004; **78**: 38-44; discussion 44-45 [PMID: 15223398 DOI: 10.1016/j.athoracsur.2004.02.005]

56 **Chung WY**, Gravante G, Al-Leswas D, Alzaraa A, Sorge R, Ong SL, Pollard C, Lloyd DM, Metcalfe MS, Dennison AR. Addition of a kidney to the normothermic ex vivo perfused porcine liver model does not increase cytokine response. *J Artif Organs* 2012; **15**: 290-294 [PMID: 22476783]

57 **Chung WY**, Gravante G, Al-Leswas D, Alzaraa A, Sorge R, Ong SL, Pollard C, Lloyd DM, Metcalfe MS, Dennison AR. The autologous normothermic ex vivo perfused porcine liver-kidney model: improving the circuit's biochemical and acid-base environment. *Am J Surg* 2012; **204**: 518-526 [PMID: 23010618]

P- Reviewers Gruttadauria S, Lai ECH, Shimizu Y
S- Editor Gou SX **L- Editor** Kerr C **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i2.155

World J Gastroenterol 2013 January 14; 19(2): 155-157
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

FIELD OF VISION

Bowel preparation prior to colonoscopy: A continual search for excellence

Matthew L Bechtold, Abhishek Choudhary

Matthew L Bechtold, Abhishek Choudhary, Division of Gastroenterology, University of Missouri, Columbia, MO 65201, United States
Matthew L Bechtold, Division of Gastroenterology and Hepatology, University of Missouri Health Sciences Center, Five Hospital Drive, Columbia, MO 65212, United States
Author contributions: Bechtold ML wrote the manuscript; Choudhary A discussed the subject and provided critical revisions to the manuscript; both authors approved the final version of the manuscript.
Correspondence to: Matthew L Bechtold, MD, FACG, Division of Gastroenterology and Hepatology, University of Missouri Health Sciences Center, Five Hospital Drive, Columbia, MO 65212, United States. bechtoldm@health.missouri.edu
Telephone: +1-57-38821013 Fax: +1-57-38844595
Received: October 12, 2012 Revised: November 15, 2012
Accepted: December 20, 2012
Published online: January 14, 2013

Abstract

Bowel preparation prior to colonoscopy is essential to maximize the benefits of colonoscopy. Numerous bowel preparations have been studied, ranging from 4 L polyethylene glycol (PEG) to split-dose regimens to 2 L PEG with an adjunct laxative (senna, bisacodyl, ascorbic acid). Due to the large volume of PEG required for adequate bowel preparation, many studies have focused on reducing this large volume to only 2 L PEG with the addition of an adjunct. Recently, a randomized controlled trial by Tajika *et al* showed that the addition of mosapride to only 1.5 L PEG was non-inferior to mosapride and 2 L PEG for bowel cleansing but did provide improvements in patient tolerance. This study offers yet another potential bowel preparation for patients undergoing colonoscopy and may trigger further studies with 1.5 L PEG with an adjunct. In this letter, we discuss the current state of bowel preparation prior to colonoscopy and offer information to guide clinicians on choosing the appropriate bowel preparation for their patients.

© 2013 Baishideng. All rights reserved.

Key words: Colonoscopy; Polyethylene glycol; Mosapride; Bowel preparation

Bechtold ML, Choudhary A. Bowel preparation prior to colonoscopy: A continual search for excellence. *World J Gastroenterol* 2013; 19(2): 155-157 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i2/155.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i2.155

INVITED COMMENTARY ON HOT ARTICLES

As clinical gastroenterologists, we read the recent article by Tajika *et al*[1] describing a new lower volume bowel preparation prior to colonoscopy with considerable interest. This article examined the use of mosapride and only 1.5 L of polyethylene glycol (PEG) for bowel preparation prior to colonoscopy. Given the continued need and search for more options for bowel preparation, we recommend reading and possibly implementing the information in this article.

Colorectal cancer is a common and devastating disease which affects many patients worldwide. Being in top three of cancers and cancer-related deaths in the United States, colorectal cancer has become a focus of gastroenterology, with much of our practice being significantly impacted by colorectal cancer screening and surveillance[2].

Over the past 10 years, colonoscopy has become the screening test of choice for colorectal cancer. Unlike other non-invasive colorectal cancer screening tools, such as fecal occult blood testing and computed tomographic (CT) colonography, colonoscopy is the only modality which encompasses both diagnostic and therapeutic potential. However, colonoscopy is dependent on adequacy of bowel preparation for complete visualization of the

Table 1 Common bowel preparations utilized prior to colonoscopy

First tier			
Split-dose PEG			
2 L the night prior and 2 L morning of colonoscopy			
Second tier			
Full-dose PEG 4 L the night prior	2 L PEG + Adjunct Ascorbic acid Bisacodyl Senna Magnesium Mosapride	Sodium phosphate (Tablet)	Sulfate prep (Na, K, Mg)
Third tier			
Miralax + Gatorade	Magnesium citrate	Enemas	1.5 L + Adjunct Mosapride

PEG: Polyethylene glycol.

colonic mucosa. If visualization is compromised, missed lesions, prolonged procedure time, and increased patient discomfort become increasingly possible, significantly impacting patients and healthcare costs[3,4]. Therefore, bowel preparation prior to colonoscopy is extremely important for an adequate colonoscopic examination.

The optimal bowel preparation prior to colonoscopy must adequately clear the fecal material to visualize the underlying mucosa but also must be tolerable to the patient. Even if the bowel preparation is exceptional at clearing the colon of feces, if it is not palatable, the patients will likely not complete the full preparation. Over the past few years, many studies have been performed with varying uses of PEG, such as split-dose PEG and PEG with adjuncts, in an effort to improve patient tolerability without decreasing the efficacy of the bowel preparation. A meta-analysis in *Gastrointestinal Endoscopy* in 2011 demonstrated that split-dose PEG (consisting of 2 L PEG the night prior and 2 L PEG the morning of the procedure), improved both the cleansing of the colon and patient tolerability as compared to full-dose PEG (4 L the night prior to colonoscopy)[5]. Subsequently, the split-dose PEG has become a very common bowel preparation and is the preferred bowel preparation of the American College of Gastroenterology. However, patients are still required to consume a large volume of PEG. Therefore, many studies have elected to reduce the volume of PEG to 2 L and add a laxative adjunct (ascorbic acid, bisacodyl, senna, magnesium, *etc*.) and monitor for bowel cleansing and patient tolerability[6-12]. However, these studies resulted in conflicting results.

In 2011, two meta-analyses were published as abstracts in the *American Journal of Gastroenterology* showing that 2 L PEG with ascorbic acid cleansed the colon as well as 4L PEG but was equally tolerated[13]; however, 2 L PEG with bisacodyl demonstrated equal bowel cleansing and improved patient tolerability[14]. Despite the results, limitations have been placed on using bisacodyl with PEG due to risk of ischemic colitis. Therefore, the search continues for an adequate low-dose PEG bowel preparation.

In the randomized controlled trial by Tajika *et al*[1] mosapride (15 mg) was utilized as an adjunct laxative to low-dose PEG (1.5 L *vs* 2 L). The study revealed that mosapride with 1.5 L PEG resulted in equal bowel cleansing and increased tolerability as compared to mosapride with 2 L PEG. Given these results, mosapride with 1.5 L PEG appears promising as an alternative bowel preparation prior to colonoscopy. However, given a few limitations within this study, the results need to be interpreted cautiously.

First, this study utilized a non-inferiority model for analysis. Most bowel preparation studies are performed using a superiority model due to limitations of the non-inferiority model. The non-inferiority model is dependent upon the margin at which non-inferiority is defined. If the margin is not accurate, the results may be significantly affected. In this study, mosapride with 1.5 L PEG was non-inferior to mosapride with 2 L PEG; however, it appears that 2 L PEG with mosapride group has many more excellent and good preparations as compared to 1.5 L PEG with mosapride group (right colon: 88 *vs* 60; left colon: 97 *vs* 82). Given this difference, especially in the right colon, it is difficult for us to believe that equality of cleansing exists for these two regimens. Therefore, this leads us to believe that the sample size estimation and the non-inferiority margin may not be as accurate as hoped. Second, although the Aronchick scale has been shown to be a valuable tool in assessing bowel preparation, it is designed for the entire colon[15]. This study used the Aronchick scale but divided it among right and left colon. Ideally, the Ottawa or Boston bowel preparation scales which utilize different segments of the colon with numerical scores may have been a better choice for bowel preparation assessment[16,17].

At this point, based upon the available literature, we believe the ideal bowel preparation prior to colonoscopy is the split-dose PEG given 2 L the night prior and 2 L the day of the colonoscopy (Table 1). However, given its large volume, it may not be suited for all patients. Furthermore, given the number of other potential options including the preparation described in this study, alternative bowel preparations are readily available and may be utilized in certain cases without adversely affecting bowel cleansing.

The choice of bowel preparation is extremely important for patients and practices. Adequate bowel preparation which is tolerable to patients will likely enhance satisfaction among patients, who would be more likely to return for another colonoscopy. It will also likely reduce the need for early repeat procedures due to poor prep quality and overall costs. Given the number of preparations available, bowel preparation prior to colonoscopy in the future may be tailored to the needs and desires of the patients. Although split-dose PEG seems to be the gold-standard at this time, other preparations may be utilized to enhance patient satisfaction without adversely affecting overall preparation quality. The choice is up to you and your patient.

REFERENCES

1 **Tajika M**, Niwa Y, Bhatia V, Kawai H, Kondo S, Sawaki A, Mizuno N, Hara K, Hijioka S, Matsumoto K, Kobayashi Y, Saeki A, Akabane A, Komori K, Yamao K. Efficacy of mosapride citrate with polyethylene glycol solution for colonoscopy preparation. *World J Gastroenterol* 2012; **18**: 2517-2525 [PMID: 22654449 DOI: 10.3748/wjg.v18.i20.2517]

2 **Siegel R**, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin* 2012; **62**: 10-29 [PMID: 22237781 DOI: 10.3322/caac.21149]

3 **Kim WH**, Cho YJ, Park JY, Min PK, Kang JK, Park IS. Factors affecting insertion time and patient discomfort during colonoscopy. *Gastrointest Endosc* 2000; **52**: 600-605 [PMID: 11060182 DOI: 10.1067/mge.2000.109802]

4 **Rex DK**, Imperiale TF, Latinovich DR, Bratcher LL. Impact of bowel preparation on efficiency and cost of colonoscopy. *Am J Gastroenterol* 2002; **97**: 1696-1700 [PMID: 12135020 DOI: 10.1111/j.1572-0241.2002.05827.x]

5 **Kilgore TW**, Abdinoor AA, Szary NM, Schowengerdt SW, Yust JB, Choudhary A, Matteson ML, Puli SR, Marshall JB, Bechtold ML. Bowel preparation with split-dose polyethylene glycol before colonoscopy: a meta-analysis of randomized controlled trials. *Gastrointest Endosc* 2011; **73**: 1240-1245 [PMID: 21628016 DOI: 10.1016/j.gie.2011.02.007]

6 **Marmo R**, Rotondano G, Riccio G, Marone A, Bianco MA, Stroppa I, Caruso A, Pandolfo N, Sansone S, Gregorio E, D' Alvano G, Procaccio N, Capo P, Marmo C, Cipolletta L. Effective bowel cleansing before colonoscopy: a randomized study of split-dosage versus non-split dosage regimens of high-volume versus low-volume polyethylene glycol solutions. *Gastrointest Endosc* 2010; **72**: 313-320 [PMID: 20561621 DOI: 10.1016/j.gie.2010.02.048]

7 **Sharma VK**, Chockalingham SK, Ugheoke EA, Kapur A, Ling PH, Vasudeva R, Howden CW. Prospective, randomized, controlled comparison of the use of polyethylene glycol electrolyte lavage solution in four-liter versus two-liter volumes and pretreatment with either magnesium citrate or bisacodyl for colonoscopy preparation. *Gastrointest Endosc* 1998; **47**: 167-171 [PMID: 9512283 DOI: 10.1016/S0016-5107(98)70351-7]

8 **Hookey LC**, Depew WT, Vanner SJ. Combined low volume polyethylene glycol solution plus stimulant laxatives versus standard volume polyethylene glycol solution: a prospective, randomized study of colon cleansing before colonoscopy. *Can J Gastroenterol* 2006; **20**: 101-105 [PMID: 16482236]

9 **DiPalma JA**, Wolff BG, Meagher A, Cleveland Mv. Comparison of reduced volume versus four liters sulfate-free electrolyte lavage solutions for colonoscopy colon cleansing. *Am J Gastroenterol* 2003; **98**: 2187-2191 [PMID: 14572566 DOI: 10.1111/j.1572-0241.2003.07690.x]

10 **Ell C**, Fischbach W, Bronisch HJ, Dertinger S, Layer P, Rünzi M, Schneider T, Kachel G, Grüger J, Köllinger M, Nagell W, Goerg KJ, Wanitschke R, Gruss HJ. Randomized trial of low-volume PEG solution versus standard PEG + electrolytes for bowel cleansing before colonoscopy. *Am J Gastroenterol* 2008; **103**: 883-893 [PMID: 18190651 DOI: 10.1111/j.1572-0241.2007.01708.x]

11 **Adams WJ**, Meagher AP, Lubowski DZ, King DW. Bisacodyl reduces the volume of polyethylene glycol solution required for bowel preparation. *Dis Colon Rectum* 1994; **37**: 229-233; discussion 233-234 [PMID: 8137669 DOI: 10.1007/BF02048160]

12 **Ker TS**. Comparison of reduced volume versus four-liter electrolyte lavage solutions for colon cleansing. *Am Surg* 2006; **72**: 909-911 [PMID: 17058733]

13 **Godfrey JD**, Clark RE, Choudhary A, Ashraf I, Matteson ML, Bechtold ML. Low-volume polyethylene glycol and ascorbic acid for bowel preparation prior to colonoscopy: A meta-analysis [abstract]. *Am J Gastroenterol* 2011; **106**: S527 (1376)

14 **Clark RE**, Godfrey JD, Choudhary AMD, Ashraf I, Matteson ML, Bechtold ML. Low-volume polyethylene glycol and bisacodyl for bowel preparation prior to colonoscopy: A meta-analysis [abstract]. *Am J Gastroenterol* 2011; **106**: S528 (1377)

15 **Aronchick CA**, Lipshutz WH, Wright SH, DuFrayne F, Bergman G. Validation of an instrument to assess colon cleansing [abstract]. *Am J Gastroenterol* 1999; **94**: 2667

16 **Rostom A**, Jolicoeur E. Validation of a new scale for the assessment of bowel preparation quality. *Gastrointest Endosc* 2004; **59**: 482-486 [PMID: 15044882 DOI: 10.1016/S0016-5107(03)02875-X]

17 **Lai EJ**, Calderwood AH, Doros G, Fix OK, Jacobson BC. The Boston bowel preparation scale: a valid and reliable instrument for colonoscopy-oriented research. *Gastrointest Endosc* 2009; **69**: 620-625 [PMID: 19136102 DOI: 10.1016/j.gie.2008.05.057]

P- Reviewer Phull PS **S- Editor** Song XX
L- Editor A **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i2.161

World J Gastroenterol 2013 January 14; 19(2): 161-164
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

FIELD OF VISION

Internal frontier: The pathophysiology of the small intestine

Haruhiko Sugimura, Satoshi Osawa

Haruhiko Sugimura, Department of Tumor Pathology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu 431-3192, Japan
Satoshi Osawa, Endoscopic and Photodynamic Medicine, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu 431-3192, Japan
Author contributions: Sugimura H wrote the manuscript; Osawa S provided the materials and contributed to discussion about the manuscript.
Correspondence to: Haruhiko Sugimura, MD, PhD, Department of Tumor Pathology, Hamamatsu University School of Medicine, 1-20-1 Handayama, Higashi-ku, Hamamatsu 431-3192, Japan. hsugimur@hama-med.ac.jp
Telephone: +81-53-4352220 Fax: +81-53-4352225
Received: September 26, 2012 Revised: October 1, 2012
Accepted: November 6, 2012
Published online: January 14, 2013

Abstract

Even though the small intestine occupies a major portion of the abdominal space and is essential for life, in most pathology textbooks any chapter on small intestinal diseases, especially in human beings, is typically shorter than those for other gastrointestinal organs. Clinical and experimental investigations of the small intestine in various clinical situations, such as nutrition management, obesity interventions, and emergency care, have elucidated several important biological problems associated with the small intestine, the last frontier of gastroenterology. In this issue, a review by Professor Basson and his team at Michigan State University sheds light on the changes in the human small intestine under various conditions based on their clinical and surgical experience. With the advent of recent innovations in enteroscopy, a form of endoscopy used to examine deep within the small intestine, the issue that they highlighted, i.e., mucosal adaptation and atrophy of the human small intestine, has emerged as a major and manageable challenge for gastroenterologists in general, including the readers of the *World Journal of Gastroenterology*.

© 2013 Baishideng. All rights reserved.

Key words: Ileum; Jejunum; Small intestine; Atrophy; Adaptation; Enteroscopy

Sugimura H, Osawa S. Internal frontier: The pathophysiology of the small intestine. *World J Gastroenterol* 2013; 19(2): 161-164 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i2/161.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i2.161

INVITED COMMENTARY ON HOT ARTICLES

Juan Rosai's Surgical Pathology (9th edition) contains 45 pages on the small intestine, compared with 80 pages on the large intestine[1]. The actual length of the small intestine is much longer than that of the large intestine, and the vital importance of the small intestine is well known. Why then are fewer pages devoted to diseases of the small intestine? Obviously, the access of surgical pathologists and gastroenterologists to the small intestine is limited, compared with access to the large intestine and appendix. Actually, the old version of Morson and Dawson's textbook Gastrointestinal Pathology[2] is fairer: the same number of pages is devoted to the small intestine and to the large intestine, probably because this book is a product of the century during which autopsies made major contributions to our knowledge base. This issue of the *World Journal of Gastroenterology* has a wide-ranging review article on the pathophysiology of the small intestine by Professor Basson and his colleagues[3]. These authors are surgeons, and a tremendous amount of data based on their own clinical experiences and those of others, as well as on animal experiments, are comprehensively discussed in their review. This review article addresses conditions such as starvation and parenteral nutrition in patients with severe trauma or pancreatitis and in patients with postoperative short-gut syndrome for various reasons including bariatric surgery for obesity. Although these

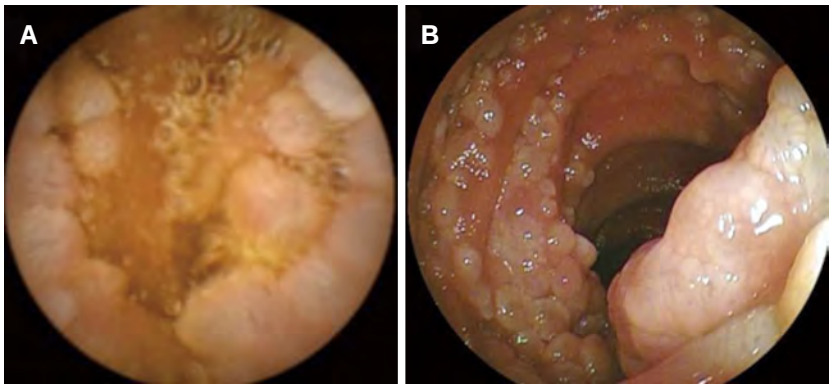


Figure 1 Endoscopy. Images obtained using capsule endoscopy (A) and double-balloon enteroscopy (B) in a 61-year-old man with follicular lymphoma who visited us because of gastrointestinal bleeding from an unknown cause and a hemoglobin concentration of 9.7 g/dL.

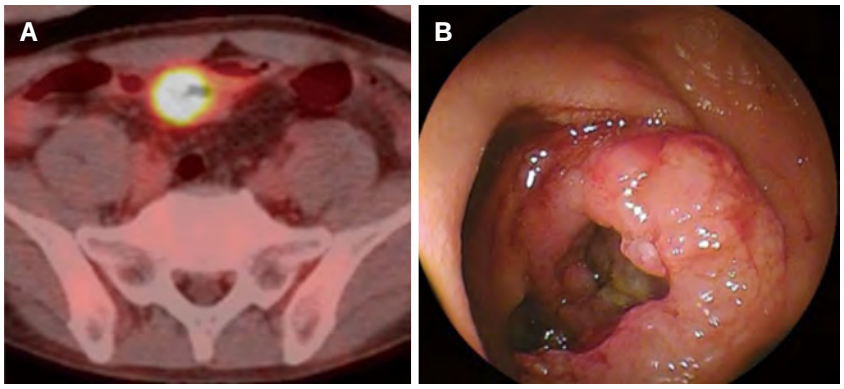


Figure 2 A mass positive by positron emission computed tomography actually was a tumor arising the mucosa of the small intestine of a 48-year-old woman who had suffered Crohn disease. A: Positron emission computed tomography/computed tomography showed an accumulation in site of wall thickening of ileum; B: Image obtained by double-balloon enteroscopy.

conditions are undoubtedly serious, recognition and explicit statements by medical professionals regarding the importance of the small intestine, and of its diseases as a "great burden of human distress", have only recently appeared in medical literature[4]. Most clinical conditions that highlight the importance of the small intestine are familiar only to experts in a particular area of medicine. These conditions are not typically a concern of non-surgical gastroenterologists, such as pathologists who are accustomed to diagnosing neoplasms of the large intestine, grading inflammatory bowel disease, and validating ischemic necrosis of the resected small intestine in cases with an acute abdomen. The small intestine is most often investigated because of its involvement in a disease originating from another organ, such as the mesothelium[5]. Changes in the mucosa of the small intestine itself are an exceptional category among the slides that pile up next to the microscope.

The situation is now changing. As avid readers of *World Journal of Gastroenterology* may have noticed[6-9], recent progress in enteroscopy has enabled areas deep within the small intestine to be reached, providing clinicians active in the wider branches of gastroenterology with the opportunity to evaluate changes in the small intestine, which have previously only been observed by surgeons. This also means that many non-surgeons must commit to the management of the small intestine, based on findings and evaluations associated with specimens of small intestine obtained using these newly developed modalities. For ordinary pathologists, for example, the concepts mainly discussed in this review, i.e., adaptation and atrophy of the small intestine, may be new and unfamiliar. These lesions were encountered only after specific "congenital or acquired disease or medical and surgical intervention" had occurred in the patients. When specimens obtained by enteroscopy become routine in the future, however, the concepts and knowledge of adaptation and atrophy of the small intestine will become an essential "must" for all practitioners including general pathologists.

One of the important conditions that the authors of this review addressed is the change in the small intestine in subjects receiving total parental nutrition. This lifesaving modality has many variations in terms of its nutritional regimen and the effects of these variations on pathophysiology, that is, on the grade of adaptation of the small intestine. These effects on the small intestinal mucosa and the consequent outcomes of individual patients have been thoroughly investigated and published in many scientific articles, but the scientific evidence in human beings remains insufficient according to the

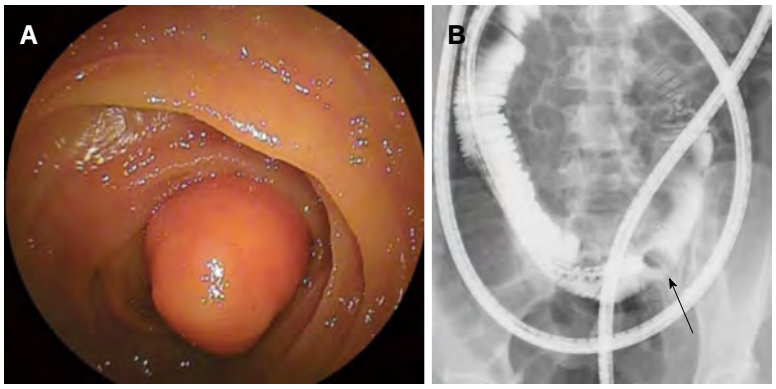


Figure 3 Identification of lipoma clarified the reason of intermittent abdominal discomfort in a 29-year-old female. Endoscopic finding (A) and selective small bowel series (B) obtained using double-balloon enteroscopy. This required surgical intervention. An arrow in the B indicates a defect by a lipoma.

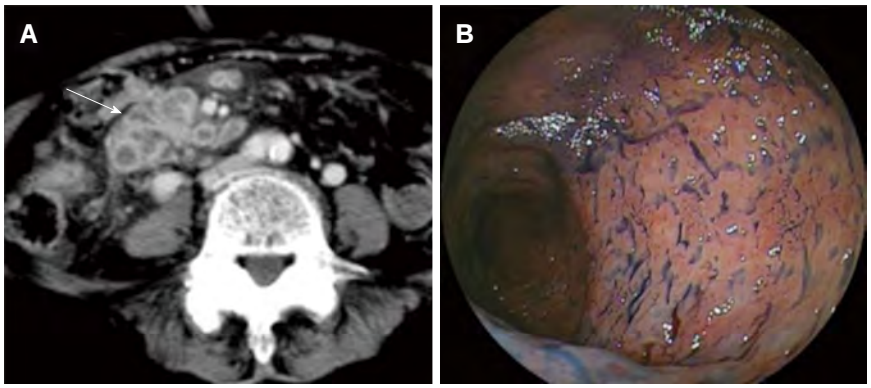


Figure 4 Intestinal tuberculosis is also revealed by double-ballon enteroscopy. A: Computed tomography showed wall thickening of the ileum with contrast enhancement (arrow); B: Double-balloon enteroscopy showed destruction of the small intestinal villi.

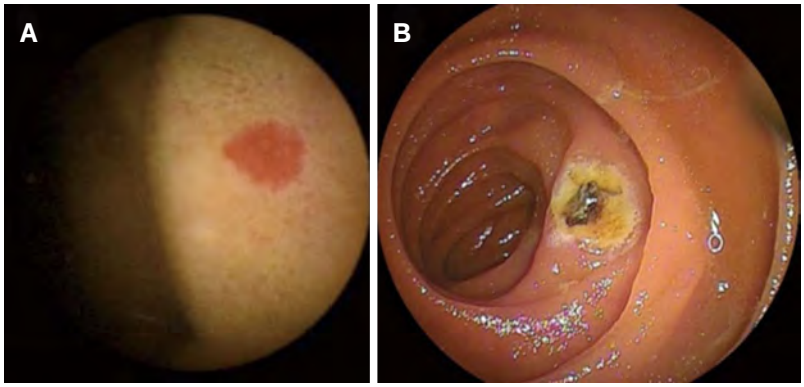


Figure 5 Angioectasia. A: Diagnosis of angioectasia was made by capsule endoscopy; B: Argon plasma coagulation successfully treated the lesion.

above-mentioned authors. The concept of mucosal adaptation[10] includes proliferation, functional augmentation, and cellular differentiation. Biochemical changes include alterations in molecules related to apoptosis, proliferation, signal transduction, and fatty acid metabolism. These meticulous frameworks in intestinal cells and tissues have so far been revealed by studies using *in vivo* and *in vitro* manipulative systems. From now on, in the era of enteroscopy, lingering questions such as "what is the reality and examples of mucosal adaptation in human clinical settings?" and "how can we validate the accumulated experimental findings in human beings and exploit them in clinical practice?" will be answered.

Small intestinal enteroscopy is now available in ordinary hospitals, thus facilitating the detection of previously unobserved pathological conditions. Capsular endoscopy, the latest wireless version of enteroscopy, has become a popular practical procedure since the publication of a seminal report on this modality a decade ago[11]. In the minds of laymen, this technique seems like a dream[12]. The use of capsular endoscopy and refined enteroscopy using a double-balloon method[13] in clinical practice have

revealed thousands of new anecdotal findings (Figure 1), and the rapid accumulation of this kind of basic knowledge of the small intestine will help to set up principles of surveillance for mucosal adaptation and atrophy of the small intestine (especially morphological changes) in various clinical conditions. For example, introduction of capsular endoscopy and a double-balloon method disclosed previously unrecognized lesions such as adenocarcinoma arising from Crohn disease in the small intestine (Figure 2)[14], submucosal lipoma (Figure 3), tuberculosis at the terminal ileum (Figure 4), and angioectasia (Figure 5). None of these lesions were accessible until the recent development of capsule endoscopy and the double balloon method. The morphology is new to pathologists and endoscopists, and these developments will critically influence the managements of the patients. The concepts that Professor Basson and colleagues have illuminated in their review will soon become an important guidepost for evaluating the histopathology of the small intestine in daily practice and for patient care by a broader range of gastroenterologists.

REFERENCES

1 **Rosai J**. Rosai and Ackerman's Surgical Pathology. 9th ed. Edinburgh: Mosby, 2004: 615-872
2 **Morson BC**, Dawson IM, Day DW, Jass JR, Price AB, Williams GT. Morson & Dawson's gastrointestinal pathology. 3 ed. London: Blackwell Scientific Publications, 1989
3 **Shaw D**, Gohil K, Basson MD. Intestinal mucosal atrophy and adaptation. *World J Gastroenterol* 2012; **18**: 6357-6375 [PMID: 23197881 DOI: 10.3748/wjg.v18.i44.6357]
4 **Tappenden KA**. Emerging therapies for intestinal failure. *Arch Surg* 2010; **145**: 528-532 [PMID: 20566971 DOI: 10.1001/archsurg.2010.102]
5 **Kim JH**, Kwon KY, Jeon YK, Nam JH, Choi C, Hyeon CL, Choi YD. Mucin-positive epithelial mesothelioma of the peritoneum: small bowel involvement. *Pathol Int* 2011; **61**: 756-761 [PMID: 22126385 DOI: 10.1111/j.1440-1827.2011.02732.x]
6 **Kav T**, Sivri B. Is enteroscopy necessary for diagnosis of celiac disease? *World J Gastroenterol* 2012; **18**: 4095-4101 [PMID: 22919241 DOI: 10.3748/wjg.v18.i31.4095]
7 **Oh TG**, Chung JW, Kim HM, Han SJ, Lee JS, Park JY, Song SY. Primary intestinal lymphangiectasia diagnosed by capsule endoscopy and double balloon enteroscopy. *World J Gastrointest Endosc* 2011; **3**: 235-240 [PMID: 22110841 DOI: 10.4253/wjge.v3.i11.235]
8 **Park SC**, Chun HJ, Kang CD, Sul D. Prevention and management of non-steroidal anti-inflammatory drugs-induced small intestinal injury. *World J Gastroenterol* 2011; **17**: 4647-4653 [PMID: 22180706 DOI: 10.3748/wjg.v17.i42.4647]
9 **Tee HP**, How SH, Kaffes AJ. Learning curve for double-balloon enteroscopy: Findings from an analysis of 282 procedures. *World J Gastrointest Endosc* 2012; **4**: 368-372 [PMID: 22912911 DOI: 10.4253/wjge.v4.i8.368]
10 **Drozdowski L**, Thomson AB. Intestinal mucosal adaptation. *World J Gastroenterol* 2006; **12**: 4614-4627 [PMID: 16937429]
11 **Iddan G**, Meron G, Glukhovsky A, Swain P. Wireless capsule endoscopy. *Nature* 2000; **405**: 417 [PMID: 10839527 DOI: 10.1038/35013140]
12 **Muñoz-Navas M**. Capsule endoscopy. *World J Gastroenterol* 2009; **15**: 1584-1586 [PMID: 19340899 DOI: 10.3748/wjg.15.1584]
13 **Yamamoto H**, Sekine Y, Sato Y, Higashizawa T, Miyata T, Iino S, Ido K, Sugano K. Total enteroscopy with a nonsurgical steerable double-balloon method. *Gastrointest Endosc* 2001; **53**: 216-220 [PMID: 11174299]
14 **Kodaira C**, Osawa S, Mochizuki C, Sato Y, Nishino M, Yamada T, Takayanagi Y, Takagaki K, Sugimoto K, Kanaoka S, Furuta T, Ikuma M. A case of small bowel adenocarcinoma in a patient with Crohn's disease detected by PET/CT and double-balloon enteroscopy. *World J Gastroenterol* 2009; **15**: 1774-1778 [PMID: 19360924 DOI: 10.3748/wjg.15.1774]

P- Reviewer Handa O **S- Editor** Song XX
L- Editor Cant MR **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i2.158

World J Gastroenterol 2013 January 14; 19(2): 158-160
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

FIELD OF VISION

Recurrent hepatitis C virus after transplant and the importance of plasma cells on biopsy

Eric R Kallwitz

Eric R Kallwitz, Section of Hepatology, Loyola University Medical Center, Maywood, IL 60153, United States
Author contributions: Kallwitz ER was responsible for the entire content, drafting and editing of the manuscript.
Correspondence to: Eric R Kallwitz, MD, Assistant Professor of Medicine, Section of Hepatology, Loyola University Medical Center, 2160 S First Avenue, Maywood, IL 60153, United States.ekallwitz@lumc.edu
Telephone: +1-708-2162538 Fax: +1-708-2166299
Received: September 19, 2012 Revised: December 14, 2012
Accepted: December 22, 2012
Published online: January 14, 2013

Abstract

Hepatitis C virus (HCV) is the leading indication for liver transplantation in the United States. It recurs universally after transplant but the rate of fibrosis and the development of graft failure is variable. Different donor and recipient features have been demonstrated to impact fibrosis. Plasma cell hepatitis, a histologic finding, is one feature associated with poor graft and patient outcomes. The pathogenic mechanism resulting in plasma cell hepatitis is poorly understood, with evidence suggesting a role for both the HCV and the immune system.A recent publication described plasma cell hepatitis in a larger context of immune medicated graft dysfunction in transplant recipients receiving interferon based therapy. This manuscript will highlight the topic of plasma cell hepatitis and provide commentary on the lack of recognition, the data regarding pathophysiologic mechanisms and the potential management options.

© 2013 Baishideng. All rights reserved.

Key words: Hepatitis C virus; Plasma cells; Biopsy; Sustained virological response

Kallwitz ER. Recurrent hepatitis C virus after transplant and the importance of plasma cells on biopsy. *World J Gastroenterol* 2013; 19(2): 158-160 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i2/158.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i2.158

INVITED COMMENTARY ON HOT ARTICLES

Hepatitis C virus (HCV) is the most common indication for liver transplantation in the United States. Recurrence of hepatitis C is nearly universal after transplantation and ensuing graft dysfunction occurs commonly. Ten percent of recipients progress to cirrhosis within 3 years of transplant[1] demonstrating that some patients develop aggressive recurrent HCV. Plasma cell hepatitis, diagnosed histologically, is one of a number of conditions associated with adverse outcomes and graft failure in patients with posttransplant HCV. Plasma cell hepatitis can develop in the context of interferon based therapy or in the absence of treatment with interferon. Levitsky *et al*[2] present a multicenter case-control study which included a large subset of patients found to have plasma cell hepatitis associated with interferon therapy for HCV.The manuscript should be read with interest by transplant hepatologists as it highlights important concepts regarding plasma cell hepatitis in patients with HCV after transplant. First, plasma cell hepatitis is under recognized. Second, the pathologic process resulting in plasma cell hepatitis is poorly understood. Finally, with a paucity of data, it is not possible to determine the best treatment for transplant recipients with this condition.

The case control series by Levitsky *et al*[2] reported that the incidence of any immune medicated graft dysfunction on interferon based therapy varied by center, ranging between 3.2%-16.3%. Persons found to have immune mediated graft dysfunction on HCV therapy had significantly worse survival, and more graft failure leading to higher rates of retransplantation. Plasma cell hepatitis was the most common manifestation of interferon induced immune mediated graft dysfunction. It is important to note that plasma cell hepatitis was also commonly identified prior to the use of interferon. The incidence of plasma cell hepatitis

Table 1 Generalized histologic and clinical features seen in post transplant patients with hepatitis C

Pathologic entity	Histologic features	Clinical and laboratory features
Plasma cell hepatitis	Plasma cells (often in sheets) Centrilobular necrosis	HCV PCR positive ANA or ASMA may be positive (often with low titers) Low level of immunosuppression Can be caused by interferon based therapy
De novo autoimmune hepatitis	Lymphoplasmacytic infiltrate Interface hepatitis	Positive ANA or ASMA Elevated immunoglobulins In persons on treatment HCV RNA often not detected (occurs in other settings in addition to HCV) Low level of immunosuppression Can be caused by interferon based therapy
Acute cellular rejection	Mixed inflammatory infiltrate Endotheliitis Nonsuppurative Cholangitis Centrilobular necrosis (variable)	Low level of immunosuppression Can be caused by interferon based therapy
Recurrent hepatitis C	Lymphocyte Aggregates Portal based fibrosis FCH is one variant (Cholestasis, Apoptosis, Fibrosis)	HCV PCR positive In FCH, markedly high viral load In FCH, high level of immunosuppression

HCV: Hepatitis C virus; FCH: Fibrosingcholestatic hepatitis; ANA: Antinuclear antibodies; ASMA: Anti-smooth muscle antibody; PCR: Polymerase chain reaction.

on pretreatment biopsies was much more common in persons who developed immune mediated graft dysfunction (36.5%) compared to the control group (7.7%, $P = 0.003$) on treatment[2]. Fourteen of the cases labeled as plasma cell hepatitis by the central pathologist were not recognized by the local pathologist who initially interpreted the liver biopsy[2]. The authors conclude that plasma cell hepatitis often predicts the development of immune mediated graft dysfunction occurring during interferon based treatment. In addition, the author's recommend that clinicians should not reduce immunosuppression doses and should not initiate interferon based therapy in those with immune features including plasma cell hepatitis on pretreatment biopsies.

The informative and interesting conclusions of this article deserve further comment. A main feature of the article from Levistky and colleagues is that plasma cell hepatitis is under recognized and is often mistaken for recurrent hepatitis C or other forms of rejection[2]. A histologic scoring system was developed in 2008[3]. Diagnostic features of plasma cell hepatitis include numerous plasma cells, often in sheets or clusters, accompanied by centrilobular necrosis. Despite the existence of standardized criteria, it is not surprising that plasma cell hepatitis is under recognized. With few publications describing plasma cell hepatitis, it is not topical to hepatologists and pathologists. Additionally, in post-transplant patients, other processes such as recurrent HCV, *de novo* autoimmune hepatitis and acute cellular rejection present alternative diagnoses. Table 1 highlights clinical and histologic features which might help distinguish the different diagnoses. It is important to realize significant overlap does exist between the pathologic process making a definite diagnosis impossible in some cases. The recent publication in a high profile journal will hopefully lead to better recognition of this disorder.

The pathogenesis of plasma cell hepatitis has yet to be defined. It has been described both as a manifestation of hepatitis C[4,5] and as a form of rejection[3]. Evidence exists for both possibilities. Prior series have shown that HCV plays a role. A two subject case series examined plasma cell hepatitis as a lymphoproliferative disorder[5]. Both patients in this series had serum or urine protein electrophoresis demonstrating a monoclonal protein and RNA probes for hepatitis C were positive within the plasma cell infiltrate[5]. An association between plasma cell hepatitis and mixed cryoglobulinemia has not been studied. In the current article, patient survival with plasma cell hepatitis was improved with a sustained virologic response to treatment for HCV[2]. Although graft failure and retransplantation occurred in some cases, five year survival was above 80% and similar to control subjects[2]. Additionally, there was a trend toward improved graft survival in cases of immune mediated graft dysfunction when hepatitis C was eradicated. In Kaplan-Meier analysis, the majority of graft loss occurred early after transplant with approximately 60% graft survival and no further graft loss occurring after two years in the group that achieved a sustained virological response (SVR)[2]. In contrast, the group that did not achieve an SVR continued to develop graft loss during the entire period of follow up and graft survival was less than 40% at five years[2]. It is additionally notable that SVR rates in series of patients with plasma cell hepatitis ranged between 40%-67%[2,3]. Given that poor outcomes are observed with plasma cell hepatitis in persons who never received interferon[3,6], prospective, randomized data are needed to compare outcome of interferon treatment versus no interferon treatment with respect to HCV eradication.

Other studies suggest the development of plasma cell hepatitis is an immune mediated event. Explanted livers of post transplant patients who later develop plasma cell hepatitis were more likely to have extensive plasma cell infiltrates[6] suggesting that immunologic predisposition exists even prior to transplant. However, not all persons with plasma cell infiltrates on explant will develop plasma cell hepatitis, and additional factors after transplant appear to play a role. There are data describing the development of plasma cell hepatitis in the setting of lowered immunosup-

pression[2,3]. In a series including 38 subjects with plasma cell hepatitis, 31 had either recently lowered immunosuppressant dosing or subtherapeutic drug levels[3]. In the series by Levitsky *et al*[2] significantly more patients with immune mediated graft dysfunction had a reduction in immunosuppression prior to interferon based therapy. Additionally, more subjects with immune mediated graft dysfunction had immunosuppression reduced during therapy[2]. One would expect interferon would have a role in the development of an immune mediated event and the contribution of interferon to the development of plasma cell hepatitis is not quite clear. In a retrospective series, interferon was not associated with the development of plasma cell hepatitis and its use did not impact outcome once plasma cell hepatitis developed[3]. In the series published by Levitsky *et al*[2] persons with existing plasma cell hepatitis had worsened immune mediated graft dysfunction after their immunosuppression was reduced and interferon was started. Increasing baseline immunosuppression prior to initiating interferon in patients with plasma cell hepatitis should be considered for future study, especially given data showing improved outcomes with augmenting immunosuppression alone[3].

With an immune predisposition, plasma cell hepatitis and *de novo* autoimmune hepatitis have overlapping features. They are nearly histologically indistinguishable, and some refer to them interchangeably[7]. A few subtle clues suggest that these processes have a different underlying pathophysiology. Case series have described *de novo* autoimmune hepatitis developing in conjunction with interferon based therapy with elevated autoimmune titers, undetected HCV RNA levels and pretreatment biopsies showing no plasma cells[8]. In addition, in transplant recipients for indications other than HCV, *de novo* autoimmune hepatitis has been shown to respond well to steroid therapy[9], whereas plasma cell hepatitis in HCV infected recipients typically does not[3].

Plasma cell hepatitis represents an important entity which is likely under reported as the result of poor recognition. Agreement on standardized nomenclature distinguishing plasma cell hepatitis from *de novo* autoimmune hepatitis in the post transplant setting may improve recognition. Plasma cell hepatitis best refers to plasma cell infiltration in the setting of post transplant hepatitis C. *De novo* autoimmune hepatitis best refers to plasma cell infiltration that occurs commonly with positive autoimmune titers, steroid responsiveness and, in the setting of interferon based therapy, may be best reserved when in a lymphoplasmacytic infiltrate develops without active viremia. As shown in Table 1, it must be recognized that overlap between the two conditions in both pathology and pathophysiologic mechanisms exist such that diagnostic certainty will not always occur.

Currently, the best management of plasma cell hepatitis that develops independent of HCV therapy is unclear. Limited data showed that augmentation of immunosuppression without the addition of prednisone may be of benefit[3]. Once on interferon based therapy, achieving an SVR was also shown to benefit patient survival[2]. The recommendation by Levitsky *et al*[2] that interferon should not be initiated in patients with plasma cell hepatitis may be overreaching based on the data presented. It also would suggest an alternate option with better outcomes existed. A practical approach may be augmenting baseline immunosuppression and a repeat liver biopsy. If the liver biopsy shows decreased immune features than interferon based therapy might be attempted. Ultimately, until there is better prospective data, responses to this entity will likely be reflective of single center experiences.

REFERENCES

1 **Berenguer M**. Natural history of recurrent hepatitis C. *Liver Transpl* 2002; **8**: S14-S18 [PMID: 12362293 DOI: 10.1053/jlts.2002.35781]

2 **Levitsky J**, Fiel MI, Norvell JP, Wang E, Watt KD, Curry MP, Tewani S, McCashland TM, Hoteit MA, Shaked A, Saab S, Chi AC, Tien A, Schiano TD. Risk for immune-mediated graft dysfunction in liver transplant recipients with recurrent HCV infection treated with pegylated interferon. *Gastroenterology* 2012; **142**: 1132-1139.e1 [PMID: 22285805 DOI: 10.1053/j.gastro.2012.01.030]

3 **Fiel MI**, Agarwal K, Stanca C, Elhajj N, Kontorinis N, Thung SN, Schiano TD. Posttransplant plasma cell hepatitis (de novo autoimmune hepatitis) is a variant of rejection and may lead to a negative outcome in patients with hepatitis C virus. *Liver Transpl* 2008; **14**: 861-871 [PMID: 18508382 DOI: 10.1002/lt.21447]

4 **Khettry U**, Huang WY, Simpson MA, Pomfret EA, Pomposelli JJ, Lewis WD, Jenkins RL, Gordon FD. Patterns of recurrent hepatitis C after liver transplantation in a recent cohort of patients. *Hum Pathol* 2007; **38**: 443-452 [PMID: 17188331 DOI: 10.1016/j.humpath.2006.08.028]

5 **Tun HW**, Krishna M, Menke DM. Hepatitis C-related posttransplant plasma cell proliferative disorder with hepatitis C virus in neoplastic plasma cells: a new posttransplant disease entity? *Transplant Proc* 2004; **36**: 2692-2696 [PMID: 15621126]

6 **Ward SC**, Schiano TD, Thung SN, Fiel MI. Plasma cell hepatitis in hepatitis C virus patients post-liver transplantation: case-control study showing poor outcome and predictive features in the liver explant. *Liver Transpl* 2009; **15**: 1826-1833 [PMID: 19938116 DOI: 10.1002/lt.21949]

7 **Fiel MI**, Schiano TD. Plasma cell hepatitis (de-novo autoimmune hepatitis) developing post liver transplantation. *Curr Opin Organ Transplant* 2012; **17**: 287-292 [PMID: 22498651 DOI: 10.1097/MOT.0b013e3283536622]

8 **Berardi S**, Lodato F, Gramenzi A, D'Errico A, Lenzi M, Bontadini A, Morelli MC, Tamè MR, Piscaglia F, Biselli M, Sama C, Mazzella G, Pinna AD, Grazi G, Bernardi M, Andreone P. High incidence of allograft dysfunction in liver transplanted patients treated with pegylated-interferon alpha-2b and ribavirin for hepatitis C recurrence: possible de novo autoimmune hepatitis? *Gut* 2007; **56**: 237-242 [PMID: 16798778 DOI: 10.1136/gut.2006.092064]

9 **Gabler WL**. National Toxicology program report on fluoride and cancer. *J Oreg Dent Assoc* 1991; **60**: 32-33 [PMID: 1856781 DOI: 10.1016/S0140-6736(97)06478-7]

P-Reviewer Shimizu Y **S- Editor** Huang XZ **L- Editor** A **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i2.165

World J Gastroenterol 2013 January 14; 19(2): 165-173
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

TOPIC HIGHLIGHT

Mansour A Parsi, MD, *Series Editor*

Endoscopic management of difficult common bile duct stones

Guru Trikudanathan, Udayakumar Navaneethan, Mansour A Parsi

Guru Trikudanathan, Department of Internal Medicine, University of Connecticut Medical Center, Farmington, CT 06269, United States
Udayakumar Navaneethan, Mansour A Parsi, Digestive Disease Institute, Cleveland Clinic, Cleveland, OH 44195, United States
Author contributions: Trikulanatham G, and Navaneethan U reviewed the literature and wrote the initial draft manuscript; Parsi MA supervised the process, revised and approved the manuscript for submission.
Correspondence to: Mansour A Parsi, MD, Digestive Disease Institute, Cleveland Clinic, 9500 Euclid Ave, Cleveland, OH 44195, United States. parsim@ccf.org
Telephone: +1-216-4454880 Fax: +1-216-4446305
Received: December 29, 2011 Revised: May 18, 2012
Accepted: May 26, 2012
Published online: January 14, 2013

Abstract

Endoscopy is widely accepted as the first treatment option in the management of bile duct stones. In this review we focus on the alternative endoscopic modalities for the management of difficult common bile duct stones. Most biliary stones can be removed with an extraction balloon, extraction basket or mechanical lithotripsy after endoscopic sphincterotomy. Endoscopic papillary balloon dilation with or without endoscopic sphincterotomy or mechanical lithotripsy has been shown to be effective for management of difficult to remove bile duct stones in selected patients. Ductal clearance can be safely achieved with peroral cholangioscopy guided laser or electrohydraulic lithotripsy in most cases where other endoscopic treatment modalities have failed. Biliary stenting may be an alternative treatment option for frail and elderly patients or those with serious co morbidities.

© 2013 Baishideng. All rights reserved.

Key words: Choledocholithiasis; Mechanical lithotripsy; Laser lithotripsy; Electrohydra-ulic lithotripsy; Difficult to remove biliary stones; Endoscopic large balloon papillary dilation; Peroral cholangioscopy

Trikudanathan G, Navaneethan U, Parsi MA. Endoscopic management of difficult common bile duct stones. *World J Gastroenterol* 2013; 19(2): 165-173 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i2/165.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i2.165

INTRODUCTION

Common bile duct (CBD) stones are seen in approximately 7%-12% of patients who undergo cholecystectomy for symptomatic cholelithiasis and are a common indication for referral to a biliary endoscopist[1]. They vary in size ranging from rather small (approximately 1-2 mm) to very large (> 3 cm). Endoscopic retrograde cholangiopancreatography (ERCP) with endoscopic sphincterotomy (ES) and basket or balloon extraction are well established therapeutic procedures for the management of CBD stones. It is estimated that nearly 85%-95% of all CBD stones can be managed effectively by these conventional endoscopic methods[2,3]. Failure to clear the bile duct renders the patient vulnerable to biliary obstruction, cholangitis and pancreatitis, thereby increasing the morbidity[4,5]. The occurrence of acute cholangitis is associated with significant mortality, especially in the elderly, underscoring the need for early intervention to clear the bile duct of stones and to relieve the obstruction to achieve adequate biliary drainage. Extraction of CBD stones is one of the most commonly performed procedures by therapeutic endoscopists. With novel advances in extraction techniques and instruments emerging routinely, it is vital to keep abreast of the new developments in order to improve the outcome. This review focuses on the alter-

native endoscopic management options for the treatment of difficult to remove CBD stones.

REVIEW CRITERIA

In July 2011, we searched MEDLINE from 1982 to the present using the Medical Subject Headings terms common bile duct stone, endoscopic retrograde cholangiopancreatography, difficult stone, endoscopy, and the key word "common bile duct stone". Full papers and abstracts in English language were considered. Important developments in research, reports from centers of excellence, and our own clinical experience in managing them, form the basis of this review article.

FACTORS ASSOCIATED WITH DIFFICULT TO TREAT BILE DUCT STONES

Multiple factors have been postulated to govern the success or failure of endoscopic extraction of CBD stones. In approximately 10%-15% of patients, managing biliary stones becomes formidable primarily due to difficulties in accessing the bile duct (periampullary diverticulum, sigmoid shaped CBD, post-gastrectomy Billroth type Ⅱ anatomy, Roux-en-Y-gastrojejunostomy), large number of stones (greater than 10), large size of stones (stones with a diameter > 15 mm which cannot be grasped with a basket), unusually shaped stones (barrel-shaped) or location of the stones (intra hepatic, cystic duct, proximal to strictures)[6]. In addition, endoscopic management becomes challenging in Mirizzi syndrome, in which stones in the cystic duct cause obstruction of the main bile duct[7]. Kim *et al*[8] prospectively evaluated the factors contributing to technical difficulty during endoscopic clearance of CBD stones. They reported that older age (> 65 years), previous gastrojejunostomy, larger stone size (≥ 15mm), impaction of stones, shorter length of the distal CBD arm (≤ 36mm), and more acute distal CBD angulation (≤ 135 degrees) are all contributors to technical difficulty for endoscopic removal of bile duct stones[8].

MANAGEMENT OF DIFFICULT STONES

In high risk patients, the risks and benefits of alternative techniques for removal of bile duct stones not amenable to conventional endoscopic techniques must be carefully balanced against each other and with surgery. The individual decision concerning the appropriate therapy is also influenced by the local expertise and the availability of the technical equipment.

CBD stones up to 1.5 cm in diameter can be extracted intact after endoscopic sphincterotomy. The rate of successful retrieval progressively declines with increasing size of the stone[9]. Larger stones especially those with a diameter ≥ 2cm may need fragmentation before removal to reduce the risk of stone impaction.

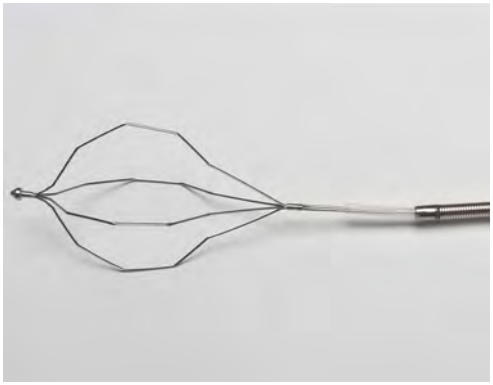


Figure 1 Example of a three-layered mechanical lithotripsy device with the basket, inner plastic sheath and an outer metal sheath. The stone is captured by the basket and crushed against the outer metal sheath.

Mechanical lithotripsy

In 1982, Riemann *et al*[10], first introduced mechanical lithotripsy (ML). ML is currently the most widely used technique for fragmentation of stones. Contemporary lithotripter baskets have a high breaking strengths and have improved the success rate of ML for extraction of large CBD stones (> 2 cm) to well over 90% without serious complications[11]. Broadly speaking, there are two types of baskets for ML. The type of basket used depends on whether lithotripsy is done on an elective ("through the scope") basis or on an emergent basis (salvage device) for basket impaction[12]. The 'through the scope' model is typically a three-layer system with the basket, inner plastic sheath, and an outer metal sheath (Figure 1). The stone is captured with the basket and the outer metal sheath is advanced to the stone which will be crushed against it. Sometimes, unexpectedly, stone and basket impaction can occur even during routine extraction of smaller stones. Under such circumstances, stone fragmentation can be done after removing the handle from the basket and the duodenoscope from the patient. An endotriptor (a spiral metal sheath) is introduced under fluoroscopic guidance, over the basket wires, and the stone is crushed after connecting the bare basket wires to the crank handle (Figure 2). The broken basket and the stone are then removed. The shaft of the endotriptor is generally shorter and thicker than that employed in standard ML[13]. Although basket impaction can occur with through the scope lithotripsy baskets; it is more commonly encountered with extraction baskets, which have thinner wires and weaker handles not suitable for fragmentation of stones.

In patients with multiple large stones, lithotripters with a sleeve system can be employed multiple times without withdrawal of the endoscope, facilitating stone fragmentation[14,15].

ML has been widely used as it is a readily available, cost effective, and simple procedure. Unfortunately the failure rate is high especially in patients with stones greater than 2.8 cm in diameter[15]. In a retrospective study the size of the stone was the only factor that significantly af-



Figure 2 Image of a mechanical lithotripter which can be used as a "salvage device" for removal of impacted baskets.

fected the success or failure of bile duct clearance. In this study of 162 patients, the cumulative probability of bile duct clearance ranged from > 90% for stones with a diameter less than 10 mm to 68% for those greater than 28 mm in diameter ($P < 0.02$)[15]. A subsequent prospective study by Garg *et al*[16] however reported that stone size alone may not be important unless considered together with the diameter of the bile duct. They concluded that the only important predictive factor that compromised the success of mechanical lithotripsy was stone impaction in the bile duct, with either an inability to pass the basket proximal to the stone or a failure of the basket to open fully around the stone to allow it to be grasped properly[16]. Although stone composition was not included in the study by Leung *et al*[12], some endoscopists believe that stones that are hard and densely calcified (visualized on a plain radiograph) resist mechanical fragmentation, resulting in an extraction failure with standard baskets. Although the stones which are molded to the shape of the bile duct may be softer, they are more difficult to crush because they may not be easily engaged by the lithotripter basket[12].

A multi-center study reported the rate of complications associated with ML to be around 3.6%[13]. Among the spectrum of complications, basket impaction or fracture of the basket wire are uniquely associated with ML. Non-surgical interventions that have been utilized in this setting include extension of sphincterotomy, awaiting spontaneous passage of the impacted basket and stone after successful stent placement, use of a second lithotripter, extracorporeal shock wave lithotripsy (ESWL), laser lithotripsy, electrohydraulic lithotripsy, and transhepatic lithotripsy and stone dislodgement[13-18]. Other complications include broken handle and perforation or injury to the bile duct[13-18].

In about 10% of the patients ML proves to be cumbersome, protracted and ineffective[6] wherein one has to resort to other methods such as electrohydraulic, or laser lithotripsy for stone fragmentation and subsequent removal.

Electrohydraulic lithotripsy

Initially used as an industrial tool for fragmenting rocks in mines, its application was extrapolated to medical use when Koch attempted fragmentation of biliary stones using this technology[19]. Electrohydraulic Lithotripsy (EHL) consists of a bipolar lithotripsy probe which discharges sparks with the aid of a charge generator in an aqueous medium. The sparks generated under water generate high-frequency hydraulic pressure waves, the energy of which is absorbed by nearby stones and results in their fragmentation[20]. The shock waves can cause inadvertent injury or perforation of the bile duct wall if the probe is not deployed close to the stone and away from the ductal wall. EHL can be performed under fluoroscopic guidance by using centering balloons or direct cholangioscopic vision[20]. The disadvantage of using only fluoroscopic guidance is related to the two dimensional imaging and the inability to confirm correct positioning of the probe. Therefore direct visualization is frequently preferred to avoid damage to the ductal wall[21]. A cholangioscope is inserted through the instrument channel of the mother scope. One or two dedicated biliary endoscopists are needed for this procedure. Continuous irrigation with water during the procedure generates a fluid medium for propagation of the shock waves and in addition offers a clear view of the stones by flushing away the debris[20].

The overall complication rate ranges from 7% to 9%[22,23], with most common complications being hemobilia, cholangitis, and less commonly, ductal perforation. Binmoeller *et al*[6], in one of the earlier large studies reported that EHL was successful in 63 of the 64 patients who had failed previous attempts of ML. Smaller published studies report stone fragmentation rates between 77%-100% for peroral EHL with minimal complications[22,24-28]. Arya *et al*[23], reported a stone fragmentation rate of 96% and final stone clearance of 90%. In a retrospective study of 94 patients who had failed stone extraction by conventional techniques, Hui *et al*[29], compared the outcomes of EHL with further endoscopy to stenting alone in a subset of elderly and infirm patients. They demonstrated that EHL and further ERCPs had a higher success rate (80%) with a low complication rate (7.7%) and recommended that elderly and frail patients should be referred to tertiary centers for EHL in order to prolong survival and decrease biliary complications[29].

The EHL equipment is compact, requires no special electricity, and is relatively inexpensive[30]. Other advantages are that the EHL procedure does not require special training or protective gear. In the United States, use of EHL for fragmentation and removal of biliary stones is quite common in centers with special interest in biliary disorders.

Laser lithotripsy

In laser lithotripsy (LL), laser light at a particular wavelength is focused on the surface of the stone to induce a wave-mediated fragmentation. The pulsed laser energy utilized in stone fragmentation is in contrast to the continuous laser energy used in tumor ablation[7]. The first successful use of pulsed laser for shock-wave lithotripsy of bile duct stones was reported in 1986[31]. Since then

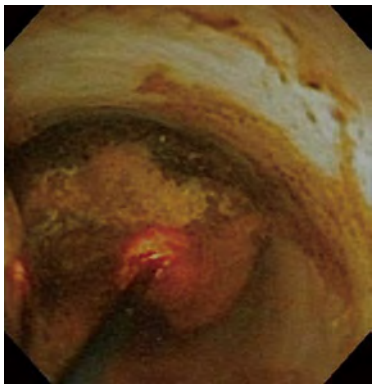


Figure 3 Laser lithotripsy of a bile duct stone under cholangioscopic guidance.

the technology has evolved and other laser types such as neodymium: yttrium-aluminum-garnet (Nd: YAG), flash lamp-pulsed dye (coumarin), the flash lamp-pulsed dye (rhodamine) with an automatic stone recognition system and the new Frequency Doubled Double Pulse Nd:YAG (FREDDY) system have been introduced[32-35]. LL is typically performed perorally under cholangioscopic or fluoroscopic guidance or by the transhepatic approach. As with EHL, LL under direct visualization using a cholangioscope is often preferred to avoid damage to the ductal wall (Figure 3).

Based on some reports, ductal clearance can be accomplished in 64% to 97% of patients by using[7,36] LL. In the majority of patients ductal clearance could be achieved in one session, although more sessions were required occasionally. LL has been demonstrated to be more effective than ESWL in terms of stone clearance rate and more rapid stone fragmentation with a shorter duration of treatment leading to a significant reduction in cost[37,38]. In some centers where the laser equipment is available, laser lithotripsy has gained popularity and has managed to replace EHL as the primary modality for fragmentation of difficult to remove stones.

A recent innovation worth mentioning is the introduction of a double-lumen basket which allows passage of a laser probe for effective laser lithotripsy after the stone is captured by the basket[39]. For a selected group of patients, this technique was shown to be feasible and effective, and the authors hope that continuous improvements in designs and construction materials would further enhance the success rate of this device.

Extracorporeal shockwave lithotripsy

In ESWL, high-pressure shock waves are generated outside the body (extracorporeal) by underwater spark gap (electrohydraulic) generated by piezoelectric crystals or electromagnetic membrane technology[30]. The shock waves are focused by elliptical transducers to the designated target through a liquid or tissue medium which prevents the energy attenuation. ESWL is performed under ultrasound or fluoroscopic guidance. Since most biliary stones are radiolucent and are not adequately visualized by fluoroscopy alone, placement of a nasobiliary tube for contrast instillation is required if ESWL is performed under fluoroscopy. In case of first generation lithotripters, the patients needed to be immersed in a water bath. Subsequent ESWL lithotripters employ water-filled compressible bags and a gel is applied to the skin surface for interface with the patient. Comparison between the lithotripters at a single center showed no significant difference in fragmentation of CBD stones[40]. General anesthesia is usually needed as the discomfort produced may not be adequately controlled by conscious sedation. The critical determining factor for success of single ESWL session is stone size and microcrystalline structure and architecture of the stone[41,42]. The presence or absence of bile duct stenosis can also influence the success of ESWL[43]. Sauerbruch *et al*[44], reported the efficacy of ESWL in achieving CBD stone fragmentation in over 90% of patients with minimal side effects.

In most institutions that already have access to ESWL for treatment of renal calculi, no other purchases of equipment needs to be made. For ESWL direct contact with the calculi is not needed, and multiple stones can be managed simultaneously[41]. ESWL can be of particular help in patients with abnormal anatomy such as those who have undergone Billroth-Ⅱ or Roux-en-Y surgeries in whom endoscopic access to the major papilla is difficult.

Although in general ESWL is tolerated well, it can be associated with adverse events such as transient biliary colic, subcutaneous ecchymosis, cardiac arrhythmia, self limited hemobilia, cholangitis, ileus and pancreatitis[7,45]. Perinephric hematoma, biliary obstruction, bowel perforation, lung injury and splenic rupture are among the rarely reported complications[42]. Multiple ESWL sessions may be required in a subset of patients to achieve ductal clearance, and endoscopic procedures between the ESWL sessions may become necessary to clear the bile duct of debris to assure drainage. The recurrence rate of CBD stones after ESWL clearance during a 1 to 2 year follow up was considerable and was around 14%[45,46]. In a randomized trial comparing fluoroscopic guided ESWL and LL, LL was preferable not only for successful stone free rate (73% *vs* 97%), but also in terms of the number of sessions needed to clear the duct (3 in ESWL *vs* 1.2 in LL) and the duration of treatment[30]. Another randomized trial comparing ultrasound guided ESWL and laser lithotripsy in the treatment of CBD stones refractory to conventional treatment clearly showed superior stone clearance rate and cost effectiveness in laser lithotripsy (52.4% *vs* 82%)[40]. However, a prospective trial comparing EHL *vs* ESWL showed no difference in success rates for clearing the CBD, duration and cost of hospitalization between the two modalities[24].

In the United States, ESWL is rarely performed for management of biliary stones and most centers prefer cholangioscopy-guided LL or EHL for this purpose.

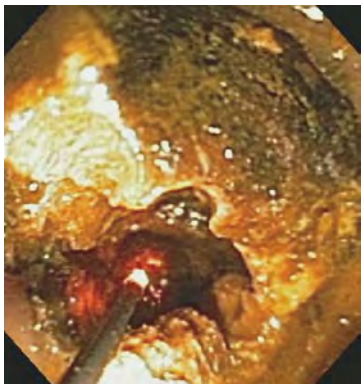


Figure 4 Direct peroral cholangioscopy guided laser lithotripsy of a bile duct stone. The red laser light makes targeting of the stone easier).

Cholangioscopy-guided lithotripsy

EHL or LL is ideally performed under direct visual control using a cholangioscope. The traditional cholangioscopy systems consist of a duodenoscope (also called "mother scope") and a dedicated cholangioscope (also called "baby scope" or "daughter scope") which is introduced into the bile duct through the accessory channel of the mother scope. The baby scope itself has an instrument channel through which the EHL or laser probe is introduced. Peroral cholangioscopy guided EHL or LL is cumbersome and can be labor intensive requiring an additional endoscopic unit and often participation of two skilled endoscopists, one to handle the duodenoscope and the other to maneuver the cholangioscope. The traditional cholangioscopes are capable of only 2-way steering, which may limit the field of view. These cholangioscopes are also extremely vulnerable to damage, requiring frequent expensive repairs. Furthermore, sharp angulations in the biliary tree may limit access into intrahepatic ducts or the cystic duct[47].

To negate these limitations, the Spyglass Direct Visualization System (Boston Scientific Corp, Natick, Mass) was designed for single operator examination of the bile ducts, 4-way steering, and dedicated irrigation channels[48,49]. In a large international multicenter study conducted at 10 centers in the United States and 5 centers in Europe, ductal clearance was successfully achieved in 71% of the patients using the Spyglass cholangioscopy system[50]. Despite its effectiveness, the spyglass system has been underutilized mainly due to its fiber optic image quality which is inferior to the video image quality offered by the new videocholangioscopes[51].

Direct peroral cholangioscopy

In direct peroral cholangioscopy (DPOC), an ultraslim upper endoscope is maneuvered across the biliary sphincter and into the bile duct for direct observation. With the introduction of high-definition ultraslim endoscopes with narrow band imaging capability, direct peroral cholangioscopy has gained popularity. This is mainly due to the many advantages of this technique. Compared to ductoscopy using a dedicated cholangioscope, direct cholangioscopy has several advantages. It offers a single operator platform, digital image quality and simultaneous irrigation and therapeutic capabilities. The most profound disadvantage of DPOC, however, is the difficulty associated with traversing the biliary sphincter to gain access to the bile duct. This is mainly due to looping of the ultraslim upper endoscope in the stomach or in the duodenum. To enhance the success rate of DPOC, specialized accessories or techniques are needed to advance the ultra slim endoscope into the proximal biliary system.

Larghi and Waxman[52] reported their experience in which the ultraslim upper endoscope was inserted with the aid of a guidewire placed during ERCP to maintain access. Additional use of manual pressure applied on the patient's abdomen has been shown to ease the passage of the ultraslim endoscope into the hilar area in some patients[53]. The main drawback encountered with passage of an ultraslim upper endoscope over a guidewire for gaining access to the bile duct is the dislodgement of the guidewire from the bile duct and also large loop formation hindering the entrance of the endoscope into the biliary system. Moon *et al*[54] demonstrated the ropeway technique using an intraductal balloon that can be anchored within an intrahepatic bile duct to advance an ultraslim upper endoscope into the biliary tree for performance of DPOC. However, withdrawal of the balloon may cause technical difficulties in maintaining access which underscores the need for other accessories to maintain the scope's position within the bile duct. The same group also reported use of an overtube balloon originally designed for double balloon enteroscopy to facilitate the introduction of an ultraslim upper endoscope into the biliary tree[55]. However the large inner diameter of the overtube (10.8 mm) compared to the outer diameter of the ultraslim upper endoscopes (5.2-6 mm), makes it difficult to manipulate both instruments and results in discomfort to the patient, looping of the endoscope in the duodenum, and difficulty in reaching the proximal bile duct[55,56].

In a recent study, we assessed utility of a novel anchoring balloon for performance of DPOC. Use of the anchoring balloon allowed consistent access to the biliary tree for performance of diagnostic and therapeutic DPOC distal to the confluence of the right and left hepatic ducts. More proximal access, however, was challenging owing to looping of the ultraslim endoscope after balloon removal.

Efforts are underway to develop the combined use of an intraductal anchoring balloon and an overtube especially designed for DPOC. Very recently, air embolism was reported following DPOC, resulting in a left sided hemiparesis[57]. Endoscopists must be conscious of the fact that air embolism could remain asymptomatic, as in regional embolism (portal venous gas) or manifest as hypoxia, shock, cardiac arrest or cerebral ischemia as noted in this case[57].

Once the hurdle of introducing an ultraslim upper endoscope into the bile duct has been overcome, a laser



Figure 5 Large diameter papillary balloon dilatation to remove bile duct stone.

or an EHL probe can easily be passed through the working channel of the ultraslim endoscope. Stones can be directly visualized before and during lithotripsy by DPOC (Figure 4). Lithotripsy is performed using this technique until the stones are satisfactorily fragmented to allow removal through the biliary sphincter. In a small study with 18 patients who had failed conventional endoscopic therapy including ML, DPOC guided EHL or LL was successful in approximately 90% of the patients with an average of 1.6 treatment sessions per patient[58].

Specialized ultraslim upper endoscopes are being designed to facilitate access to the biliary tree for DPOC[59]. Ultraslim upper endoscopes can be passed through the nasal cavity for performance of DPOC. Direct transnasal cholangioscopy has been reported for successful extraction of CBD stones[60].

Endoscopic papillary balloon dilatation

Endoscopic papillary balloon dilation (EPBD) was introduced as an alternative to endoscopic sphincterotomy for removal of bile duct stones in 1980's[61]. In an initial report involving 10 patients with CBD stones, biliary sphincteroplasty to 15 mm allowed removal of CBD stones in 6 patients[61]. In the other 4 patients, ductal clearance required a combination of biliary sphincterotomy and mechanical lithotripsy. The use of large diameter papillary balloon dilatation (up to 20 mm in diameter) for management of difficult to remove biliary stones was reported by Ersoz *et al*[62] in 2003. They reported a high success rate for stone removal. However, their complication rate was also high. Several reports have suggested that EPBD is associated with risk of severe pancreatitis which raises safety concern of this procedure[61,63]. In our institution, we use EPBD selectively and try to avoid its use in those with high risk of post ERCP pancreatitis (Figure 5).

Since those initial reports, multiple studies have shown that EPBD alone or in combination with other techniques can be of use for management of difficult to remove biliary stones[62-68]. EPBD is especially attractive in patients who are at risk for bleeding after endoscopic sphincterotomy or in those with altered anatomy in whom a full sphincterotomy cannot be successfully achieved.

Some authors have suggested that the stone recurrence rate may also be higher with EPBD than with endoscopic sphincterotomy and mechanical lithotripsy[69]. However the results of a Japanese multicentric trial with a mean follow up of 6.7 years demonstrated that there is lesser risk of stone recurrence following EPBD when compared with sphincterotomy[70]. Further, a recent meta-analysis which included 15 randomized trials comparing EPBD and endoscopic sphincterotomy showed reduced risk of bleeding and infections and is especially indicated in older patients, those who are at risk for infection and coagulopathy[71]. Despite its effectiveness, EPBD has been associated with serious complications[63]. A higher risk of post ERCP pancreatitis has been observed which has been attributed to the inadequately loosened sphincter of Oddi and the intra mucosal hemorrhage and inflammation/edema around the papilla. This may cause compression of the pancreatic duct and may accentuate the risk of pancreatitis[72]. In this regard, a randomized controlled trial demonstrated that a 5-min dilation time as opposed to the conventional 1-min time resulted in an adequately loosened sphincter of Oddi and consequently reduced the risk of post ERCP pancreatitis and improved its efficacy[73]. The rate of these complications can be reduced by strict patient selection, avoidance of forced procedures, optimal dilation duration and immediate conversion to an alternative procedure if any difficulty is encountered during EPBD.

Endoscopic biliary stenting

In very old patients and those with serious co-morbidities where other endoscopic or surgical procedures may confer unacceptably high risks, endoscopic biliary stenting is a useful alternative[69]. Biliary drainage by stenting is mandatory if ductal clearance cannot be achieved during ERCP or in between procedures in patients who require more than one session for ductal clearance. CBD stones have been reported to reduce in size in 60% of patients within one to two years after biliary stenting[69].

Mechanical irritation of the stent on the stone is postulated to be one of the mechanisms.

In a study involving 28 geriatric patients with CBD stones refractory to conventional endoscopic removal, endoscopic biliary stent placement combined with oral ursodeoxycholic acid and terpene therapy for a mean of six months led to significant reduction in the size of CBD stone[74]. Subsequently, endoscopic stone removal was successfully performed in 26 of 28 patients with a mean of 1.7 ERCP procedures. This combination therapy may be of use for treatment of difficult to remove CBD stones in a subset of patients with significant co-morbidities and intolerance to prolonged endoscopic treatment modalities.

In conclusion, the past several years have witnessed the emergence of new technologies and techniques for management of difficult to remove biliary stones. Treat-

ment of such stones is generally accomplished using a multimodal approach combining conventional techniques such as endoscopic sphincterotomy, use of extraction balloons and baskets and mechanical lithotripsy, with newer techniques such as cholangioscopy guided laser or electrohydraulic lithotripsy. Recent advances in the development of videocholangioscopes, single operator catheter-based cholangioscopes and specially-designed ultrathin upper endoscopes for DPOC have made lithotripsy under direct visual guidance safer, more reliable, and more routine. Future studies will certainly shed more light on the safety of different modalities for stone extraction and will help determine the best management approach for different subgroup of patients with difficult to remove bile duct stones.

REFERENCES

1 **Freitas ML**, Bell RL, Duffy AJ. Choledocholithiasis: evolving standards for diagnosis and management. *World J Gastroenterol* 2006; **12**: 3162-3167 [PMID: 16718834]
2 **Samardzic J**, Latic F, Kraljik D, Pitlovic V, Mrkovic H, Miskic D, Latic A, Delibegovic S. Treatment of common bile duct stones--is the role of ERCP changed in era of minimally invasive surgery? *Med Arh* 2010; **64**: 187-188 [PMID: 20645517]
3 **Strömberg C**, Nilsson M. Nationwide study of the treatment of common bile duct stones in Sweden between 1965 and 2009. *Br J Surg* 2011; **98**: 1766-1774 [PMID: 21935910 DOI: 10.1002/bjs.7690]
4 **Cairns SR**, Dias L, Cotton PB, Salmon PR, Russell RC. Additional endoscopic procedures instead of urgent surgery for retained common bile duct stones. *Gut* 1989; **30**: 535-540 [PMID: 2714686 DOI: 10.1136/gut.30.4.535]
5 **Parsi MA**, Stevens T, Dumot JA, Zuccaro G. Endoscopic therapy of recurrent acute pancreatitis. *Cleve Clin J Med* 2009; **76**: 225-233 [PMID: 19339638 DOI: 10.3949/ccjm.76a.08017]
6 **Binmoeller KF**, Brückner M, Thonke F, Soehendra N. Treatment of difficult bile duct stones using mechanical, electrohydraulic and extracorporeal shock wave lithotripsy. *Endoscopy* 1993; **25**: 201-206 [PMID: 8519238 DOI: 10.1055/s-2007-1010293]
7 **McHenry L**, Lehman G. Difficult bile duct stones. *Curr Treat Options Gastroenterol* 2006; **9**: 123-132 [PMID: 16539873 DOI: 10.1007/s11938-006-0031-6]
8 **Kim HJ**, Choi HS, Park JH, Park DI, Cho YK, Sohn CI, Jeon WK, Kim BI, Choi SH. Factors influencing the technical difficulty of endoscopic clearance of bile duct stones. *Gastrointest Endosc* 2007; **66**: 1154-1160 [PMID: 17945223 DOI: 10.1016/j.gie.2007.04.033]
9 **Lauri A**, Horton RC, Davidson BR, Burroughs AK, Dooley JS. Endoscopic extraction of bile duct stones: management related to stone size. *Gut* 1993; **34**: 1718-1721 [PMID: 8282260 DOI: 10.1136/gut.34.12.1718]
10 **Riemann JF**, Seuberth K, Demling L. Clinical application of a new mechanical lithotripter for smashing common bile duct stones. *Endoscopy* 1982; **14**: 226-230 [PMID: 7140657 DOI: 10.1055/s-2007-1021626]
11 **Schneider MU**, Matek W, Bauer R, Domschke W. Mechanical lithotripsy of bile duct stones in 209 patients--effect of technical advances. *Endoscopy* 1988; **20**: 248-253 [PMID: 3168938 DOI: 10.1055/s-2007-1018186]
12 **Leung JW**, Tu R. Mechanical lithotripsy for large bile duct stones. *Gastrointest Endosc* 2004; **59**: 688-690 [PMID: 15114312 DOI: 10.1016/S0016-5107(04)00174-9]
13 **Thomas M**, Howell DA, Carr-Locke D, Mel Wilcox C, Chak A, Raijman I, Watkins JL, Schmalz MJ, Geenen JE, Catalano MF. Mechanical lithotripsy of pancreatic and biliary stones: complications and available treatment options collected from expert centers. *Am J Gastroenterol* 2007; **102**: 1896-1902 [PMID: 17573790 DOI: 10.1111/j.1572-0241.2007.01350.]
14 **Chung SC**, Leung JW, Leong HT, Li AK. Mechanical lithotripsy of large common bile duct stones using a basket. *Br J Surg* 1991; **78**: 1448-1450 [PMID: 1773322 DOI: 10.1002/bjs.1800781214]
15 **Cipolletta L**, Costamagna G, Bianco MA, Rotondano G, Piscopo R, Mutignani M, Marmo R. Endoscopic mechanical lithotripsy of difficult common bile duct stones. *Br J Surg* 1997; **84**: 1407-1409 [PMID: 9361600 DOI: 10.1002/bjs.1800841019]
16 **Garg PK**, Tandon RK, Ahuja V, Makharia GK, Batra Y. Predictors of unsuccessful mechanical lithotripsy and endoscopic clearance of large bile duct stones. *Gastrointest Endosc* 2004; **59**: 601-605 [PMID: 15114300 DOI: 10.1016/S0016-5107(04)00295-0]
17 **Shim CS**. How Should Biliary Stones be Managed? *Gut Liver* 2010; **4**: 161-172 [PMID: 20559517]
18 **Shim CS**. How Should Biliary Stones be Managed? *Gut Liver* 2010; **4**: 161-172 [PMID: 20559517 DOI: 10.5009/gnl.2010.4.2.161]
19 **Koch H**, Stolte M, Walz V. Endoscopic lithotripsy in the common bile duct. *Endoscopy* 1977; **9**: 95-98 [PMID: 891486 DOI: 10.1055/s-0028-1098497]
20 **Seitz U**, Bapaye A, Bohnacker S, Navarrete C, Maydeo A, Soehendra N. Advances in therapeutic endoscopic treatment of common bile duct stones. *World J Surg* 1998; **22**: 1133-1144 [PMID: 9828721 DOI: 10.1007/s002689900532]
21 **Yoo KS**, Lehman GA. Endoscopic management of biliary ductal stones. *Gastroenterol Clin North Am* 2010; **39**: 209-27, viii [PMID: 20478483 DOI: 10.1016/j.gtc.2010.02.008]
22 **Blind PJ**, Lundmark M. Management of bile duct stones: lithotripsy by laser, electrohydraulic, and ultrasonic techniques. Report of a series and clinical review. *Eur J Surg* 1998; **164**: 403-409 [PMID: 9696440]
23 **Arya N**, Nelles SE, Haber GB, Kim YI, Kortan PK. Electrohydraulic lithotripsy in 111 patients: a safe and effective therapy for difficult bile duct stones. *Am J Gastroenterol* 2004; **99**: 2330-2334 [PMID: 15571578 DOI: 10.1111/j.1572-0241.2004.40251.]
24 **Adamek HE**, Buttmann A, Wessbecher R, Kohler B, Riemann JF. Clinical comparison of extracorporeal piezoelectric lithotripsy (EPL) and intracorporeal electrohydraulic lithotripsy (EHL) in difficult bile duct stones. A prospective randomized trial. *Dig Dis Sci* 1995; **40**: 1185-1192 [PMID: 7781432 DOI: 10.1007/BF02065522]
25 **Adamek HE**, Maier M, Jakobs R, Wessbecher FR, Neuhauser T, Riemann JF. Management of retained bile duct stones: a prospective open trial comparing extracorporeal and intracorporeal lithotripsy. *Gastrointest Endosc* 1996; **44**: 40-47 [PMID: 8836715]
26 **Leung JW**, Chung SS. Electrohydraulic lithotripsy with peroral choledochoscopy. *BMJ* 1989; **299**: 595-598 [PMID: 2508816 DOI: 10.1136/bmj.299.6699.595]
27 **Yasuda K**, Nakajima M, Cho E, Mukai H, Kawai K. Comparison of peroral and percutaneous cholangioscopy. *Endoscopy* 1989; **21** Suppl 1: 347-350 [PMID: 2606084 DOI: 10.1055/s-2007-1012988]
28 **Moon JH**, Cha SW, Ryu CB, Kim YS, Hong SJ, Cheon YK, Cho YD, Kim YS, Lee JS, Lee MS, Shim CS, Kim BS. Endoscopic treatment of retained bile-duct stones by using a balloon catheter for electrohydraulic lithotripsy without cholangioscopy. *Gastrointest Endosc* 2004; **60**: 562-566 [PMID: 15472679 DOI: 10.1016/S0016-5107(04)02012-7]
29 **Hui CK**, Lai KC, Ng M, Wong WM, Yuen MF, Lam SK, Lai CL, Wong BC. Retained common bile duct stones: a comparison between biliary stenting and complete clearance of stones by electrohydraulic lithotripsy. *Aliment Pharmacol Ther* 2003; **17**: 289-296 [PMID: 12534415 DOI: 10.1046/

j.1365-2036.2003.01415.]
30 **DiSario J**, Chuttani R, Croffie J, Liu J, Mishkin D, Shah R, Somogyi L, Tierney W, Song LM, Petersen BT. Biliary and pancreatic lithotripsy devices. *Gastrointest Endosc* 2007; **65**: 750-756 [PMID: 17383651 DOI: 10.1016/j.gie.2006.10.002]
31 **Lux G**, Ell C, Hochberger J, Müller D, Demling L. The first successful endoscopic retrograde laser lithotripsy of common bile duct stones in man using a pulsed neodymium-YAG laser. *Endoscopy* 1986; **18**: 144-145 [PMID: 2874019 DOI: 10.1055/s-2007-1018356]
32 **Ponchon T**, Gagnon P, Valette PJ, Henry L, Chavaillon A, Thieulin F. Pulsed dye laser lithotripsy of bile duct stones. *Gastroenterology* 1991; **100**: 1730-1736 [PMID: 1673442]
33 **Ell C**, Hochberger J, May A, Fleig WE, Bauer R, Mendez L, Hahn EG. Laser lithotripsy of difficult bile duct stones by means of a rhodamine-6G laser and an integrated automatic stone-tissue detection system. *Gastrointest Endosc* 1993; **39**: 755-762 [PMID: 8293896 DOI: 10.1016/S0016-5107(93)70259-X]
34 **Kim TH**, Oh HJ, Choi CS, Yeom DH, Choi SC. Clinical usefulness of transpapillary removal of common bile duct stones by frequency doubled double pulse Nd: YAG laser. *World J Gastroenterol* 2008; **14**: 2863-2866 [PMID: 18473411 DOI: 10.3748/wjg.14.2863]
35 **Hochberger J**, Bayer J, Maiss J, Tex S, Hahn EG. [Clinical results with a new frequency-doubled, double pulse Nd: YAG laser (FREDDY) for lithotripsy in complicated choledocholithiasis]. *Biomed Tech (Berl)* 1998; **43** Suppl: 172 [PMID: 9859311 DOI: 10.1515/bmte.1998.43.s1.172]
36 **Hochberger J**, Bayer J, May A, Mühldorfer S, Maiss J, Hahn EG, Ell C. Laser lithotripsy of difficult bile duct stones: results in 60 patients using a rhodamine 6G dye laser with optical stone tissue detection system. *Gut* 1998; **43**: 823-829 [PMID: 9824611 DOI: 10.1136/gut.43.6.823]
37 **Neuhaus H**, Zillinger C, Born P, Ott R, Allescher H, Rösch T, Classen M. Randomized study of intracorporeal laser lithotripsy versus extracorporeal shock-wave lithotripsy for difficult bile duct stones. *Gastrointest Endosc* 1998; **47**: 327-334 [PMID: 9609422 DOI: 10.1016/S0016-5107(98)70214-7]
38 **Jakobs R**, Adamek HE, Maier M, Krömer M, Benz C, Martin WR, Riemann JF. Fluoroscopically guided laser lithotripsy versus extracorporeal shock wave lithotripsy for retained bile duct stones: a prospective randomised study. *Gut* 1997; **40**: 678-682 [PMID: 9203950]
39 **Lee JE**, Moon JH, Choi HJ, Song AR, Jung EK, Cheon YK, Cho YD, Lee JS, Lee MS. Endoscopic treatment of difficult bile duct stones by using a double-lumen basket for laser lithotripsy--a case series. *Endoscopy* 2010; **42**: 169-172 [PMID: 19998219 DOI: 10.1055/s-0029-1215353]
40 **den Toom R**, Nijs HG, van Blankenstein M, Laméris JS, Schröder FH, Terpstra OT. Extracorporeal shock wave treatment of common bile duct stones: experience with two different lithotriptors at a single institution. *Br J Surg* 1991; **78**: 809-813 [PMID: 1873707 DOI: 10.1002/bjs.1800780714]
41 **White DM**, Correa RJ, Gibbons RP, Ball TJ, Kozarek RJ, Thirlby RC. Extracorporeal shock-wave lithotripsy for bile duct calculi. *Am J Surg* 1998; **175**: 10-13 [PMID: 9445230 DOI: 10.1016/S0002-9610(97)00234-1]
42 **Tandan M**, Reddy DN, Santosh D, Reddy V, Koppuju V, Lakhtakia S, Gupta R, Ramchandani M, Rao GV. Extracorporeal shock wave lithotripsy of large difficult common bile duct stones: efficacy and analysis of factors that favor stone fragmentation. *J Gastroenterol Hepatol* 2009; **24**: 1370-1374 [PMID: 19702905 DOI: 10.1111/j.1440-1746.2009.05919.x]
43 **Amplatz S**, Piazzi L, Felder M, Comberlato M, Benvenuti S, Zancanella L, Di Fede F, de'Guelmi A, Bertozzo A, Farris P, Grasso T, Mega A, Chilovi F. Extracorporeal shock wave lithotripsy for clearance of refractory bile duct stones. *Dig Liver Dis* 2007; **39**: 267-272 [PMID: 17275426 DOI: 10.1016/j.dld.2006.11.003]
44 **Sauerbruch T**, Stern M. Fragmentation of bile duct stones by extracorporeal shock waves. A new approach to biliary calculi after failure of routine endoscopic measures. *Gastroenterology* 1989; **96**: 146-152 [PMID: 2642439 DOI: 10.1111/j.1572-0241.2004.30151.x]
45 **Parsi MA**, Stevens T, Lopez R, Vargo JJ. Extracorporeal shock wave lithotripsy for prevention of recurrent pancreatitis caused by obstructive pancreatic stones. *Pancreas* 2010; **39**: 153-155 [PMID: 19820418 DOI: 10.1097/MPA.0b013e3181bb1733]
46 **Conigliaro R**, Camellini L, Zuliani CG, Sassatelli R, Mortilla MG, Bertoni G, Formisano D, Bedogni G. Clearance of irretrievable bile duct and pancreatic duct stones by extracorporeal shockwave lithotripsy, using a transportable device: effectiveness and medium-term results. *J Clin Gastroenterol* 2006; **40**: 213-219 [PMID: 16633122 DOI: 10.1097/00004836-200603000-00008]
47 **Chathadi KV**, Chen YK. New kid on the block: development of a partially disposable system for cholangioscopy. *Gastrointest Endosc Clin N Am* 2009; **19**: 545-555 [PMID: 19917460 DOI: 10.1016/j.giec.2009.06.001]
48 **Chen YK**. Preclinical characterization of the Spyglass peroral cholangiopancreatoscopy system for direct access, visualization, and biopsy. *Gastrointest Endosc* 2007; **65**: 303-311 [PMID: 17258991 DOI: 10.1016/j.gie.2006.07.048]
49 **Chen YK**, Pleskow DK. SpyGlass single-operator peroral cholangiopancreatoscopy system for the diagnosis and therapy of bile-duct disorders: a clinical feasibility study (with video). *Gastrointest Endosc* 2007; **65**: 832-841 [PMID: 17466202 DOI: 10.1016/j.gie.2007.01.025]
50 **Chen YK**, Parsi MA, Binmoeller KF, Hawes RH, Pleskow DK, Slivka A, Haluszka O, Petersen BT, Sherman S, Devière J, Meisner S, Stevens PD, Costamagna G, Ponchon T, Peetermans JA, Neuhaus H. Single-operator cholangioscopy in patients requiring evaluation of bile duct disease or therapy of biliary stones (with videos). *Gastrointest Endosc* 2011; **74**: 805-814 [PMID: 21762903 DOI: 10.1016/j.gie.2011.04.016]
51 **Parsi MA**. Peroral cholangioscopy in the new millennium. *World J Gastroenterol* 2011; **17**: 1-6 [PMID: 21218076 DOI: 10.3748/wjg.v17.i1.1]
52 **Larghi A**, Waxman I. Endoscopic direct cholangioscopy by using an ultra-slim upper endoscope: a feasibility study. *Gastrointest Endosc* 2006; **63**: 853-857 [PMID: 16650553 DOI: 10.1016/j.gie.2005.07.050]
53 **Park do H**, Park BW, Lee HS, Park SH, Park JH, Lee SH, Kim HS, Kim SJ. Peroral direct cholangioscopic argon plasma coagulation by using an ultraslim upper endoscope for recurrent hepatoma with intraductal nodular tumor growth (with videos). *Gastrointest Endosc* 2007; **66**: 201-203 [PMID: 17531236 DOI: 10.1016/j.gie.2006.11.037]
54 **Moon JH**, Ko BM, Choi HJ, Hong SJ, Cheon YK, Cho YD, Lee JS, Lee MS, Shim CS. Intraductal balloon-guided direct peroral cholangioscopy with an ultraslim upper endoscope (with videos). *Gastrointest Endosc* 2009; **70**: 297-302 [PMID: 19394010 DOI: 10.1016/j.gie.2008.11.019]
55 **Choi HJ**, Moon JH, Ko BM, Hong SJ, Koo HC, Cheon YK, Cho YD, Lee JS, Lee MS, Shim CS. Overtube-balloon-assisted direct peroral cholangioscopy by using an ultra-slim upper endoscope (with videos). *Gastrointest Endosc* 2009; **69**: 935-940 [PMID: 19327480 DOI: 10.1016/j.gie.2008.08.043]
56 **Tsou YK**, Lin CH, Tang JH, Liu NJ, Cheng CL. Direct peroral cholangioscopy using an ultraslim endoscope and overtube balloon-assisted technique: a case series. *Endoscopy* 2010; **42**: 681-684 [PMID: 20669079 DOI: 10.1055/s-0030-1255616]
57 **Efthymiou M**, Raftopoulos S, Antonio Chirinos J, May GR. Air embolism complicated by left hemiparesis after direct cholangioscopy with an intraductal balloon anchoring system. *Gastrointest Endosc* 2012; **75**: 221-223 [PMID: 21470606 DOI: 10.1016/j.gie.2011.01.038]

58 **Moon JH**, Ko BM, Choi HJ, Koo HC, Hong SJ, Cheon YK, Cho YD, Lee MS, Shim CS. Direct peroral cholangioscopy using an ultra-slim upper endoscope for the treatment of retained bile duct stones. *Am J Gastroenterol* 2009; **104**: 2729-2733 [PMID: 19623165 DOI: 10.1038/ajg.2009.435]

59 **Itoi T**, Moon JH, Waxman I. Current status of direct peroral cholangioscopy. *Dig Endosc* 2011; **23** Suppl 1: 154-157 [PMID: 21535223 DOI: 10.1111/j.1443-1661.2011.01114.x]

60 **Seo MS**, Moon JH, Choi HJ, Kim HK, Cheon YK, Cho YD, Lee MS. Bile-Duct Stone Removal under Direct Transnasal Cholangioscopy Using an Ultraslim Upper Endoscope. *Gut Liver* 2010; **4**: 428-429 [PMID: 20981228 DOI: 10.5009/gnl.2010.4.3.428]

61 **Staritz M**, Ewe K, Meyer zum Büschenfelde KH. Endoscopic papillary dilatation, a possible alternative to endoscopic papillotomy. *Lancet* 1982; **1**: 1306-1307 [PMID: 6123047 DOI: 10.1016/S0140-6736(82)92873-2]

62 **Ersoz G**, Tekesin O, Ozutemiz AO, Gunsar F. Biliary sphincterotomy plus dilation with a large balloon for bile duct stones that are difficult to extract. *Gastrointest Endosc* 2003; **57**: 156-159 [PMID: 12556775 DOI: 10.1067/mge.2003.52]

63 **Disario JA**, Freeman ML, Bjorkman DJ, Macmathuna P, Petersen BT, Jaffe PE, Morales TG, Hixson LJ, Sherman S, Lehman GA, Jamal MM, Al-Kawas FH, Khandelwal M, Moore JP, Derfus GA, Jamidar PA, Ramirez FC, Ryan ME, Woods KL, Carr-Locke DL, Alder SC. Endoscopic balloon dilation compared with sphincterotomy for extraction of bile duct stones. *Gastroenterology* 2004; **127**: 1291-1299 [PMID: 15520997 DOI: 10.1053/j.gastro.2004.07.017]

64 **Itoi T**, Itokawa F, Sofuni A, Kurihara T, Tsuchiya T, Ishii K, Tsuji S, Ikeuchi N, Moriyasu F. Endoscopic sphincterotomy combined with large balloon dilation can reduce the procedure time and fluoroscopy time for removal of large bile duct stones. *Am J Gastroenterol* 2009; **104**: 560-565 [PMID: 19174779 DOI: 10.1038/ajg.2008.67]

65 **Stefanidis G**, Viazis N, Pleskow D, Manolakopoulos S, Theocharis L, Christodoulou C, Kotsikoros N, Giannousis J, Sgouros S, Rodias M, Katsikani A, Chuttani R. Large balloon dilation vs. mechanical lithotripsy for the management of large bile duct stones: a prospective randomized study. *Am J Gastroenterol* 2011; **106**: 278-285 [PMID: 21045816 DOI: 10.1038/ajg.2010.421]

66 **Heo JH**, Kang DH, Jung HJ, Kwon DS, An JK, Kim BS, Suh KD, Lee SY, Lee JH, Kim GH, Kim TO, Heo J, Song GA, Cho M. Endoscopic sphincterotomy plus large-balloon dilation versus endoscopic sphincterotomy for removal of bile-duct stones. *Gastrointest Endosc* 2007; **66**: 720-76; quiz 768, 771 [PMID: 17905013 DOI: 10.1016/j.gie.2007.02.033]

67 **Attasaranya S**, Cheon YK, Vittal H, Howell DA, Wakelin DE, Cunningham JT, Ajmere N, Ste Marie RW, Bhattacharya K, Gupta K, Freeman ML, Sherman S, McHenry L, Watkins JL, Fogel EL, Schmidt S, Lehman GA. Large-diameter biliary orifice balloon dilation to aid in endoscopic bile duct stone removal: a multicenter series. *Gastrointest Endosc* 2008; **67**: 1046-1052 [PMID: 18178208 DOI: 10.1016/j.gie.2007.08.047]

68 **May GR**, Cotton PB, Edmunds SE, Chong W. Removal of stones from the bile duct at ERCP without sphincterotomy. *Gastrointest Endosc* 1993; **39**: 749-754 [PMID: 8293895 DOI: 10.1111/j.1572-0241.2004.30151.x]

69 **Lee DK**, Jahng JH. Alternative methods in the endoscopic management of difficult common bile duct stones. *Dig Endosc* 2010; **22** Suppl 1: S79-S84 [PMID: 20590778 DOI: 10.1111/j.1443-1661.2010.00960.x]

70 **Yasuda I**, Fujita N, Maguchi H, Hasebe O, Igarashi Y, Murakami A, Mukai H, Fujii T, Yamao K, Maeshiro K, Tada T, Tsujino T, Komatsu Y. Long-term outcomes after endoscopic sphincterotomy versus endoscopic papillary balloon dilation for bile duct stones. *Gastrointest Endosc* 2010; **72**: 1185-1191 [PMID: 20869711]

71 **Weinberg BM**, Shindy W, Lo S. Endoscopic balloon sphincter dilation (sphincteroplasty) versus sphincterotomy for common bile duct stones. *Cochrane Database Syst Rev* 2006; (**4**): CD004890 [PMID: 17054222 DOI: 10.1002/14651858.CD004890.pub2]

72 **Baron TH**, Harewood GC. Endoscopic balloon dilation of the biliary sphincter compared to endoscopic biliary sphincterotomy for removal of common bile duct stones during ERCP: a metaanalysis of randomized, controlled trials. *Am J Gastroenterol* 2004; **99**: 1455-1460 [PMID: 15307859]

73 **Liao WC**, Lee CT, Chang CY, Leung JW, Chen JH, Tsai MC, Lin JT, Wu MS, Wang HP. Randomized trial of 1-minute versus 5-minute endoscopic balloon dilation for extraction of bile duct stones. *Gastrointest Endosc* 2010; **72**: 1154-1162 [PMID: 20869710 DOI: 10.1016/j.gie.2010.07.009]

74 **Han J**, Moon JH, Koo HC, Kang JH, Choi JH, Jeong S, Lee DH, Lee MS, Kim HG. Effect of biliary stenting combined with ursodeoxycholic acid and terpene treatment on retained common bile duct stones in elderly patients: a multicenter study. *Am J Gastroenterol* 2009; **104**: 2418-2421 [PMID: 19568225 DOI: 10.1038/ajg.2009.303]

P- Reviewer Al Mofleh IA **S- Editor** Gou SX
L- Editor A **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i2.174

World J Gastroenterol 2013 January 14; 19(2): 174-184
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

GUIDELINES FOR BASIC SCIENCE

Prognostic value of innate and adaptive immunity in colorectal cancer

Fabio Grizzi, Paolo Bianchi, Alberto Malesci, Luigi Laghi

Fabio Grizzi, Paolo Bianchi, Luigi Laghi, Laboratory of Molecular Gastroenterology, Humanitas Clinical and Research Center, 20089 Milan, Italy
Alberto Malesci, Division of Gastroenterology, Humanitas Clinical and Research Center, 20089 Milan, Italy
Alberto Malesci, Department of Translational Medicine, University of Milan, 20089 Milan, Italy
Author contributions: Grizzi F conceived and wrote this manuscript; Bianchi P, Malesci A and Laghi L revised and editing this manuscript.
Supported by Ministry of Education, University and Research (MIUR), Target Project Oncologia 2006; Alliance Against Cancer; and the Italian Association for Cancer Research Grant Project, No. IG5256
Correspondence to: Fabio Grizzi, PhD, Laboratory of Molecular Gastroenterology, Humanitas Clinical and Research Center, Via Manzoni 56, Rozzano, 20089 Milan, Italy. fabio.grizzi@humanitasresearch.it
Telephone: +39-2-82245161 Fax: +39-2-82244590
Received: April 4, 2012 Revised: July 12, 2012
Accepted: July 18, 2012
Published online: January 14, 2013

Abstract

Colorectal cancer (CRC) remains one of the major public health problems throughout the world. Originally depicted as a multi-step dynamical disease, CRC develops slowly over several years and progresses through cytologically distinct benign and malignant states, from single crypt lesions through adenoma, to malignant carcinoma with the potential for invasion and metastasis. Moving from histological observations since a long time, it has been recognized that inflammation and immunity actively participate in the pathogenesis, surveillance and progression of CRC. The advent of immunohistochemical techniques and of animal models has improved our understanding of the immune dynamical system in CRC. It is well known that immune cells have variable behavior controlled by complex interactions in the tumor microenvironment. Advances in immunology and molecular biology have shown that CRC is immunogenic and that host immune responses influence survival. Several lines of evidence support the concept that tumor stromal cells, are not merely a scaffold, but rather they influence growth, survival, and invasiveness of cancer cells, dynamically contributing to the tumor microenvironment, together with immune cells. Different types of immune cells infiltrate CRC, comprising cells of both the innate and adaptive immune system. A relevant issue is to unravel the discrepancy between the inhibitory effects on cancer growth exerted by the local immune response and the promoting effects on cancer proliferation, invasion, and dissemination induced by some types of inflammatory cells. Here, we sought to discuss the role played by innate and adaptive immune system in the local progression and metastasis of CRC, and the prognostic information that we can currently understand and exploit.

© 2013 Baishideng. All rights reserved.

Key words: Colorectal cancer; Immunity; Inflammation; Prognosis; Metastasis

Grizzi F, Bianchi P, Malesci A, Laghi L. Prognostic value of innate and adaptive immunity in colorectal cancer. *World J Gastroenterol* 2013; 19(2): 174-184 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i2/174.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i2.174

INTRODUCTION

Despite progresses in our biological and clinical knowledge, colorectal cancer (CRC) remains one of the major public health problems throughout the world[1]. By its frequency, CRC ranks third in men and women worldwide[1]. In addition, CRC continues to be one of the most common fatal types of cancer. Originally depicted as a multi-step dynamical disease, CRC develops slowly over several years and progresses through cytologically distinct benign and malignant states, from single crypt lesions through

adenoma, to malignant carcinoma with the potential for invasion and metastasis[2,3]. According to the theory of multi-step carcinogenesis, colorectal epithelial cells accumulate a number of molecular changes to eventually become fully malignant[4,5]. In spite of unifying theories, genetic and epigenetic events during the carcinogenesis process differ considerably from tumor to tumor. Thus, CRC is not a single disease; rather it encompasses different molecular and pathological entities with a wide range of clinical behaviors[6]. At the molecular level, CRC is the tip of an iceberg the basis of which encloses a complex array of gene alterations, affecting supra-molecular processes. Essentially, like individual fingerprints, each tumor arises and behaves in a unique fashion that is unlikely to be exactly recapitulated by any other tumor. Nevertheless, molecular changes allows for a categorization of CRC, which is largely accepted, although likely to oversimplistic. It has been demonstrated that main genetic and epigenetic features, such as microsatellite instability (MSI), chromosomal instability, CpG island methylator phenotype (CIMP) or global DNA hypomethylation, lead to alterations of gene function on a genome-wide scale. It is known that activation of oncogenes, including *KRAS*, *BRAF*, *PIK3CA* and *TP53*, affects intracellular signaling pathways[6,7]. The suppressor pathway is disrupted in CRC with chromosomal instability occurring in the majority of CRCs (approximately 85%), which have a molecular profile characterized by specific chromosomal amplifications and transformations, aneuploidy, and loss of heterozygosity[6-8]. Differently, CRCs of the mutator pathway (approximately 15%) have a defective DNA mismatch repair system, which leads to accumulation of thousands of unrepaired mutations[8]. This inability to repair DNA slippage errors and mismatches can easily be demonstrated because it results in variability in the length of DNA microsatellites, formed by repetitive sequences, that is MSI. It is accepted that MSI CRCs have a heterogeneous histological appearance, better prognosis due to a reduced metastatic potential, and a different response to chemotherapy[9-14].

Histopathological examination reveals that likely other solid tumors, CRC are associated with diverse immune cell infiltrates[15-20], and that in the cancer context, epithelial cells coexist with extracellular matrix components and non-neoplastic cell types, including fibroblasts, myofibroblasts, adipocytes, endothelial cells, pericytes, which collectively form the tumor stroma. Several lines of evidence support the concept that tumor stromal cells, are not merely a scaffold, but rather they influence growth, survival, and invasiveness of cancer cells, dynamically contributing to the tumor microenvironment, together with immune cells[20-25]. The types of immune system cells that are found infiltrating CRC consist of cells of the innate immune system i.e., macrophages, neutrophils, mast cells and natural killer cells, as well as cells associated with an adaptive immune response i.e., T and B lymphocytes. Although it is commonly thought that an immune response localized to the tumor inhibit cancer growth, it is clear that some types of tumor-associated inflammation may also exert an opposite action, at least at some point of CRC natural history[26] (Figure 1). Here we sought to briefly review these two contradictory aspects of the immune response to CRC.

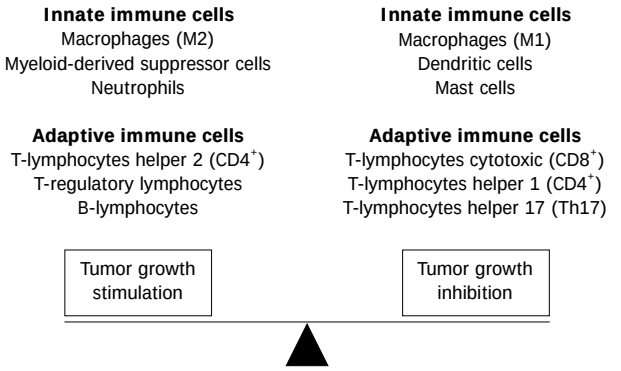


Figure 1 Immune cells have variable behavior controlled by complex interactions in the tumor microenvironment. Although it is commonly thought that an immune response localized to the tumor inhibit cancer growth, it is now clear that some types of tumor-associated inflammation can exert an opposite action.

INNATE IMMUNITY AND COLORECTAL CANCER

It is well known that innate immunity represents the body's first defense or "gut reaction" to an abnormal situation, such as cancer, and does not involve specific recognition of immunogenic peptides, or antigens[20]. Aside T and B lymphocytes , innate immune cells orchestrate an inflammatory environment that may function to either stimulate or inhibit cancer growth[20]. Various innate immune cells have been implicated in CRC development and progression[15,27,28]. Among these, macrophages, are a primary source of secreted pro-inflammatory cytokines, and are generally distinguished as type 1 (M1) or type 2 (M2)[29-31]. M1s generally have an interleukin (IL) 12^{low}IL-10^{high} phenotype, show impaired expression of reactive nitrogen intermediates, poor antigen presentation and have tumoricidal capacity, while show high expression of angiogenic factors [including Vascular-endothelial growth factor (VEGF) epidermal-growth factor (EGF) and semaphorin 4D], metalloproteases (MMPs) and cathepsins as well as of the growth arrest-specific protein 6 (GAS6)[29-32]. Additionally, M1s can support T-helper 1 (Th1) adaptive immunity[33]. Conversely, M2s secrete immunosuppressive cytokines and promote tumor growth[22,34]. It has been shown that cancer cells shape their interaction with macrophages by escaping phagocytosis and by promoting a M2-like polarization throughout chemokines and polarizing cytokines including chemokine (C-C motif) ligand 2 (CCL2), colony stimulating factor 1 (CSF1), macrophage slowing factor (MSF), tumor necrosis factor-alpha (TNF-α, IL-10 and transforming growth factor-β (TGF-β).

Among the cells with M2 phenotype, the tumor-associated macrophages (TAM) have been shown as capable of secreting proteases that enhance invasion and metastases, together with a range of cytokines inhibiting an adaptive tumor-specific immune response, and angiogenic factors that increase neovascularity. In patients with CRC, macrophages are usually found located around necrotic areas of tumor and the advancing tumor margin[28]. Their role in CRC still remains controversial. While, it was originally thought that the main function of TAMs was a direct cytotoxic effects on tumor cells, phagocytosis apoptotic/necrotic cell debris, and present tumor-associated antigens to T lymphocytes, current evidences suggest that inflammation and TAMs can also promote tumor growth and metastasis. Kang *et al*[35] have recently highlighted an association between intra-tumoral TAM densities with CRC malignant aggressiveness. Additionally, increased frequencies of intra-tumoral TAM have been associated with high levels of MMP type 2 and 9 expression in CRC cells[36,37]. These findings are in accord with a previous cell-line study showing that co-culturing of tumor cells with macrophages enhances cancer cell migration, invasiveness, and MMP-2 and MMP-9 secretion[35].

Kaler *et al*[38] have recently established that macrophages promote Wnt signaling pathway in CRC cells and thus enhance their proliferation, and demonstrated that macrophages exert their pro-tumorigenic activity mainly through the release of IL-1β. The same authors demonstrated that tumor necrosis factor related apoptosis inducing ligand (TRAIL) induced apoptosis of CRC cells is inhibited by macrophage derived IL-1β, and showed that macrophages and recombinant IL-1β counteract TRAIL-induced apoptosis through activation of Wnt signaling and stabilization of the nuclear transcription factor Snail in tumor cells.

A number of studies have also shown that macrophages can release a vast diversity of cytokines, proteolytic enzymes, growth factors, and inflammatory mediators that may directly influence and stimulate the growth and migration of tumor cells[29-33]. Li *et al*[39] first reported that IL-6 released by macrophage directly promotes CRC cell progression. They showed that monocyte/macrophage-derived IL-6 enhances migration of HT-29 CRC cells *in vitro*[39]. Although, how IL-6 enhances HT-29 cell migration still remains unknown, it has been suggested that TGF-β may be involved in this process. Using monoclonal antibodies to neutralize IL-10 in macrophage supernatant, Li *et al*[39] found that the IL-6-mediated effects on HT-29 CRC cells were all enhanced. Therefore, the interaction between IL-6 and IL-10 released from macrophage is indeed involved in CRC progression and prognosis. The above findings support the fact that TAMs play a regulatory role in the tumor microenvironment by modulating secretion of cytokines such as IL-6 and IL-10, thereby causing cancer cells to manipulate their microenvironment to facilitate cancer growth[40,41].

TAM activation and maturation is under the influence of the tumor microenvironment. TAMs retain a relatively immature phenotype, characterized by a low expression of differentiation-associated antigens in hypoxic microenvironments[35,42,43]. Conversely to an M2 phenotype with pro-tumoral functions in CRC[35], it has been shown that the macrophages, especially those secreting IL-12 and IL-23, infiltrating the tumor front are positively correlated with a favorable outcome, which implicates TAMs as possessing anti-tumor functions. Forssell *et al*[44] stained with the pan-monocyte/macrophage marker CD68 a series of CRC specimens and showed that the higher macrophage infiltration along the tumor invasive front correlated with improved survival in colon cancer compared to rectal cancer. They concluded that a dense macrophage infiltration at the tumor front positively influences prognosis in colon cancer and that the degree of cell-to-cell contact may influence the balance between pro-tumorigenic and anti-tumorigenic properties of macrophages. High levels of tissue macrophages have been also associated with earlier disease stage, absence of nodal and lymphovascular metastases and an overall better prognosis. Zhou *et al*[45] by analyzing the relationship between the density of TAMs and the potential of hepatic metastasis and survival have shown that a higher density of macrophages along the invasive front of CRC was associated with a higher 5-year survival rate. In addition, according to Forssell's scoring system that defines CD68 hot-spots as small areas among which the infiltration of macrophages was considerably above the average level of CD68-positive cells, the highest CD68 hot-spot was associated with both the incidence of hepatic metastasis and the interval between colon resection and the occurrence of hepatic metastasis[45].

The mechanisms behind the anti-tumor effects of TAMs have still not been fully elucidated, and seem potentially be ascribed to the M1 phenotype, which is in part controlled by the $CD4^+$T lymphocytes and the death of cancer cells[45-47]. It has been ascertained that recruitment of TAMs contributes to the development of an adaptive immune response against cancer, and the balance between antigen availability and clearance through phagocytosis and subsequent degradation of senescent or apoptotic cells.

It is undeniable that the discrepancies in results between different studies may be due to a number of factors related to the location of the TAMs and the assessment methods employed. Recent studies have reported that different macrophage phenotypes localized to different regions of the carcinoma have variable effects on tumor cells[18,19]. Furthermore, evidences have shown that the relationship between TAMs and tumor progression is tumor type-dependent.

Other innate immune system cells can be detected in CRC microenvironment, including mast cells (MCs), neutrophils, natural killer (NK) cells, and eosinophils.

Nagtegaal *et al*[48] have shown that peritumoral MCs prevent local and distant recurrence, with improved survival as a consequence. The significant benefit of MCs on

tumor progression in CRC was also highlighted by Gounaris *et al*[49] who reported that depletion of MCs, either by drugs either in MC-deficient mice, led to remission of existing polyps. Similar to other immune cell types, high numbers of MCs are associated with earlier CRC disease stage and have been proposed as an independent prognostic marker for improved survival[50]. It has also been reported that the number of MCs progressively decreases from normal mucosa through premalignant conditions and the lowest numbers are seen in cancers. Because of their location, MCs may prevent the metastasis of carcinomas that are restricted to the sub-mucosa. *In vivo* studies have observed that destruction of lymphatic vessels in the peritumoral infiltrate is always accompanied by MCs. This led to the hypothesis that MCs degranulate in the sub-mucosa when they come into contact with the inflammatory infiltrate or CRC cells, thus leading to the destruction of lymphatic vessels and thereby preventing further metastasis. Gulubova and Vlaykova[51] proposed the MCs density along the invasive front of the primary CRC as a helpful tool for prognosis of patients after surgical therapy. In their study, it has been shown that patients with low MCs density had significantly better prognosis compared to those with high MCs density. In addition, Blatner *et al*[52] reported that in CRC, MCs contribute to systemic regulatory T-cell dysfunction. MCs have an intricate interaction with T-regulatory cells that controls the functions of both cell types in a reciprocal manner. MCs play also an important role in allograft acceptance, where they are required to sustain the peripheral tolerance mediated by T-regulatory cells. These latter can inhibit MCs differentiation and hinder degranulation by contact-dependent mechanisms and production of soluble factors, such as IL-10. Conversely, the activation and subsequent degranulation of MCs breaks peripheral tolerance. MCs degranulation or direct cell contact and secretion of IL-6 promote Th-17 conversion of T-regulatory cells with loss of both forkhead box P3 transcription factor (Foxp3) expression and T-cell-suppressive properties.

Neutrophils may form up to 15% of the inflammatory infiltrate associated with CRCs and this proportion increases within areas of tumor necrosis[28]. In patients with rectal cancer, high concentrations of neutrophils have been shown as independent predictors of improved prognosis especially when microscopic abscesses form[28]. However, an elevated neutrophil/lymphocyte ratio was, however, found by Halazun *et al*[53] led to a poorer survival time and higher rate of recurrence in CRC patients undergoing surgery for liver metastasis.

NK cells are granular lymphocytes that form part of the innate cellular immune response[28]. In CRC, high numbers of NK cells in the inflammatory infiltrate has been associated with better prognosis[28]. The number of NK cells decreases with increasing cancer stage. Additionally, it has been shown that the ratio of NK cells in the peripheral blood is an important prognostic indicator in CRC patients and it is of interest to note that 5-fluorouracil-based chemotherapy increases the number of NK cells[54,55].

Aside the above innate immune cell types, dendritic cells (DCs), antigen-presenting cells that are critical to the stimulation of effective anti-tumor adaptive immune responses, can become defective in the tumor microenvironment and aid in tumor immune evasion by failing to stimulate T lymphocytes. It has been suggested that the presence of DCs may be of significant benefit in patients with CRC. Xie *et al*[56] also demonstrated that the presence of DCs was found predominantly in early compared to later disease stages and mostly located in tumor surrounding tissue. Suzuki *et al*[57] showed the presence of mature $CD83^+$ DCs at the cancer invasive front and by light and electron microscopy demonstrated their aggregation into clusters with lymphocytes, the majority of which were $CD45RO^+$ T lymphocytes. They concluded that mature $CD83^+$ DCs at the invasive margin promote T-cell activation for the generation of tumor specific immunity.

Although, there is growing evidence that the host innate immune system has a critical role in regulating carcinogenesis, the specific receptors involved and the importance of their interaction with commensal bacteria remain to be elucidated. Two major classes of innate immune receptors, the Toll-like receptors and Nod-like receptors, many of which are upstream of nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), have been investigated[58,59]. Particularly, the toll-like receptors have been implicated in promoting colon tumorigenesis. Fukata *et al*[60] have shown that toll-like receptor-4 (TLR4) is over-expressed in human colitis-associated cancer and, that mice deficient in TLR4 are markedly protected against colitis-associated neoplasia. A high TLR4 expression in the tumor microenvironment has recently been reported as a possible marker of disease progression in CRC[60]. Conversely, the role of nod-like receptors in regulating colorectal tumorigenesis remains unclear. Chen *et al*[61] reported an increased intestinal permeability associated with enhanced inflammatory cytokine production and epithelial cell proliferation in Nod1-deficient mice. As the depletion of the gut microbiota suppressed tumor development in Nod1-deficient mice, a link should exist between commensal bacteria and host innate Nod1 signaling pathway involved in the development of inflammation-mediated CRC.

ADAPTIVE IMMUNITY AND COLORECTAL CANCER

It is today well recognized that cells of the adaptive immune system are recruited in CRC and colitis-associated tumours (CAC), where they have either pro- and anti-tumorigenic roles. T lymphocytes participate in inflammation, cancer development and progression, as well as in anticancer immunity[20-22]. In CAC the adaptive immune system seems to have mainly a pro-tumorigenic role, while in CRC it may play a double-faced role, being the

balance between immune-surveillance (carried out by $CD8^+$ and $CD4^+$ T lymphocytes) and tumour-promoting inflammation (by various sub-types of T lymphocytes) to change over time, and eventually dictating disease progression. It has been suggested that immune-surveillance might mediate the early recognition and elimination of transformed cells and aberrant crypt foci, keeping small tumours in a dormant state[62]. Immune-surveillance is retained important during the metastatic process, when small numbers or isolated tumoral cells travel and can be attacked by antitumor immune cells not inhibited by factors in the tumour microenvironment. Galon *et al*[18] suggested that once human CRC become clinically detectable, the adaptive immune response plays a role in preventing tumour recurrence and metastasis. Independently, Chiba *et al*[63] and Banerjea *et al*[64] reported that intra-tumoral T cells modify the tumour stroma or CRC cells in a way that attenuate the metastatic potential.

Cytotoxic T lymphocytes ($CD8^+$ T cells, or CTL) constitute one of the leading effector of antitumor immunity. In order for $CD8^+$ T cells to recognize antigens, these need to be exposed on the tumour cells in association with the human leukocyte antigen (HLA) class I proteins[65]. Upon encounter of a tumour cell antigen/HLA I complex for which their T cell receptor (TCR) is specific, $CD8^+$ T lymphocytes clonally expand and differentiate[65]. Once activated, cytotoxic T lymphocytes can mediate specific destruction of tumour cells through the release of lytic components *via* cell-cell interaction[65,66]. Perforin, a cytolytic protein found in the granules of CD8 T-cells and NK cells, and enzymatic proteases, including granzyme B, are secreted determining cell death by disruption of the cell membrane and activation of the apoptotic pathway respectively. $CD4^+$ T cells, which only respond to antigens presented by the HLA class Ⅱ proteins expressed by DCs, are important for antitumor immunity. On the peculiar cytokine profile induced, $CD4^+$ T lymphocytes are mainly subdivided in Th1 or Th2 lymphocytes[67]. Th1 cells secrete cytokines such as interferon-gamma (IFN-γ) and TNF-α, and support cytotoxic T lymphocytes by producing IL-2, required for $CD8^+$ T cells proliferation. Conversely, Th2 cells principally secrete IL-10, IL-4, and IL-5, and limit cytotoxic T lymphocytes proliferation.

Regulatory T cells (Treg cells) have been defined as a T-cell population that functionally suppresses an immune response by influencing the activity of another cell type[68]. Treg cells have been categorized into two main classes based on their ontogeny: naturally occurring Treg (nTreg), which develop in the thymus and are present in mice and healthy humans from an early postnatal period, and Treg which can arise in the periphery (or *in vitro*)[69]. nTreg are characterized by their high expression of CD25 ($CD4^+CD25^+$) and co-expression of the FOXP3[69]. Needham *et al*[70] reported that depletion of intra-tumoral Tregs enhances antitumor immunity and tumour rejection in mouse models. Clarke *et al*[71] have shown that depletion of Tregs in the peripheral blood of patients with CRC was recently shown to unmask $CD4^+$ T-cell responses to tumour antigens. It is known that self-tumour antigens may also induce the preferential proliferation of $CD4^+CD25^+FOXP3^+$ Treg cells. Tregs function to maintain immune homeostasis and limit acute inflammation. These cells interfere with T cell priming and can affect the antitumor function of effector cells *via* secretion of TGF-β and IL-10. A number of investigations suggest that Tregs infiltrating the tumour may adversely affect prognosis[72-75]. Increased levels of Treg cells have been, however, associated with a favourable prognosis in CRC[76-79]. The mechanisms of this dual effect, which seems dependent on the tissue type, are not well understood, although it is hypothesized that Tregs might suppress inflammation induced by growth promoting innate immune system cells.

Although the role of B lymphocytes in cancer has been overshadowed by the interest in developing T-cell-mediated cellular responses, it is now apparent that B lymphocytes can play a complementary role in the host response against tumour. B lymphocytes represent a cell population that express clonally diverse cell surface Ig receptors recognizing specific antigenic epitopes. In addition to the role of B lymphocytes in antibody production, these cells mediate/regulate several other functions fundamental for immune homeostasis. Of significant importance is the antigen-presenting role of B lymphocytes in the initiation of T-cell immune responses. Moreover, B lymphocytes can play a significant role in infection and autoimmunity as regulatory cells (indicated as Bregs) via the elaboration of suppressive cytokines, such as IL-10, TGF-β, or IL-4. The role played by B cells in cancer immunology remains still complex and somewhat controversial. Depending upon their state of activation, B lymphocytes have had divergent roles on T-cell differentiation and effector function. Oversimplifying, resting B lymphocytes have been reported to suppress T-cell-mediated antitumor immunity, by acting on both $CD4^+$ and $CD8^+$ T lymphocytes. In contrast, a number of reports suggest the efficacy of activated B lymphocytes in cellular immunotherapy of malignancies. In particular, activated B lymphocytes have been reported to enhance the ability to generate tumour-infiltrating lymphocytes in vitro involving anti-CD3 and IL-2.

The therapeutic targeting of tumours or components of the immune system with molecule-specific monoclonal antibodies (mAb) is now considered a viable treatment option for cancer patients. One of the currently applied antibodies in clinics is represented by rituximab (Rituxan) that targets B cells for elimination by binding the B cell-associated marker CD20. Interestingly, it has been recently developed a C57BL/6 TRAIL-sensitive tumour model with the aim of being able to use gene-targeted mice to better evaluate the innate and adaptive immune cells contributing to the tumoricidal activity of the MD5-1 mAb in more clinically relevant established tumours. C57BL/6 gene-targeted or immune cell-depleted mice were used to examine the antitumor activity of

MD5-1 against the TRAIL-sensitive mouse MC38 colon adenocarcinoma. It has been shown that an intact B cell compartment is critical for the therapeutic activity of MD5-1 against established tumours. B cells were confirmed to trigger tumour cell apoptosis by FcR-mediated cross-linking of the MD5-1 mAb *in vitro* and *in vivo* B lymphocytes were critical for directly triggering MD5-1-mediated tumour cell apoptosis.

Although the role of B-cells in human CRCs is still not completely characterized, B-cell-deficient mice exhibit spontaneous regression of MC38 colon carcinoma cells. Studies involving BCR transgenic mice indicated that B lymphocytes might inhibit antitumor T lymphocytes responses by antigen-nonspecific mechanisms. Shah *et al* investigated the role of B lymphocytes in tumour immunity by studying immune responses of mice genetically lacking B lymphocytes to primary tumours. They highlight that although the effects of B lymphocytes on anti-tumour response warrant further study, adoptive transfer of CD40(-/-) B lymphocytes into B lymphocytes-deficient mice resulted in restored growth of MC38 colon carcinoma cells suggesting additional factors other than CD40 are involved in dampening anti-tumour responses.

PROGNOSTIC INFORMATION OF IMMUNE CELL INFILTRATE

In contrast to infiltration of cells responsible for chronic inflammation, the presence of high numbers of T lymphocytes has been reported to be a positive prognostic factor in several cancers. The first reports on the beneficial effect of lymphocytic infiltration in CRC appeared already in the Eighties. They were later confirmed until recent most studies highlighting a prominent function for memory T lymphocytes and $CD8^+$ T lymphocytes in predicting disease-free survival and overall survival. In general terms, it has been suggested that prognosis in patients with cancer is positively affected by (1) the presence of a tumour gene signature consistent with a type I adaptive immune response (i.e., increased antigen presentation, IFN-γ signalling, and TCR signalling); and (2) the presence of T cells that penetrate through tumour stroma and deeply infiltrate the parenchyma to become intra-tumoral T cells[20]. Thus, besides a Th-1 response signature, the other key feature of an effective immune response is the ability of T cells to reach the site of the tumour and to infiltrate it. Because T-cell infiltration is not spatially homogeneous in CRC, attention has been focused on the predictive values of T lymphocytes located in the center of the tumor (CT), along the invasive margin (IM) and in lymphoid aggregate mainly detectable in proximity of the tumor (these aggregates are called tertiary lymphoid structures)[80,81].

In a large series of CRCs, Pagès *et al*[82] assessed the immune component of the tumoral microenvironment by a combination of high-throughput gene expression and immunophenotypic analyses, and evaluated its possible influence on tumour dissemination. They found an association between evidence of an immune reaction within the tumour and the absence of tumour local invasion of vascular, lymphatic, and neural structures (collectively referred to as VELIPI). CRCs without VELIPI were associated with an enhanced immune cell infiltration and an increase of mRNA expression of adaptive Th1 effector T-cell markers [CD8, T-box transcription factor 21 (T-bet), IFN regulatory factor-1 (IRF-1), IFN-γ, granulysin, and granzyme B]. The immunohistochemical analysis of adaptive immune markers i.e., CD3, CD8, granzyme B, and CD45RO, by tissue microarrays prepared from the CT and from its IM revealed a statistically significant correlation between the density of these immune cells and outcome for all patients but those with metastatic disease at diagnosis. Further, the combined analysis of both tumour regions improved the accuracy of survival prediction compared with single-region analysis[18].

Independently, Deschoolmeester *et al*[83] showed that the presence of a pronounced lymphocytic infiltration within the tumour is associated with improved survival. They found that $CD3^+$ and $CD8^+$ T lymphocytes within tumour nests and of $CD3^+$ in the stroma had a major impact on the patients' overall survival. The improved survival associated with infiltration of T lymphocytes has been suggested to result from the effective suppression of micrometastases. Therefore, the densities of $CD8^+$ T cells within the primary tumour might be a potential marker of the presence of a systemic immunosurveillance mechanism. In addition, tumour cells secrete substances in the stromal compartment, which might be recognized by the immune system that subsequently attack the tumour. A weak adaptive immune reaction correlated with a very poor prognosis even in patients with early tumour invasion. Conversely, a high density of adaptive immune cells correlated with a highly favourable prognosis whatever the local extent of the tumour and the regional lymph node invasion.

In mice, targeted disruptions of genes that encode critical components of the immune system (i.e., mice lacking: IFN-γ or its receptor, signal transducer and activator of transcription-1 mediating IFN-γ receptor signalling, perforin, recombination activating gene-2, or IL-12) induce an increased susceptibility of the host to tumours[84]. These findings make it possible to hypothesize an immune-mediated control of tumour development by the adaptive compartment.

A number of studies have reported that MSI, CIMP, BRAF mutation, PIK3CA mutation, and tumour LINE-1 hypomethylation are associated with CRC prognosis and that lymphocytic infiltration is associated with many of these molecular variables. The associations of a prognostic biomarker with a given disease, strongly suggests its stage-dependency as outcome predictor. This is best exemplified by MSI CRC, whose overall prognostic advantage is associated with a low frequency of stage Ⅲ and Ⅳ cases at diagnosis as compared to microsatellite stable counterpart. Most MSI CRCs show a pronounced

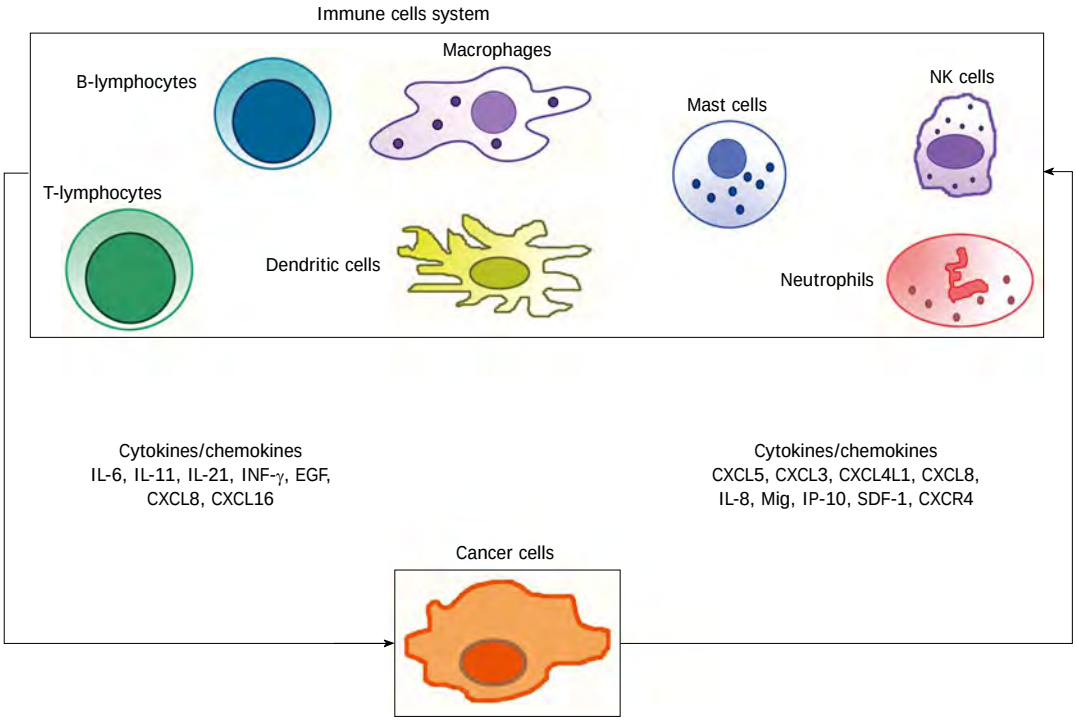


Figure 2 Anti- and pro-tumor immune response involves the interaction of several cell types of the adaptive as well as of the innate immune system, and an intricate network of products (i.e., cytokines and chemokines). IL: Interleukin; INF-γ: Intracellular interferon-γ; EGF: Epidermal-growth factor; NK cells: Natural killer cells.

intra-tumoral inflammatory reaction (in fact a criterion for MSI testing), the mechanistic explanation of which, however, is still incompletely understood. Within these tumours, infiltrating lymphocytes have been identified as predominantly activated $CD8^+$ T cells. The presence of these cytotoxic T lymphocytes has been attributed to the inherently greater production of abnormal peptides as a result of unreliable DNA repair in MSI-positive tumours. It is known that truncated peptides produced by frameshift mutations due to MSI may be immunogenic and contribute to the host immune response. However, little is still known about the interrelationship between tumour-infiltrating T lymphocytes, MSI status, and other tumour molecular features. It is indubitable that to define the prognostic effect of tumour-infiltrating T-cells independently of those potential confounders, large studies of CRC with extensive molecular characterization are needed. Additionally, caution is needed before incorporating tumour-infiltrating T cells into tumour staging. To minimise the risk of inappropriate tumour down-staging at diagnosis, survival data need to be confirmed in independent series of patients studied in the past decade. Moreover, the association has to be conclusively proven between low densities of tumour-infiltrating T cells and the clinical detection of metachronous metastases, which remains the most appropriate outcome measure for recognising a role of the local immune response in micrometastasis suppression. Recently, Laghi *et al*[85] investigated the relationship between the density of $CD3^+$ T infiltrating lymphocytes along the tumour invasive margin, and the occurrence of metachronous distant-organ metastases after potentially curative resection, in a large, consecutive series of patients with deeply invading (pT3 or pT4) MSI-typed CRC, and no evidence of distant organ metastasis at diagnosis. They found that large areas of $CD3^+$ cells at the invasive front of pT3 or pT4 CRCs are associated with a low risk of metachronous metastasis and consequently a survival advantage, only in patients with node-negative cancers, but not in patients whose cancers involved lymphnodes. Of interest, the prognostic advantage conferred by a high density of $CD3^+$ cells was independent of tumour microsatellite status in patients with stage Ⅱ CRC. CD3-immunostaining of CRC tissue might therefore be useful for selecting stage Ⅱ patients who, because they are at very low risk for cancer progression, could be spared adjuvant treatments.

Nosho *et al*[86] examined the prognostic role of tumour-infiltrating T-cell subsets in a database of 768 CRCs from two prospective cohort studies. They concurrently assessed the densities of $CD3^+$, $CD8^+$, $CD45RO^+$, and $FOXP3^+$ lymphocytes as well as other relevant molecular and pathological features, therefore making possible to evaluate the independent effect of each T-cell subset density on patient survival. They found that the density of $CD45RO^+$ cells, but not that of $CD3^+$, $CD8^+$, or $FOXP3^+$ cells, was an independent prognostic biomarker of longer survival in CRC patients. In contrast, Salama *et al*[76] by analysing T-cell infiltrates in 967 CRCs including 593 stage Ⅱ and 374 stage Ⅲ cases, reported that $FOXP3^+$ lymphocytes density had stronger prognostic significance than $CD8^+$ and $CD45RO^+$ cells, and predicted a better outcome. $FOXP3^+$ lymphocytes were

found not associated with any histopathologic features. At multivariate analysis, stage, vascular invasion, and $FOXP3^+$ cell density in tumoral tissue were independent prognostic indicators. These results led Salama *et al*[76] to conclude that the inclusion of $FOXP3^+$ cell density may help to improve the prognostication of early-stage CRC, although these authors do not explored this parameter with tumor stage.

CONCLUSION

It is accepted that human cancer is a complex dynamical disease[87,88]. It is also now well recognized that cancers are not just composed of malignant cells, but that they are microenvironment consisting of many cell types, including a range of immune cells. It is indubitable that the antitumor immune response involves the interaction of several cell types and products (Figure 2), of the adaptive as well as of the innate immune system. It is also clear that CRC can escape immune surveillance using several strategies. The molecular profiles of the function and interaction of innate and adaptive immune cells, and the definition of tumor antigens have all led to build the basis of the knowledge of how the immune system modulates tumor growth and inhibition. The challenge remains to determine not only how the "rejection" pathway initiates in human malignancy, but also how that rejection is maintained. It is indubitable that the analysis of the type, quantity, location and the functions of the immune infiltrate becomes a primary step in understanding CRC natural history, and, in a clinical perspective, its prognostic determinants. A comprehensive analysis of all components of the lymphocytic infiltrates in the context of their localization, organization and impact at various steps of tumor progression remains largely, if not entirely, to be reported to prospective studies. In parallel, understanding the mechanisms of efficient immune reactions, the place where they are initiated, the cells and key cytokines and chemokines involved (Figure 2), and their impact at different stages of the disease should provide new tools and goals for more effective and less toxic targeted therapies.

REFERENCES

1 **Deschoolmeester V**, Baay M, Specenier P, Lardon F, Vermorken JB. A review of the most promising biomarkers in colorectal cancer: one step closer to targeted therapy. *Oncologist* 2010; **15**: 699-731 [PMID: 20584808 DOI: 10.1634/theoncologist.2010-0025]

2 **Michor F**, Iwasa Y, Lengauer C, Nowak MA. Dynamics of colorectal cancer. *Semin Cancer Biol* 2005; **15**: 484-493 [PMID: 16055342 DOI: 10.1016/j.semcancer.2005.06.005]

3 **Jass JR**. Colorectal cancer: a multipathway disease. *Crit Rev Oncog* 2006; **12**: 273-287 [PMID: 17425506]

4 **Fearon ER**, Vogelstein B. A genetic model for colorectal tumorigenesis. *Cell* 1990; **61**: 759-767 [PMID: 2188735 DOI: 10.1016/0092-8674(90)90186-I]

5 **Vogelstein B**, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL. Genetic alterations during colorectal-tumor development. *N Engl J Med* 1988; **319**: 525-532 [PMID: 2841597 DOI: 10.1056/NEJM198809013190901]

6 **Ogino S**, Chan AT, Fuchs CS, Giovannucci E. Molecular pathological epidemiology of colorectal neoplasia: an emerging transdisciplinary and interdisciplinary field. *Gut* 2011; **60**: 397-411 [PMID: 21036793 DOI: 10.1136/gut.2010.217182]

7 **Pino MS**, Chung DC. The chromosomal instability pathway in colon cancer. *Gastroenterology* 2010; **138**: 2059-2072 [PMID: 20420946 DOI: 10.1053/j.gastro.2009.12.065]

8 **Boland CR**, Goel A. Microsatellite instability in colorectal cancer. *Gastroenterology* 2010; **138**: 2073-2087.e3 [PMID: 20420947 DOI: 10.1053/j.gastro.2009.12.064]

9 **Imai K**, Yamamoto H. Carcinogenesis and microsatellite instability: the interrelationship between genetics and epigenetics. *Carcinogenesis* 2008; **29**: 673-680 [PMID: 17942460 DOI: 10.1093/carcin/bgm228]

10 **Hyde A**, Fontaine D, Stuckless S, Green R, Pollett A, Simms M, Sipahimalani P, Parfrey P, Younghusband B. A histology-based model for predicting microsatellite instability in colorectal cancers. *Am J Surg Pathol* 2010; **34**: 1820-1829 [PMID: 21107088 DOI: 10.1097/PAS.0b013e3181f6a912]

11 **Boland CR**, Thibodeau SN, Hamilton SR, Sidransky D, Eshleman JR, Burt RW, Meltzer SJ, Rodriguez-Bigas MA, Fodde R, Ranzani GN, Srivastava S. A National Cancer Institute Workshop on Microsatellite Instability for cancer detection and familial predisposition: development of international criteria for the determination of microsatellite instability in colorectal cancer. *Cancer Res* 1998; **58**: 5248-5257 [PMID: 9823339]

12 **Malesci A**, Laghi L, Bianchi P, Delconte G, Randolph A, Torri V, Carnaghi C, Doci R, Rosati R, Montorsi M, Roncalli M, Gennari L, Santoro A. Reduced likelihood of metastases in patients with microsatellite-unstable colorectal cancer. *Clin Cancer Res* 2007; **13**: 3831-3839 [PMID: 17606714 DOI: 10.1158/1078-0432.CCR-07-0366]

13 **Gryfe R**, Kim H, Hsieh ET, Aronson MD, Holowaty EJ, Bull SB, Redston M, Gallinger S. Tumor microsatellite instability and clinical outcome in young patients with colorectal cancer. *N Engl J Med* 2000; **342**: 69-77 [PMID: 10631274 DOI: 10.1056/NEJM200001133420201]

14 **Carethers JM**, Smith EJ, Behling CA, Nguyen L, Tajima A, Doctolero RT, Cabrera BL, Goel A, Arnold CA, Miyai K, Boland CR. Use of 5-fluorouracil and survival in patients with microsatellite-unstable colorectal cancer. *Gastroenterology* 2004; **126**: 394-401 [PMID: 14762775 DOI: 10.1053/j.gastro.2003.12.023]

15 **McLean MH**, Murray GI, Stewart KN, Norrie G, Mayer C, Hold GL, Thomson J, Fyfe N, Hope M, Mowat NA, Drew JE, El-Omar EM. The inflammatory microenvironment in colorectal neoplasia. *PLoS One* 2011; **6**: e15366 [PMID: 21249124 DOI: 10.1371/journal.pone.0015366]

16 **Peddareddigari VG**, Wang D, Dubois RN. The tumor microenvironment in colorectal carcinogenesis. *Cancer Microenviron* 2010; **3**: 149-166 [PMID: 21209781 DOI: 10.1007/s12307-010-0038-3]

17 **Halama N**, Michel S, Kloor M, Zoernig I, Pommerencke T, von Knebel Doeberitz M, Schirmacher P, Weitz J, Grabe N, Jäger D. The localization and density of immune cells in primary tumors of human metastatic colorectal cancer shows an association with response to chemotherapy. *Cancer Immun* 2009; **9**: 1 [PMID: 19226101]

18 **Galon J**, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, Tosolini M, Camus M, Berger A, Wind P, Zinzindohoué F, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Pagès F. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. *Science* 2006; **313**: 1960-1964 [PMID: 17008531 DOI: 10.1126/science.1129139]

19 **Galon J**, Fridman WH, Pagès F. The adaptive immunologic microenvironment in colorectal cancer: a novel perspective. *Cancer Res* 2007; **67**: 1883-1886 [PMID: 17332313 DOI:

10.1158/0008-5472.CAN-06-4806]
20 **Disis ML**. Immune regulation of cancer. *J Clin Oncol* 2010; **28**: 4531-4538 [PMID: 20516428 DOI: 10.1200/JCO.2009.27.2146]
21 **Mantovani A**, Romero P, Palucka AK, Marincola FM. Tumour immunity: effector response to tumour and role of the microenvironment. *Lancet* 2008; **371**: 771-783 [PMID: 18275997 DOI: 10.1016/S0140-6736(08)60241-X]
22 **Mantovani A**, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature* 2008; **454**: 436-444 [PMID: 18650914 DOI: 10.1038/nature07205]
23 **Bissell MJ**, Radisky D. Putting tumours in context. *Nat Rev Cancer* 2001; **1**: 46-54 [PMID: 11900251 DOI: 10.1038/35094059]
24 **Mueller MM**, Fusenig NE. Friends or foes - bipolar effects of the tumour stroma in cancer. *Nat Rev Cancer* 2004; **4**: 839-849 [PMID: 15516957 DOI: 10.1038/nrc1477]
25 **Bhowmick NA**, Neilson EG, Moses HL. Stromal fibroblasts in cancer initiation and progression. *Nature* 2004; **432**: 332-337 [PMID: 15549095 DOI: 10.1038/nature03096]
26 **Ferrone C**, Dranoff G. Dual roles for immunity in gastrointestinal cancers. *J Clin Oncol* 2010; **28**: 4045-4051 [PMID: 20644090 DOI: 10.1200/JCO.2010.27.9992]
27 **Saleh M**, Trinchieri G. Innate immune mechanisms of colitis and colitis-associated colorectal cancer. *Nat Rev Immunol* 2011; **11**: 9-20 [PMID: 21151034 DOI: 10.1038/nri2891]
28 **Salama P**, Platell C. Host response to colorectal cancer. *ANZ J Surg* 2008; **78**: 745-753 [PMID: 18844901 DOI: 10.1111/j.1445-2197.2008.04642.x]
29 **Biswas SK**, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. *Nat Immunol* 2010; **11**: 889-896 [PMID: 20856220 DOI: 10.1038/ni.1937]
30 **Mantovani A**, Sica A. Macrophages, innate immunity and cancer: balance, tolerance, and diversity. *Curr Opin Immunol* 2010; **22**: 231-237 [PMID: 20144856 DOI: 10.1016/j.coi.2010.01.009]
31 **Sica A**, Larghi P, Mancino A, Rubino L, Porta C, Totaro MG, Rimoldi M, Biswas SK, Allavena P, Mantovani A. Macrophage polarization in tumour progression. *Semin Cancer Biol* 2008; **18**: 349-355 [PMID: 18467122 DOI: 10.1016/j.semcancer.2008.03.004]
32 **Martinez FO**, Gordon S, Locati M, Mantovani A. Transcriptional profiling of the human monocyte-to-macrophage differentiation and polarization: new molecules and patterns of gene expression. *J Immunol* 2006; **177**: 7303-7311 [PMID: 17082649]
33 **Mantovani A**, Sica A, Locati M. New vistas on macrophage differentiation and activation. *Eur J Immunol* 2007; **37**: 14-16 [PMID: 17183610 DOI: 10.1002/eji.200636910]
34 **Rigo A**, Gottardi M, Zamò A, Mauri P, Bonifacio M, Krampera M, Damiani E, Pizzolo G, Vinante F. Macrophages may promote cancer growth via a GM-CSF/HB-EGF paracrine loop that is enhanced by CXCL12. *Mol Cancer* 2010; **9**: 273 [PMID: 20946648 DOI: 10.1186/1476-4598-9-273].]
35 **Kang JC**, Chen JS, Lee CH, Chang JJ, Shieh YS. Intratumoral macrophage counts correlate with tumor progression in colorectal cancer. *J Surg Oncol* 2010; **102**: 242-248 [PMID: 20740582 DOI: 10.1002/jso.21617]
36 **Papadopoulou S**, Scorilas A, Arnogianaki N, Papapanayiotou B, Tzimogiani A, Agnantis N, Talieri M. Expression of gelatinase-A (MMP-2) in human colon cancer and normal colon mucosa. *Tumour Biol* 2001; **22**: 383-389 [PMID: 11786732 DOI: 10.1159/000050641]
37 **Herszényi L**, Sipos F, Galamb O, Solymosi N, Hritz I, Miheller P, Berczi L, Molnár B, Tulassay Z. Matrix metalloproteinase-9 expression in the normal mucosa-adenoma-dysplasia-adenocarcinoma sequence of the colon. *Pathol Oncol Res* 2008; **14**: 31-37 [PMID: 18347934 DOI: 10.1007/s12253-008-9004-5]
38 **Kaler P**, Galea V, Augenlicht L, Klampfer L. Tumor associated macrophages protect colon cancer cells from TRAIL-induced apoptosis through IL-1beta-dependent stabilization of Snail in tumor cells. *PLoS One* 2010; **5**: e11700 [PMID: 20661477 DOI: 10.1371/journal.pone.0011700]
39 **Li YY**, Hsieh LL, Tang RP, Liao SK, Yeh KY. Interleukin-6 (IL-6) released by macrophages induces IL-6 secretion in the human colon cancer HT-29 cell line. *Hum Immunol* 2009; **70**: 151-158 [PMID: 19272324 DOI: 10.1016/j.humimm.2009.01.004]
40 **Herbeuval JP**, Lelievre E, Lambert C, Dy M, Genin C. Recruitment of STAT3 for production of IL-10 by colon carcinoma cells induced by macrophage-derived IL-6. *J Immunol* 2004; **172**: 4630-4636 [PMID: 15034082]
41 **Li YY**, Chang JW, Hsieh LL, Yeh KY. Neutralization of interleukin (IL)-10 released by monocytes/macrophages enhances the up-regulatory effect of monocyte/macrophage-derived IL-6 on expressions of IL-6 and MUC1, and migration in HT-29 colon cancer cells. *Cell Immunol* 2010; **265**: 164-171 [PMID: 20851386 DOI: 10.1016/j.cellimm.2010.07.014]
42 **Lewis C**, Murdoch C. Macrophage responses to hypoxia: implications for tumor progression and anti-cancer therapies. *Am J Pathol* 2005; **167**: 627-635 [PMID: 16127144 DOI: 10.1016/S0002-9440(10)62038-X]
43 **Mantovani A**, Schioppa T, Porta C, Allavena P, Sica A. Role of tumor-associated macrophages in tumor progression and invasion. *Cancer Metastasis Rev* 2006; **25**: 315-322 [PMID: 16967326 DOI: 10.1007/s10555-006-9001-7]
44 **Forssell J**, Oberg A, Henriksson ML, Stenling R, Jung A, Palmqvist R. High macrophage infiltration along the tumor front correlates with improved survival in colon cancer. *Clin Cancer Res* 2007; **13**: 1472-1479 [PMID: 17332291 DOI: 10.1158/1078-0432.CCR-06-2073]
45 **Zhou Q**, Peng RQ, Wu XJ, Xia Q, Hou JH, Ding Y, Zhou QM, Zhang X, Pang ZZ, Wan DS, Zeng YX, Zhang XS. The density of macrophages in the invasive front is inversely correlated to liver metastasis in colon cancer. *J Transl Med* 2010; **8**: 13 [PMID: 20141634 DOI: 10.1186/1479-5876-8-13]
46 **Umemura N**, Saio M, Suwa T, Kitoh Y, Bai J, Nonaka K, Ouyang GF, Okada M, Balazs M, Adany R, Shibata T, Takami T. Tumor-infiltrating myeloid-derived suppressor cells are pleiotropic-inflamed monocytes/macrophages that bear M1- and M2-type characteristics. *J Leukoc Biol* 2008; **83**: 1136-1144 [PMID: 18285406 DOI: 10.1189/jlb.0907611]
47 **DeNardo DG**, Barreto JB, Andreu P, Vasquez L, Tawfik D, Kolhatkar N, Coussens LM. CD4(+) T cells regulate pulmonary metastasis of mammary carcinomas by enhancing protumor properties of macrophages. *Cancer Cell* 2009; **16**: 91-102 [PMID: 19647220 DOI: 10.1016/j.ccr.2009.06.018]
48 **Nagtegaal ID**, Marijnen CA, Kranenbarg EK, Mulder-Stapel A, Hermans J, van de Velde CJ, van Krieken JH. Local and distant recurrences in rectal cancer patients are predicted by the nonspecific immune response; specific immune response has only a systemic effect--a histopathological and immunohistochemical study. *BMC Cancer* 2001; **1**: 7 [PMID: 11481031 DOI: 10.1186/1471-2407-1-7]
49 **Gounaris E**, Erdman SE, Restaino C, Gurish MF, Friend DS, Gounari F, Lee DM, Zhang G, Glickman JN, Shin K, Rao VP, Poutahidis T, Weissleder R, McNagny KM, Khazaie K. Mast cells are an essential hematopoietic component for polyp development. *Proc Natl Acad Sci USA* 2007; **104**: 19977-19982 [PMID: 18077429 DOI: 10.1073/pnas.0704620104]
50 **Nielsen HJ**, Hansen U, Christensen IJ, Reimert CM, Brünner N, Moesgaard F. Independent prognostic value of eosinophil and mast cell infiltration in colorectal cancer tissue. *J Pathol* 1999; **189**: 487-495 [PMID: 10629548 DOI: 10.1002/(SICI)1096-9896(199912)189:]
51 **Gulubova M**, Vlaykova T. Prognostic significance of mast cell number and microvascular density for the survival of patients with primary colorectal cancer. *J Gastroenterol Hepatol* 2009; **24**: 1265-1275 [PMID: 17645466 DOI: 10.1111/j.1440-1746.2007.05009.x]

52 **Blatner NR**, Bonertz A, Beckhove P, Cheon EC, Krantz SB, Strouch M, Weitz J, Koch M, Halverson AL, Bentrem DJ, Khazaie K. In colorectal cancer mast cells contribute to systemic regulatory T-cell dysfunction. *Proc Natl Acad Sci USA* 2010; **107**: 6430-6435 [PMID: 20308560 DOI: 10.1073/pnas.0913683107]

53 **Halazun KJ**, Aldoori A, Malik HZ, Al-Mukhtar A, Prasad KR, Toogood GJ, Lodge JP. Elevated preoperative neutrophil to lymphocyte ratio predicts survival following hepatic resection for colorectal liver metastases. *Eur J Surg Oncol* 2008; **34**: 55-60 [PMID: 17448623 DOI: 10.1016/j.ejso.2007.02.014]

54 **Vesely P**, Tousková M, Melichar B. Phenotype of peripheral blood leukocytes and survival of patients with metastatic colorectal cancer. *Int J Biol Markers* 2005; **20**: 126-133 [PMID: 16011044]

55 **Holcombe RF**, Jacobson J, Dakhil SR, Stewart RM, Betzing KS, Kannan K, Macdonald JS. Association of immune parameters with clinical outcome in stage III colon cancer: results of Southwest Oncology Group Protocol 9009. *Cancer Immunol Immunother* 1999; **48**: 533-539 [PMID: 10602891 DOI: 10.1007/s002620050602]

56 **Xie ZJ**, Jia LM, He YC, Gao JT. Morphological observation of tumor infiltrating immunocytes in human rectal cancer. *World J Gastroenterol* 2006; **12**: 1757-1760 [PMID: 16586547]

57 **Suzuki A**, Masuda A, Nagata H, Kameoka S, Kikawada Y, Yamakawa M, Kasajima T. Mature dendritic cells make clusters with T cells in the invasive margin of colorectal carcinoma. *J Pathol* 2002; **196**: 37-43 [PMID: 11748640 DOI: 10.1002/path.1018]

58 **Akira S**, Uematsu S, Takeuchi O. Pathogen recognition and innate immunity. *Cell* 2006; **124**: 783-801 [PMID: 16497588 DOI: 10.1016/j.cell.2006.02.015]

59 **Franchi L**, McDonald C, Kanneganti TD, Amer A, Núñez G. Nucleotide-binding oligomerization domain-like receptors: intracellular pattern recognition molecules for pathogen detection and host defense. *J Immunol* 2006; **177**: 3507-3513 [PMID: 16951308]

60 **Fukata M**, Hernandez Y, Conduah D, Cohen J, Chen A, Breglio K, Goo T, Hsu D, Xu R, Abreu MT. Innate immune signaling by Toll-like receptor-4 (TLR4) shapes the inflammatory microenvironment in colitis-associated tumors. *Inflamm Bowel Dis* 2009; **15**: 997-1006 [PMID: 19229991 DOI: 10.1002/ibd.20880]

61 **Chen GY**, Shaw MH, Redondo G, Núñez G. The innate immune receptor Nod1 protects the intestine from inflammation-induced tumorigenesis. *Cancer Res* 2008; **68**: 10060-10067 [PMID: 19074871 DOI: 0008-5472.CAN-08-2061]

62 **Terzić J**, Grivennikov S, Karin E, Karin M. Inflammation and colon cancer. *Gastroenterology* 2010; **138**: 2101-2114.e5 [PMID: 20420949 DOI: 10.1053/j.gastro.2010.01.058]

63 **Chiba T**, Ohtani H, Mizoi T, Naito Y, Sato E, Nagura H, Ohuchi A, Ohuchi K, Shiiba K, Kurokawa Y, Satomi S. Intraepithelial CD8+ T-cell-count becomes a prognostic factor after a longer follow-up period in human colorectal carcinoma: possible association with suppression of micrometastasis. *Br J Cancer* 2004; **91**: 1711-1717 [PMID: 15494715]

64 **Banerjea A**, Bustin SA, Dorudi S. The immunogenicity of colorectal cancers with high-degree microsatellite instability. *World J Surg Oncol* 2005; **3**: 26 [PMID: 15890075 DOI: 10.1186/1477-7819-3-26]

65 **Paschen A**, Eichmuller S, Schadendorf D. Identification of tumor antigens and T-cell epitopes, and its clinical application. *Cancer Immunol Immunother* 2004; **53**: 196-203 [PMID: 14689239 DOI: 10.1007/s00262-003-0479-3]

66 **Loose D**, Van de Wiele C. The immune system and cancer. *Cancer Biother Radiopharm* 2009; **24**: 369-376 [PMID: 19538060 DOI: 10.1089/cbr.2008.0593]

67 **Barnas JL**, Simpson-Abelson MR, Yokota SJ, Kelleher RJ, Bankert RB. T cells and stromal fibroblasts in human tumor microenvironments represent potential therapeutic targets. *Cancer Microenviron* 2010; **3**: 29-47 [PMID: 21209773 DOI: 10.1007/s12307-010-0044-5]

68 **Zou W**. Regulatory T cells, tumour immunity and immunotherapy. *Nat Rev Immunol* 2006; **6**: 295-307 [PMID: 16557261 DOI: 10.1038/nri1806]

69 **Saurer L**, Mueller C. T cell-mediated immunoregulation in the gastrointestinal tract. *Allergy* 2009; **64**: 505-519 [PMID: 19210347 DOI: 10.1111/j.1398-9995.2009.01965.x]

70 **Needham DJ**, Lee JX, Beilharz MW. Intra-tumoural regulatory T cells: a potential new target in cancer immunotherapy. *Biochem Biophys Res Commun* 2006; **343**: 684-691 [PMID: 16563349 DOI: 10.1016/j.bbrc.2006.03.018]

71 **Clarke SL**, Betts GJ, Plant A, Wright KL, El-Shanawany TM, Harrop R, Torkington J, Rees BI, Williams GT, Gallimore AM, Godkin AJ. CD4+CD25+FOXP3+ regulatory T cells suppress anti-tumor immune responses in patients with colorectal cancer. *PLoS One* 2006; **1**: e129 [PMID: 17205133 DOI: 10.1371/journal.pone.0000129]

72 **Khan AR**, Dovedi SJ, Wilkinson RW, Pritchard DI. Tumor infiltrating regulatory T cells: tractable targets for immunotherapy. *Int Rev Immunol* 2010; **29**: 461-484 [PMID: 20839911 DOI: 10.3109/08830185.2010.508854]

73 **Curiel TJ**, Coukos G, Zou L, Alvarez X, Cheng P, Mottram P, Evdemon-Hogan M, Conejo-Garcia JR, Zhang L, Burow M, Zhu Y, Wei S, Kryczek I, Daniel B, Gordon A, Myers L, Lackner A, Disis ML, Knutson KL, Chen L, Zou W. Specific recruitment of regulatory T cells in ovarian carcinoma fosters immune privilege and predicts reduced survival. *Nat Med* 2004; **10**: 942-949 [PMID: 15322536 DOI: 10.1038/nm1093]

74 **Hiraoka N**, Onozato K, Kosuge T, Hirohashi S. Prevalence of FOXP3+ regulatory T cells increases during the progression of pancreatic ductal adenocarcinoma and its premalignant lesions. *Clin Cancer Res* 2006; **12**: 5423-5434 [PMID: 17000676 DOI: 10.1158/1078-0432.CCR-06-0369]

75 **Kobayashi N**, Hiraoka N, Yamagami W, Ojima H, Kanai Y, Kosuge T, Nakajima A, Hirohashi S. FOXP3+ regulatory T cells affect the development and progression of hepatocarcinogenesis. *Clin Cancer Res* 2007; **13**: 902-911 [PMID: 17289884 DOI: 10.1158/1078-0432.CCR-06-2363]

76 **Salama P**, Phillips M, Grieu F, Morris M, Zeps N, Joseph D, Platell C, Iacopetta B. Tumor-infiltrating FOXP3+ T regulatory cells show strong prognostic significance in colorectal cancer. *J Clin Oncol* 2009; **27**: 186-192 [PMID: 19064967 DOI: 10.1200/JCO.2008.18.7229]

77 **Correale P**, Rotundo MS, Del Vecchio MT, Remondo C, Migali C, Ginanneschi C, Tsang KY, Licchetta A, Mannucci S, Loiacono L, Tassone P, Francini G, Tagliaferri P. Regulatory (FoxP3+) T-cell tumor infiltration is a favorable prognostic factor in advanced colon cancer patients undergoing chemo or chemoimmunotherapy. *J Immunother* 2010; **33**: 435-441 [PMID: 20386463 DOI: 10.1097/CJI.0b013e3181d32f01]

78 **Suzuki H**, Chikazawa N, Tasaka T, Wada J, Yamasaki A, Kitaura Y, Sozaki M, Tanaka M, Onishi H, Morisaki T, Katano M. Intratumoral CD8(+) T/FOXP3 (+) cell ratio is a predictive marker for survival in patients with colorectal cancer. *Cancer Immunol Immunother* 2010; **59**: 653-661 [PMID: 19908042 DOI: 10.1007/s00262-009-0781-9]

79 **Frey DM**, Droeser RA, Viehl CT, Zlobec I, Lugli A, Zingg U, Oertli D, Kettelhack C, Terracciano L, Tornillo L. High frequency of tumor-infiltrating FOXP3(+) regulatory T cells predicts improved survival in mismatch repair-proficient colorectal cancer patients. *Int J Cancer* 2010; **126**: 2635-2643 [PMID: 19856313]

80 **Pagès F**, Galon J, Dieu-Nosjean MC, Tartour E, Sautès-Fridman C, Fridman WH. Immune infiltration in human tumors: a prognostic factor that should not be ignored. *Oncogene* 2010; **29**: 1093-1102 [PMID: 19946335 DOI: 10.1038/onc.2009.416]

81 **Zlobec I**, Lugli A. Invasive front of colorectal cancer: dynamic interface of pro-/anti-tumor factors. *World J Gastro-*

enterol 2009; **15**: 5898-5906 [PMID: 20014453 DOI: 10.3748/wjg.15.5898]
82 **Pagès F**, Berger A, Camus M, Sanchez-Cabo F, Costes A, Molidor R, Mlecnik B, Kirilovsky A, Nilsson M, Damotte D, Meatchi T, Bruneval P, Cugnenc PH, Trajanoski Z, Fridman WH, Galon J. Effector memory T cells, early metastasis, and survival in colorectal cancer. *N Engl J Med* 2005; **353**: 2654-2666 [PMID: 16371631 DOI: 10.1056/NEJMoa051424]
83 **Deschoolmeester V**, Baay M, Van Marck E, Weyler J, Vermeulen P, Lardon F, Vermorken JB. Tumor infiltrating lymphocytes: an intriguing player in the survival of colorectal cancer patients. *BMC Immunol* 2010; **11**: 19 [PMID: 20385003 DOI: 10.1186/1471-2172-11-19]
84 **Dunn GP**, Old LJ, Schreiber RD. The three Es of cancer immunoediting. *Annu Rev Immunol* 2004; **22**: 329-360 [PMID: 15032581 DOI: 10.1146/annurev.immunol.22.012703.104803]
85 **Laghi L**, Bianchi P, Miranda E, Balladore E, Pacetti V, Grizzi F, Allavena P, Torri V, Repici A, Santoro A, Mantovani A, Roncalli M, Malesci A. CD3+ cells at the invasive margin of deeply invading (pT3-T4) colorectal cancer and risk of post-surgical metastasis: a longitudinal study. *Lancet Oncol* 2009; **10**: 877-884 [PMID: 19656725 DOI: 10.1016/S1470-2045(09)70186-X]
86 **Nosho K**, Baba Y, Tanaka N, Shima K, Hayashi M, Meyerhardt JA, Giovannucci E, Dranoff G, Fuchs CS, Ogino S. Tumour-infiltrating T-cell subsets, molecular changes in colorectal cancer, and prognosis: cohort study and literature review. *J Pathol* 2010; **222**: 350-366 [PMID: 20927778 DOI: 10.1002/path.2774]
87 **Grizzi F**, Chiriva-Internati M. Cancer: looking for simplicity and finding complexity. *Cancer Cell Int* 2006; **6**: 4 [PMID: 16480511 DOI: 10.1186/1475-2867-6-4]
88 **Grizzi F**, Di Ieva A, Russo C, Frezza EE, Cobos E, Muzzio PC, Chiriva-Internati M. Cancer initiation and progression: an unsimplifiable complexity. *Theor Biol Med Model* 2006; **3**: 37 [PMID: 17044918 DOI: 10.1186/1742-4682-3-37]

P- Reviewers Braet F, Ho YH, Zheng S **S- Editor** Gou SX
L- Editor A **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i2.185

World J Gastroenterol 2013 January 14; 19(2): 185-198
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

REVIEW

Gastrointestinal radiation injury: Symptoms, risk factors and mechanisms

Abobakr K Shadad, Frank J Sullivan, Joseph D Martin, Laurence J Egan

Abobakr K Shadad, Laurence J Egan, Department of Gastroenterology, University Hospital Galway, University Hospital Galway, 34562 Galway, Ireland
Abobakr K Shadad, Laurence J Egan, Department of Pharmacology and Therapeutics, School of Medicine, University Hospital Galway, 34562 Galway, Ireland
Frank J Sullivan, Joseph D Martin, Department of Radiation Oncology, National University of Ireland Galway, University Hospital Galway, 34562 Galway, Ireland
Author contributions: Shadad AK wrote the review; Sullivan FJ, Martin JD and Egan LJ revised the contents.
Correspondence to: Laurence J Egan, Professor, Department of Pharmacology and Therapeutics, School of Medicine, University Hospital Galway, 34562 Galway,
Ireland. laurence.egan@nuigalway.ie
Telephone: +353-91-495370 Fax: +353-91-495572
Received: December 4, 2011 Revised: March 31, 2012
Accepted: December 15, 2012
Published online: January 14, 2013

Abstract

Ionising radiation therapy is a common treatment modality for different types of cancer and its use is expected to increase with advances in screening and early detection of cancer. Radiation injury to the gastrointestinal tract is important factor working against better utility of this important therapeutic modality. Cancer survivors can suffer a wide variety of acute and chronic symptoms following radiotherapy, which significantly reduces their quality of life as well as adding an extra burden to the cost of health care. The accurate diagnosis and treatment of intestinal radiation injury often represents a clinical challenge to practicing physicians in both gastroenterology and oncology. Despite the growing recognition of the problem and some advances in understanding the cellular and molecular mechanisms of radiation injury, relatively little is known about the pathophysiology of gastrointestinal radiation injury or any possible susceptibility factors that could aggravate its severity. The aims of this review are to examine the various clinical manifestations of post-radiation gastrointestinal symptoms, to discuss possible patient and treatment factors implicated in normal gastrointestinal tissue radiosensitivity and to outline different mechanisms of intestinal tissue injury.

© 2013 Baishideng. All rights reserved.

Key words: Radiation enteritis; Radiation proctitis; Symptoms; Pathophysiology; Risk factors

Shadad AK, Sullivan FJ, Martin JD, Egan LJ. Gastrointestinal radiation injury: Symptoms, risk factors and mechanisms. *World J Gastroenterol* 2013; 19(2): 185-198 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i2/185.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i2.185

INTRODUCTION

Radiation delivery methods

Radiation therapy can be delivered by three main methods. External beam radiotherapy is a method in which radiation beam is delivered from outside the body to the target tumour through two or three-dimensional beam arrays using linear accelerators. Advances in planning and delivery techniques such as 3-dimensional simulation and intensity modulated radiation therapy are associated with a reduced risk of normal tissue toxicity and allow a higher radiation dose to be used compared to conventional two dimensional methods[1-4]. Enhanced target definition of both tumours and surrounding normal tissues and combining beams of varying intensity in intensity modulated radiation therapy allow for better dose delivery and improved tumour control with less toxicity[5,6]. Quantitative dose tracking to both normal tissues and tumors in the form of dose-volume histograms[7] provide a graphic display of a simulated radiation treatment plan and generate valuable information on the dose distribution within the volume of interest[8]. These are now common planning

tools in modern external beam radiation delivery. Brachytherapy is an internal form of radiation therapy where the radiation sources are implanted within or in close proximity to the target tumour to deliver a high dose of localized radiation. This procedure is a highly effective dose delivery method for certain tumours such as prostate and gynaecological cancers. The third method of the radiation therapy is the systemic administration of radioactive particles which is termed radioisotope therapy. In this method radioactive particles (radionuclides) are injected into the blood stream to be adsorbed specifically by the targeted tissue such as thyroid gland[9]. Gastrointestinal radiation injury most commonly occurs following external beam therapy.

A highly precise radiation delivery can be achieved through newer techniques such as image guided radiotherapy techniques which allow verification of the target position on a daily basis to account for internal target motion[10]. Stereotactic radiation therapy[11] can focus a narrow radiation beams on a small target such as early cancer or metastatic lesions[12].

TREATMENT RELATED RISK FACTORS FOR GASTROINTESTINAL INJURY

Radiation dose, fractionation and field size

Radiation dose is a major determinant of the severity of acute and late normal tissue toxicity[13-20], the desired optimal radiation dose is defined as the dose that maximizes the difference between "tumor" and "normal tissue" damage within the sigmoid shape dose-effect relationship curve[11]. With respect to the gastrointestinal tract, the severity of toxicity is reported as Grades of severity to different symptoms or clinical manifestations ranging from minor symptomatic changes to severe life threatening complications. Multiple toxicity grading systems have been developed to assess adverse events of cancer treatment[21]. Generally, Grade 1 and 2 radiation injury are frequent and they are often requiring no treatment although they can cause a considerable effect on patient quality of life. Examples of commonly used toxicity grading system to assess radiation injury severity are the Radiation Therapy Oncology Group[22] and the Common Terminology Criteria for Adverse Events grading system (Table 1)[21]. Radiation dose per fraction and altered fractionation schedules are important factors linked to increased risk of intestinal radiation toxicity[23]. The radiosensitivity of the cell depends on two factors, the intrinsic radiosensitivity which is linearly related to the radiation dose and it is represents the initial slope of the cell survival curve (alpha). The second factor is (beta) which represents the curvature of the cell survival curve and it is a factor of dose-per fraction and dose-rate variations in radiobiology[24].

The alpha/beta ratio represents the dose at which the linear and quadratic components of the Linear-Quadratic model contribute equally to cell killing and has been shown to have a connection to early and late radiation response[25]. In radiotherapy of tumors with long turnover time such as prostate cancer, the alpha/beta ratio is smaller than that of early reacting normal tissues. In this case, hypo-fractionation will be a better strategy for radiotherapy than the many small fractions used for other tumors[24,26,27].

A data analysis of 918 head and neck cancer patients reported a variable prevalence of mucositis between patients treated with continuous hyperfractionated accelerated radiotherapy (CHART) and patients received conventional fractionation radiotherapy. The incidence of Grade 3 confluent mucositis reported after CHART was 75% compared to 44% following conventional fractionation radiotherapy[28].

Modification of the radiation delivery regimes through

Table 1 Example of some gastrointestinal symptoms grades following radiation injury

Grade	Gastrointestinal symptoms
	Nausea
1	Loss of appetite without alteration in eating habits
2	Oral intake decreased without significant weight loss, dehydration or malnutrition; IV fluids indicated < 24 h
3	Inadequate oral caloric or fluid intake; IV fluids, tube feedings, or TPN indicated ⩾ 24 h
4	Life-threatening consequences
5	Death
	Anorexia
1	Loss of appetite without alteration in eating habits
2	Oral intake altered without significant weight loss or malnutrition; oral nutritional supplements indicated
3	Associated with significant weight loss or malnutrition (e.g., inadequate oral caloric and/or fluid intake); IV fluids, tube feedings or TPN indicated
4	Life-threatening consequences
5	Death
	Haemorrhage-GI
1	Mild, intervention (other than iron supplements) not indicated
2	Symptomatic and medical intervention or minor cauterization indicated
3	Transfusion, interventional radiology, endoscopic, or operative intervention indicated; radiation therapy (i.e., hemostasis of bleeding site)
4	Life-threatening consequences; major urgent intervention indicated
5	Death
	Ulceration-GI
1	Asymptomatic, radiographic or endoscopic findings only
2	Symptomatic; altered GI function (e.g., altered dietary habits, oral supplements); IV fluids indicated < 24 h
3	Symptomatic and severely altered GI function (e.g., inadequate oral caloric or fluid intake); IV fluids, tube feedings, or TPN indicated ⩾ 24 h
4	Life-threatening consequences
5	Death
	Incontinence anal
1	Occasional use of pads required
2	Daily use of pads required
3	Interfering with ADL; operative intervention indicated
4	Permanent bowel diversion indicated
5	Death

According to the Common Terminology Criteria for Adverse Events system v 3.0. IV: Intravenous; GI: Gastrointestinal; ADL: Activities of daily living; TPN: Total parenteral nutrition.

Table 2 Summary of risk factors for gastrointestinal radiation injury

Risk factors	
Radiation techniques	Treatment volume, total dose, fractionation dose and schedules
Combined modality therapies	Surgery Chemotherapy: Particularly concurrent
Medical co-morbidities	Vascular disease, connective tissue disease, inflammatory bowel disease, HIV
Genetic susceptibility	Single nucleotide polymorphism, ataxia telangiectasia

HIV: Human immunodeficiency virus.

hypofractionation was also suggested to be safer than conventionally fractionated conformal radiotherapy in a randomized study of prostate cancer radiotherapy[29].

Treatment field size and intestinal volume irradiated are important factor and a key determinant of radiation toxicity. Bowel toxicity was found to be directly related to the volume of small intestine irradiated[13]. Moreover, irradiation to a larger volume of small intestine was reported to increase the operative mortality in rectal cancer patients treated with anterior and posterior field irradiation technique[30,31]. Furthermore, the impact of the field volume has been demonstrated in a randomized study of prostate cancer radiotherapy. Patients who were treated with conformal shielding with 48% less volume irradiation had less rectal toxicity at 5 years than patients treated with conventional radiotherapy, despite identical radiation dose[32,33] (Table 2).

Combined modality approaches

Combined modality therapy increases the risk of radiation toxicity. Surgery or concurrent chemotherapy is linked to an increased incidence of radiation toxicity. Previous abdominal surgery increases the risk of radiation toxicity[34]. Anatomical changes that increases intestinal exposure to radiation such as postoperative small intestine prolapse into the pelvic cavity[13,35] or surgical adhesions that fix intestinal segments within the radiation field can all predispose part of the intestine to receive higher doses of radiation[36]. Combining prostatectomy with radiotherapy can increase rectal toxicity during prostate cancer treatment[37]. An analysis of acute toxicity was performed in 405 prostate patients in The European Organization for Research and Treatment of Cancer randomized trial 22863. In those patients it was reported that among other factors, previous genitourinary surgery was found to be predictive of lower gastrointestinal radiation toxicity[38].

Combining chemotherapy with radiation has been reported to increase the rate of acute intestinal toxicity, while the long term effect of this combination is not clear[20,39]. Oral mucositis was reported in more than 90% of patients treated with a combined chemo-radiotherapy regime for head and neck cancer[40,41] in comparison to another study which reported an incidence of oral mucositis in 62% of patients treated with radiotherapy alone[42]. Concurrent chemotherapy with radiation has also been reported to increase the risk of oesophageal radiation injury by 12-fold[43].

In a study of cervical cancer patients treated with chemoradiotherapy, the incidence of Grade 3 late intestinal toxicity increased from 10% to 26% in patients treated with both mitomycin and fluorouracil compared to a fluorouracil alone group, suggesting a possible role for the type of chemotherapeutic agent used as determinant of severity of gastrointestinal toxicity[44].

Different mechanisms have been suggested to explain the sensitizing effect of adding chemotherapy in increasing the risk of intestinal radiation injury. Examples of possible mechanisms are alterations in cell cycle kinetics, or synchronization of replicating cell populations. Halopyrimidines such as fluorouracil, fluorodeoxyuridine and iododeoxyuridine may sensitize tumors both by inhibiting effective DNA repair and by increasing the amount of radiation induced DNA damage[45-47].

SYMPTOMS RESULTING FROM RADIATION INJURY TO THE GASTROINTESTINAL TRACT

Acute and chronic gastrointestinal radiation injury

During external beam radiotherapy, ionising radiation enters and exits the body and therefore affects normal tissues surrounding the target tumour. The gastrointestinal tract which extends over a large surface area and any part of the gastrointestinal tract that falls within the radiation field can be affected, resulting in acute and chronic symptoms of gastrointestinal radiation injury (Table 3).

Clinical manifestations of gastrointestinal radiation injury can present during or soon following radiotherapy. These symptoms are related to acute mucosal injury and inflammation. Delayed symptoms occur a few months or years after radiotherapy and are attributed to a chronic process of transmural fibrosis and vascular sclerosis. Typically symptoms are considered "acute" if they occur within the course of treatment or up to 90 d following treatment. These are generally reversible. Chronic side effects are much less common and occur more than 90 d post radiation; they are less likely to reverse. The onset of delayed symptoms has been reported as much as after 30 years following radiotherapy[48].

Mouth, pharynx, and oesophagus

During radiotherapy for head and neck or thoracic cancer, the upper gastrointestinal tract falls within the radiation field. Irradiation to this area results in acute mucosal injury causing mucositis and ulceration which manifests within the first two weeks in 30%-60% of patients, causing dysphagia and odynophagia[49]. In a study of 254 non-small-cell lung cancer patients, acute toxicity of Grade 2 or worse has been reported in 78% of patients[50]. Mucositis is debilitating, can be a dose limiting side effect and

Table 3 Acute and chronic manifestations of gastrointestinal radiation injury

Clinical manifestations	Radiation tolerance dose TD5/5, TD50/5 (Gy)	Gastrointestinal organ
Oral mucositis occurs in > 90% of patients with concurrent chemotherapy[40] Xerostomia and altered saliva composition	Parotid gland: TD5/5 (32) TD50/5 (46)[60,179,180]	Mouth, salivary glands, hypopharynx, parotid
Acute Grade 3-4 oesophageal injuries occur in 46%with concurrent chemotherapy[181]	TD5/5 (55-60)	
Dose > 58 Gy predicts Grade 3-5 acute oesophagitis[54] 60 Gy resulted in Grade 3 toxicity in 42%[182] Radiation can lead to late stricture and/or perforation of the oesophagus[53,60]	TD50/5 (68-72)[60,179]	Oesophagus
40 Gy: Severe late toxicity in 7% including ulceration, gastritis and small-bowel obstruction/perforation[183]	TD5/5; (50-60) TD50/5 (65-70)[60,179]	Stomach
Elevated liver enzymes in 5%[184]	TD5/5; (30-50)	Liver Small intestine
(31.3-37 Gy resulted in RILD in 9.4%[66,185]	TD50/5; (40-55)[60,179]	
45 Gy cause 5% Grade 3-4 toxicity and 14% with concurrent chemotherapy[71]	TD5/5; (40-50)	
Diarrhoea, abdominal pain in 20-70%[72] Transmural fibrosis leading to obstruction in 5%-10%[68,77-78]	TD50/5; (55-60)[60,179,180]	
Intestinal fistulation occurs at a rate of 0.6% to 4.8%[68,79] 50 Gy 5 year estimate of small bowel obstruction is 11%[186]		Colon and rectum
Colitis in 25%-50% of patients[186]	Colon	
Grade 2-3 acute proctitis 40%[91]	TD5/5; (45-55)	
Chronic rectal symptoms in 6.7%-31%[91]	TD50/5 (55-65)[60,179,180]	
Acute symptoms of anus and rectal injury occur in up to 75% of patients during radiotherapy[187]	Rectum TD5/5; (60-61.38) TD50/5 (80-81.38)[60,179,180]	

TD5/5: Radiation dose associated with 5% of patients' risk of delayed toxicity in 5 years; TD50/5: The radiation dose associated with 50% of patients' risk of delayed toxicity in 5 years. RILD: Radiation-induced liver disease.

is difficult to treat. Severe symptoms may require therapy interruption, or the provision of an alternative nutritional route to avoid dehydration and malnutrition. Therefore, elective percutaneous endoscopic gastrostomy tube insertion before radiotherapy is a recognized practice and it is associated with improved quality of life and a lower rate of hospital admissions[51,52]. In severe cases, acute radiation oesophagitis can lead to more serious complications such as significant bleeding or oesophageal perforation[53].

Clinical predictors for acute oesophageal toxicity include maximum radiation dose. A maximum dose of > 58 Gy was reported to predict the risk of Grade 3-5 oesophageal toxicity in 207 non-small-cell lung cancer patients treated with 3-dimensional conformal radiotherapy[54] while a dose of 50 Gy was significantly associated with Grade 2 or worse oesophagitis in another cohort 36 non-small-cell lung cancer patients[55]. In both studies patients received concurrent chemotherapy which is also a risk factor for oesophageal toxicity. The volume of the irradiated oesophagus has also been suggested to predict acute oesophageal toxicity[55]. In another series of 208 non-small-cell lung cancer patients treated with three dimensional conformal radiotherapy, concurrent chemotherapy and maximal point dose to the oesophagus > 60 Gy were found to be significantly associated with the risk of Grade 3-5 oesophageal injury[56]. The Quantitative Analyses of Normal Tissue Effects in the Clinic paper by Werner-Wasik *et al*[57] published in 2010 reviewed the published data on the dose-volume effect and concluded that it was not possible to identify the best threshold volumetric parameter for oesophageal irradiation given the variety of the volumetric metrics in the published data.

Delayed symptoms of oesophageal injury can manifest after several months following radiotherapy and include chronic ulceration, fistulisation or chronic dysphagia. Dysphagia can be secondary to tissue fibrosis and stricture formation or due to motility disorder induced by muscular or nerve injuries. Delayed oesophageal toxicity has been reported in 17% of non-small-cell lung cancer patients[50] and the median and maximal time to the onset of late toxicity was 5 and 40 mo respectively after radiotherapy.

Stomach and duodenum

Gastric injury during radiotherapy occurs when the stomach falls within the radiation field of an adjacent tumour. Nausea, vomiting, dyspepsia and abdominal pain has been reported to occur early after radiotherapy to the upper abdomen in 50% of patients[58]. These symptoms result from acute mucosal injury causing erosions and ulceration of gastric and duodenal mucosa.

Later symptoms include chronic dyspepsia and abdominal pain due to chronic ulceration secondary to mucosal injury[59]. Rarely, gastric wall fibrosis can lead to gastric outlet obstruction. The radiation dose associated with 5% and 50% of patients risk of delayed gastric toxicity in 5 years (TD5/5 and TD50/5) have been estimated at 50 Gy and 65 Gy respectively for gastric ulceration or perforation. An accurate data on dose-volume constrain for partial gastric irradiation is not available. However, the threshold dose of 45 Gy to the whole stomach has been associated with ulceration in 5%-7% of patients[60,61].

Liver injury

Following irradiation to the liver, radiation induced liver disease can occur in patients with normal pre-radiotherapy liver function, causing anicteric hepatomegaly and mild alkaline phosphatase serum level elevation. A more

severe derangement of liver function occurs in patients with pre-existing liver disease[62]. Radiation induced liver disease can progress to fibrosis, cirrhosis, and liver failure[63]. Abdominal imaging with computed tomography scan or magnetic resonance imaging can be helpful to show low-attenuation injury areas or areas of atrophy in the irradiated liver segment[64].

Risk factors for radiation induced liver disease include baseline liver dysfunction, Hepatitis B virus carrier status[65], mean dose > 30 Gy for partial liver radiotherapy, concurrent chemotherapy and volume of liver irradiated[66,67].

Small intestine

The small intestine receives irradiation during radiotherapy of pelvic or abdominal malignancies. The degree of injury depends on the radiation dose and the volume of intestinal segment that falls within the radiation field[68,69]. Significant correlation has been suggested between the volume of irradiated small bowel and the likelihood of acute toxicity, regardless of the radiation dose delivered[70]. Other predictors of acute small intestine toxicity include the use of concurrent chemotherapy. This effect has been reported in 186 cervical cancer patients who received 45 Gy preoperative pelvic radiotherapy alone where 5% of patients experienced Grade 3-4 toxicity in comparison to 14% of 183 patients who received radiotherapy and weekly cisplatin[71]. The relatively fixed portions of the small intestine such as the duodenum and the terminal ileum are at increased risk of radiation toxicity as they are more susceptible to receive higher doses of radiation than the mobile parts of small intestine.

The radiation dose associated with delayed small bowel toxicity have been estimated by Emami *et al*[60]. The TD5/5 and TD50/5 doses for one third of small bowel irradiation were estimated at 50 Gy and 60 Gy respectively. The TD5/5 and TD50/5 for the whole-organ irradiation were 40 Gy and 55 Gy respectively. These doses estimates remained as a guide for two decades and more recent data were consistent with these ranges[60,61].

Clinically, nausea, vomiting and abdominal pain are early symptoms that can occur during the first two weeks following abdominal radiotherapy, and may be mediated by the release of inflammatory cytokines following radiotherapy. Diarrhoea and abdominal pain occur during the first two weeks of radiotherapy to abdominal or pelvic malignancies in 20% to 70% of patients[72]. This may be a result of direct radiation injury to the small intestinal mucosa causing epithelial atrophy, and reduced mucosal blood flow[73].

The acute symptoms usually settle within three weeks after completion of radiotherapy and the intestinal epithelium regenerates from stem cells at the base of the crypts.

Delayed symptoms of radiation small intestinal injury manifest months to years after radiotherapy with symptoms of diarrhoea, recurrent abdominal pains and malabsorption.

Chronic diarrhoea following radiotherapy can result from different pathophysiological processes such as bile salt malabsorption, bacterial overgrowth, fat malabsorption, rapid intestinal transit or lactose intolerance[74]. More chronic symptoms occur as a result of pathological abnormalities to the intestinal vascular compartment resulting in intestinal ischemia as well as progressive intestinal fibrosis leading to structural abnormalities such as strictures and fistulation. Bacterial overgrowth contributes to malabsorption and diarrhoea, particularly in patients with intestinal strictures[73,75].

Intestinal obstruction can complicate 5% to 10% of severe small intestinal radiation injury[76]. The rate of severe small intestinal complications following radiotherapy for rectal cancer can vary considerably depending on the tumor and treatment characteristics, Reports indicate rates of 0.8% to 13% for small intestinal obstruction[68,77,78] and 0.6% to 4.8% for intestinal fistulation[68,79]. Patients with severe small intestinal injury have a poor prognosis since surgery to manage strictures is complex and has been associated with poor outcomes[73,80].

Colon and rectum

During abdominal and pelvic radiotherapy, the colon and rectum are commonly affected as their anatomical locations fall within the radiation field of a variety of cancers. The fixed portions of the colon, the caecum and the rectum are at greater risk of receiving higher doses of radiation than the remainder of the colon.

Acute radiation injury to the rectum and anal canal can result in a diversity of symptoms such as abdominal pain, diarrhoea, tenesmus, rectal pain, urgency, rectal discharge, incontinence, and fresh rectal bleeding. These symptoms occur primarily as a consequence of direct mucosal damage[81-83]. Acute radiation injury to the colon can be severe and in 5%-15% can lead to therapy interruption or treatment plan alteration[84].

A recent study showed that 47% of women who received radiotherapy for cervical or endometrial cancer reported symptoms of radiation intestinal injury affecting quality of life within 3 mo following therapy completion[85]. These results are consistent with a previous structured questionnaire study[86] which showed that 53% of patients had reported bowel symptoms significantly affecting their quality of life, whilst 81% of patients in the study described new-onset gastrointestinal problems after receiving radiotherapy.

The recent data on dose-volume effect in radiation induced rectal injury was reviewed by Michalski *et al*[87]. The incidence of Grade > 2 injury from different studies was variable according to dose, treatment parameters and scale used in each study. Among the recent studies reported, an incidence of 13.5% and 16% of Grade > 2 rectal injury. Identified predictors for Grade > 2 rectal injury or rectal bleeding include the volume of the rectum irradiated and a total radiation dose > 60 Gy during 3-dimensional conformal radiotherapy. The effect of concurrent chemotherapy has been observed in the

European Organization for Research and Treatment of Cancer study where patients received 45 Gy preoperative radiotherapy or radiotherapy and 5-flurouracil (5-FU). Grade > 2 diarrhoea occurred in 17% of radiotherapy alone group compared to 38% of radiotherapy and 5-FU group[88].

Delayed symptoms of radiation colonic injury are insidious and usually follow a progressive course. They can manifest after a latent period of few months or years. One study has reported a 15% incidence of bowel toxicity 20 years after receiving radiotherapy in a cervical cancer cohort[89]. Severe life threatening complications occur after radiotherapy such as fistulation, sepsis, perforation or bleeding at a rate between 4%-8% within 5-10 year time after radiotherapy[90]. Patients suffer from a variety of symptoms, such as abdominal pain, changing bowel habits with intermittent diarrhoea. Constipation can result from altered colonic motility due to fibrosis and stricture formation. An abnormal bowel transit can manifest as recurrent pain and increased risk of obstruction or pseudo-obstruction secondary to fecal loading. Excessive fibrosis can cause loss of ano-rectal compliance and manifests as urgency and frequency[73]. Faecal incontinence has been reported in up to 20% of patients and significantly reduces patients' quality of life[86,91].

Unlike radiation injury to the small bowel, radiation injury to the colon does not compromise nutrient absorption and malabsorption is uncommon[73].

Adverse effects of radiation to the pelvis primarily affect the colon and the rectum. However, other organs can be irradiated causing increased morbidity for example injury to the urinary tract or the genital system resulting in symptoms affecting quality of life were reported in 30% of patients[92-95]. A rare but serious complication of prostate brachytherapy is recto-vesical fistula which occurs with a low frequency of 1 in 250 to 1 in 1000 patients implanted[96-98].

Radiation exposure increases the risk of a secondary malignancy. Patients who received radiotherapy were shown to have significantly higher risk of developing second cancers both overall and in the areas that were exposed to the radiation field[99]. In an analysis of testicular cancer survivors which included 28 843 men, the risk of a second cancer was estimated. The patterns of second cancer suggested that many factors may be involved, including previous treatment received, but the precise roles of different factors is still to be clarified. It has been reported that secondary leukaemia was associated with both radiotherapy and chemotherapy, whereas excess cancers of the stomach, bladder, and possibly pancreas were associated mainly with radiotherapy[100]. This risk also includes colorectal cancer, which can occur more than 10 to 20 years after radiation exposure[99,101].

PATIENT RELATED RISK FACTORS

Patient factors and individual variations

Individual patient phenotypic factors have been suggested to influence the susceptibility to intestinal radiation injury. It was reported that older patient age is associated with an increased risk of developing reduced organ function after radiotherapy[102-104]. Body habitus has been reported as another susceptibility factor, where thin patients with narrow antero-posterior diameter can suffer an increased risk of intestinal radiation toxicity compared to normal individuals[36]. Smoking status has been associated with risk of chronic intestinal toxicity[20,105] as well as previous history of surgery[13,35,36].

Medical co-morbidities

Vascular disease: Co-morbid vascular disease such as hypertension, diabetes mellitus and atherosclerosis were suggested to predispose patients to an increased vascular injury following radiation and subsequent intestinal wall ischemia and impaired tissue repair[74]. The microocclusive vascular disease in addition to increased blood viscosity in diabetes mellitus were suggested to predispose to intestinal tissue ischemia[106,107]. One study investigated the possible effect of diabetes mellitus during prostate cancer radiotherapy. The study reported higher rates of late genitourinary/gastrointestinal toxicities in diabetic patients than in non-diabetics (34% and 23% respectively). It was also noticed that diabetics developed complications earlier than the non-diabetics (10 mo and 24 mo respectively)[108].

Inflammatory bowel disease: Co-morbid inflammatory bowel disease (IBD) is considered in some institutions as a relative contraindication to radiotherapy for concerns of greater risk of acute and late side effects[109-111]. Intolerance to radiotherapy in IBD patients has been demonstrated in case reports[112,113] and in a larger retrospective analysis where the incidence of severe acute and late events was 21% and 29% respectively[114,115]. However, in a large retrospective analysis in patients with colorectal cancer, the data on treatment modality received for 170 colorectal cancer patients with history of IBD found no significant difference in cancer treatment modalities between patients with or without history of IBD. This observation points out that a history of IBD was not a barrier to receive radiotherapy treatment in this patient group[116].

It has been postulated that co-morbid IBD and intestinal inflammation may alter the acute tissue response to radiotherapy through inflammatory mediators, growth factors and cytokine cascades produced at the site of intestinal injury. Some mediators and cytokines were suggested to decrease the sensitivity to radiation injury e.g., fibroblast growth factor 2, prostaglandin-E2, tumor necrosis factor-α and interleukin (IL)-1 and IL-11. However, others were suggested to have mixed effects e.g., IL-12 protecting bone marrow-derived cells but sensitizing intestinal epithelial cells to radiation injury[117-123].

Collagen vascular diseases: Collagen vascular diseases (CVD) increases the risk of both acute and chronic radia-

tion toxicity, as has been reported by Chon *et al*[105] in 4 different studies in patients with and without CVD. On the other hand, radiation may cause an acute exacerbation of systemic symptoms in patients with CVD[124], possibility through release of fibroblast-triggering mediators by the inflammatory cells[105].

Human immunodeficiency virus infection: It has been reported that human immunodeficiency virus (HIV) infection induces a state of radiosensitivity because severe mucositis was observed in HIV patients who received radiotherapy for the treatment of Kaposi sarcoma[125,126]. Support for this hypothesis was found by an increased radiosensitivity of skin fibroblasts of HIV patients with Kaposi sarcoma compared to healthy control[127]. It was also noted in a study involving 59 HIV positive patients that T-lymphocytes of HIV infected individuals were considerably more sensitive to X-rays compared to that of HIV negative donors[128]. Housri *et al*[129] reviewed the recent evidence and suggested recommendations for radiotherapy in HIV patients, based on the strength of the best available evidence, and classified according to Strength of Recommendation Taxonomy. There was no conclusive evidence to support the need for special precautions for HIV patients during radiotherapy[130].

Genotypic variations

It has been suggested that patient's genotype may impact their individual susceptibility to radiation toxicity. This can occur through inherited germ-line mutations in genes involved in DNA damage detection, DNA repair or cell cycle regulation[131-133]. Recently the term Radiogenomics has been introduced to refer to the science that aims to predict clinical radiosensitivity and to optimize radiotherapy treatment from individual genetic profiles[134].

Genetic variations are thought to be a key determinant of normal tissue radiosensitivity and may account for up to 80% of the inter-individual variations in normal tissue reaction to radiotherapy[135,136]. Support for this hypothesis was provided in a study of breast cancer radiotherapy, which reported the incidence and time to development of radiation-induced telangiectasia[137]. The results of the study revealed a wide range of variation suggesting that patient-related factors can explain 81%-90% of the patient-to-patient variation in telangiectasia level seen after radiotherapy despite similar radiation treatment given. The results further supported reports of other studies[138,139].

The state of extreme tissue radiosensitivity which has been identified in patients with germ-line mutations in genes involved in DNA damage detection or DNA repair e.g., Nijmegen breakage syndrome, Fanconi's anemia and Ataxia telangiectasia has supported the potential role of genetic variations as an important determinant of individual's radiation response. Nevertheless, this risk is probably confined to patients and carriers of those mutant genes and is not known to be relevant to other patients receiving radiotherapy[31,45,140,141].

Candidate gene studies, [single nucleotide polymorphism (SNP) association studies] have investigated the role of many genes which have been linked to different elements of the mechanisms related to the pathogenesis of radiation toxicity. Genes investigated include those involved in DNA repair such as *ATM*, *BRCA1*, *BRCA2*[142,143], apoptosis such as *TP53, BCL2*[144,145], antioxidant enzymes such as *SOD1*[146], and growth factors *FGF2*[147,148] and *VEGF*[147,148]. In this regard, an association has been suggested between candidate SNPs in the genes *TGFB1*, *SOD2*, *XRCC3*, *XRCC1* and late radiation toxicity in breast cancer patients[133,149]. Similarly, SNP association studies in pelvic tissue have suggested correlations between some risk genes such as *XRCC1*, *XRCC3*, *TGFB1*, *OGG1* and an increased risk of developing late gastrointestinal and genitourinary radiation toxicity following radiotherapy[150-152].

MECHANISMS OF RADIATION INJURY

Ionising radiation induces double strand breaks in DNA. This triggers activation of a signalling pathway that leads to activation of tumour suppressor p53. Depending on the extent of the DNA damage, which depends on the radiation dose, and on other factors in the cellular milieu, p53 activation leads to cell cycle arrest and DNA double strand break repair, or apoptosis. In cancer radiotherapy, apoptosis of tumour cells is the desired outcome. However, intestinal crypt epithelial cells are quite sensitive to radiation and the killing of these cells leads to mucosal injury[153,154]. Specifically, when the dose of radiation is sufficient to kill all of the epithelial stem cells in a crypt, then as the epithelial cells migrate up the crypt and are eventually shed into the intestinal lumen; the crypt cannot be repopulated with epithelial cells, and consequently involutes. When this happens to a large proportion of crypts in a region of intestine, normal barrier function is lost which leads to the exposure of the normally sterile lamina propria to luminal microbes. This triggers an acute inflammatory response associated with immune cellular infiltrates; T lymphocytes, macrophages and neutrophils causing loss of epithelial cells as well as degradation of the extracellular matrix in the lamina propria due to enzymes and mediators released by the immune cells[155]. A further damage to the mucosal and submucosal tissues are caused by reactive oxygen metabolites which are produced in large amounts by activated leukocytes in the inflamed mucosa and this can induce significant damage to various cellular components, including structural and regulatory proteins, carbohydrates, lipids, DNA, and RNA[31].

During radiotherapy, ionising radiation kills crypt epithelial stem cells. As a result, crypts involute and epithelial barrier integrity is lost. This provides access of luminal microbes and their products to innate immune cells in the lamina propria, with activation of immune cells. An impaired recognition of bacterial translocation can further exacerbate the inflammatory process and promote

stricture formation by two possible mechanisms. The bacterial wall antigens could causes a secondary excessive up regulation of pro-inflammatory transcription factors, such as nuclear factor kappaB[156]. This might be followed by prolonged macrophage activation and induction of NADPH oxidase expression[157] leading to a further increased in oxygen radical secretion to eradicate bacteria leading to further tissue destruction[155]. Meanwhile translocated bacteria could directly stimulate neighboring mesenchymal cells via pattern recognition receptors leading to increased activation of immune cells[156,158].

The acute inflammatory process continues but eventually, after the cessation of radiation, through poorly understood mechanisms, crypts start to regenerate and this restores normal epithelial barrier function, which is followed by resolution of inflammation[159]. In some patients this inflammatory process becomes exaggerated for unknown reasons with severe ulceration and inflammatory process runs a chronic course characterised by extensive fibrosis and intestinal ischemia[160,161]. Recent observations in animal models of radiation injury indicate that repair after radiation may depend on the recruitment of mesenchymal stem cells from the bone marrow to the site of radiation injury. Mesenchymal stem cell mobilization and engraftment is thought to be induced by cytokines and potentially specific homing induced by chemokines, all of these are released by inflammation[162,163].

The final pathological outcome of the radiation injury in the intestinal tissue will depend on a complex crosstalk between various cellular components of the tissue within the extracellular matrix which eventually determine the process of tissue recovery or long term complications[73,164].

Radiation injury to the vascular compartment is thought to be a key feature in the pathological processes of intestinal radiation injury as well as an important determinant of both acute and chronic effects after radiotherapy[165,166]. It has been regarded as a major component in the initiation, progression and maintenance of delayed intestinal tissue damage and enhanced fibrosis which lead to loss of mucosal function and stricture formation[167,168].

Endothelial cell apoptosis has been implicated as the primary lesion leading to epithelial stem cell dysfunction and subsequent intestinal tissue damage following radiotherapy. Support for this hypothesis was found by identifying a state of radioresisitance following inhibition of endothelial apoptosis in experimental mice. Radiation-induced crypt damage, organ failure, and death from radiation injury were all prevented when the endothelial apoptosis was inhibited pharmacologically, by the administration of fibroblast growth factor or genetically by deletion of the acid sphingomyelinase gene[169]. However, subsequent studies challenged this concept by demonstrating an epithelial cell apoptosis at lower radiation doses which is insufficient to cause endothelial cell death. This result has been enforced further by experiments using high dose Boron therapy radiation, specifically targeted to the endothelium. The results showed no effect on epithelial stem cell survival[168,170].

Formation of new blood vessels (angiogenesis) is a crucial requirement for tumour growth and survival[171]. The tumour vasculature is prone to hypoxia which results in further production of proangiogenic factors by tumour cells. Angiogenesis inhibitors target tumour endothelial cells and cause inhibition of new vessel formation and a transient tumour hypoxia[172,173]. Although tumour hypoxia has been linked to increasing tumour radio-resistance, studies have shown that the administration of angiogenesis inhibitors improves tumour oxygenation and response to radiotherapy[174-177]. Different mechanism has been suggested for the radio-sensitising effect of combining angiogenesis inhibitors with radiotherapy[173]. Mazeron *et al*[178] has recently reviewed the clinical trials on angiogenesis inhibitors, but despite the promising value of these new agents, the biological basis for their synergistic effect and the safety and efficacy of these agents are still to be determined.

CONCLUSION

Intestinal radiation injury is a significant clinical issue which is expected to increase in prevalence due to improved survival of cancer patients as well as to increased availability of radiotherapy as an affordable treatment option. Radiotherapy treatment can cause a wide variety of gastrointestinal side effects. Following radiation injury to the gastrointestinal tract, symptoms can present acutely or after a long period of time. Although severe intestinal injury is less common with the development of advanced radiotherapy planning and delivery techniques, a less severe degree of injury is common and continues to affect a considerable proportion of patients, significantly reduces their quality of life, ands an extra burden to the cost of health care. The accurate diagnosis and management of intestinal radiation injury represents a clinical challenge to practicing physicians in both gastroenterology and oncology. Despite the growing recognition of the problem and some advances in understanding the cellular and molecular mechanisms of radiation injury, relatively little is known about the pathophysiology of intestinal radiation injury or the exact factors that aggravate it. Patient and treatment related risk factors have been suggested although the exact influence posed by these factors is still to be better characterized. Combined modality therapies for cancer are commonly used and they increase the risk of radiation toxicity and further add to the problem. Medical co-morbid diseases such as vascular disease, inflammatory bowel disease and collagen vascular disease can pose a significant risk that can affect patient suitability to receive radiotherapy treatment. Genotypic variations can influence the risk of gastrointestinal radiation injury but future research findings on this area are needed to assess their clinical importance. A better understanding of the pathophysiology of radiation injury may provide the opportunity to develop more effective preventive and therapeutic strategies.

ACKNOWLEDGMENTS

The Health Research Board, Ireland.

REFERENCES

1 **Meyer J**, Czito B, Yin FF, Willett C. Advanced radiation therapy technologies in the treatment of rectal and anal cancer: intensity-modulated photon therapy and proton therapy. *Clin Colorectal Cancer* 2007; **6**: 348-356 [PMID: 17311699 DOI: 10.3816/CCC.2007.n.003]

2 **Meyer JJ**, Willett CG, Czito BG. Emerging role of intensity-modulated radiation therapy in anorectal cancer. *Expert Rev Anticancer Ther* 2008; **8**: 585-593 [PMID: 18402525 DOI: 10.1586/14737140.8.4.585]

3 **Willett CG**. Technical advances in the treatment of patients with rectal cancer. *Int J Radiat Oncol Biol Phys* 1999; **45**: 1107-1108 [PMID: 10613301]

4 **Melian E**, Mageras GS, Fuks Z, Leibel SA, Niehaus A, Lorant H, Zelefsky M, Baldwin B, Kutcher GJ. Variation in prostate position quantitation and implications for three-dimensional conformal treatment planning. *Int J Radiat Oncol Biol Phys* 1997; **38**: 73-81 [PMID: 9212007]

5 **Ling CC**, Burman C, Chui CS, Kutcher GJ, Leibel SA, LoSasso T, Mohan R, Bortfeld T, Reinstein L, Spirou S, Wang XH, Wu Q, Zelefsky M, Fuks Z. Conformal radiation treatment of prostate cancer using inversely-planned intensity-modulated photon beams produced with dynamic multileaf collimation. *Int J Radiat Oncol Biol Phys* 1996; **35**: 721-730 [PMID: 8690638]

6 **Fraass BA**, Kessler ML, McShan DL, Marsh LH, Watson BA, Dusseau WJ, Eisbruch A, Sandler HM, Lichter AS. Optimization and clinical use of multisegment intensity-modulated radiation therapy for high-dose conformal therapy. *Semin Radiat Oncol* 1999; **9**: 60-77 [PMID: 10196399]

7 **Drzymala RE**, Mohan R, Brewster L, Chu J, Goitein M, Harms W, Urie M. Dose-volume histograms. *Int J Radiat Oncol Biol Phys* 1991; **21**: 71-78 [PMID: 2032898]

8 **Niemierko A**, Goitein M. Dose-volume distributions: a new approach to dose-volume histograms in three-dimensional treatment planning. *Med Phys* 1994; **21**: 3-11 [PMID: 8164585 DOI: 10.1118/1.597361]

9 **Clarke SE**. Radionuclide therapy in oncology. *Cancer Treat Rev* 1994; **20**: 51-71 [PMID: 8293428 DOI: 10.1016/0305-7372(94)90010-8]

10 **Chung HT**, Xia P, Chan LW, Park-Somers E, Roach M. Does image-guided radiotherapy improve toxicity profile in whole pelvic-treated high-risk prostate cancer? Comparison between IG-IMRT and IMRT. *Int J Radiat Oncol Biol Phys* 2009; **73**: 53-60 [PMID: 18501530 DOI: 10.1016/j.ijrobp.2008.03.015]

11 **Ikushima H**. Radiation therapy: state of the art and the future. *J Med Invest* 2010; **57**: 1-11 [PMID: 20299738]

12 **Lo SS**, Fakiris AJ, Chang EL, Mayr NA, Wang JZ, Papiez L, Teh BS, McGarry RC, Cardenes HR, Timmerman RD. Stereotactic body radiation therapy: a novel treatment modality. *Nat Rev Clin Oncol* 2010; **7**: 44-54 [PMID: 19997074 DOI: 10.1038/nrclinonc.2009.188]

13 **Letschert JG**, Lebesque JV, de Boer RW, Hart AA, Bartelink H. Dose-volume correlation in radiation-related late small-bowel complications: a clinical study. *Radiother Oncol* 1990; **18**: 307-320 [PMID: 2244018 DOI: 10.1016/0167-8140(90)90111-9]

14 **Mak AC**, Rich TA, Schultheiss TE, Kavanagh B, Ota DM, Romsdahl MM. Late complications of postoperative radiation therapy for cancer of the rectum and rectosigmoid. *Int J Radiat Oncol Biol Phys* 1994; **28**: 597-603 [PMID: 8113102]

15 **Baglan KL**, Frazier RC, Yan D, Huang RR, Martinez AA, Robertson JM. The dose-volume relationship of acute small bowel toxicity from concurrent 5-FU-based chemotherapy and radiation therapy for rectal cancer. *Int J Radiat Oncol Biol Phys* 2002; **52**: 176-183 [PMID: 11777636]

16 **Gunnlaugsson A**, Kjellén E, Nilsson P, Bendahl PO, Willner J, Johnsson A. Dose-volume relationships between enteritis and irradiated bowel volumes during 5-fluorouracil and oxaliplatin based chemoradiotherapy in locally advanced rectal cancer. *Acta Oncol* 2007; **46**: 937-944 [PMID: 17851844 DOI: 10.1080/02841860701317873]

17 **Huang EY**, Sung CC, Ko SF, Wang CJ, Yang KD. The different volume effects of small-bowel toxicity during pelvic irradiation between gynecologic patients with and without abdominal surgery: a prospective study with computed tomography-based dosimetry. *Int J Radiat Oncol Biol Phys* 2007; **69**: 732-739 [PMID: 17531397 DOI: 10.1016/j.ijrobp.2007.03.060]

18 **Tho LM**, Glegg M, Paterson J, Yap C, MacLeod A, McCabe M, McDonald AC. Acute small bowel toxicity and preoperative chemoradiotherapy for rectal cancer: investigating dose-volume relationships and role for inverse planning. *Int J Radiat Oncol Biol Phys* 2006; **66**: 505-513 [PMID: 16879928 DOI: 10.1016/j.ijrobp.2006.05.005]

19 **Minsky BD**, Conti JA, Huang Y, Knopf K. Relationship of acute gastrointestinal toxicity and the volume of irradiated small bowel in patients receiving combined modality therapy for rectal cancer. *J Clin Oncol* 1995; **13**: 1409-1416 [PMID: 7751886]

20 **Theis VS**, Sripadam R, Ramani V, Lal S. Chronic radiation enteritis. *Clin Oncol (R Coll Radiol)* 2010; **22**: 70-83 [PMID: 19897345 DOI: 10.1016/j.clon.2009.10.003]

21 **Trotti A**, Colevas AD, Setser A, Rusch V, Jaques D, Budach V, Langer C, Murphy B, Cumberlin R, Coleman CN, Rubin P. CTCAE v3.0: development of a comprehensive grading system for the adverse effects of cancer treatment. *Semin Radiat Oncol* 2003; **13**: 176-181 [PMID: 12903007 DOI: 10.1016/S1053-4296(03)00031-6]

22 **Cox JD**, Stetz J, Pajak TF. Toxicity criteria of the Radiation Therapy Oncology Group (RTOG) and the European Organization for Research and Treatment of Cancer (EORTC) *Int J Radiat Oncol Biol Phys* 1995; **31**: 1341-1346 [PMID: 7713792]

23 **Denham JW**. Influence of dose-rate on inflammatory damage and adhesion molecule expression after abdominal radiation in the rat. *Int J Radiat Oncol Biol Phys* 2000; **47**: 1460-1461 [PMID: 10939886]

24 **Fowler JF**. The radiobiology of prostate cancer including new aspects of fractionated radiotherapy. *Acta Oncol* 2005; **44**: 265-276 [PMID: 16076699 DOI: 10.1080/02841860410002824]

25 **Thames HD**, Bentzen SM, Turesson I, Overgaard M, Van den Bogaert W. Time-dose factors in radiotherapy: a review of the human data. *Radiother Oncol* 1990; **19**: 219-235 [PMID: 2281152]

26 **Fowler JF**, Chappell RJ, Ritter MA. The prospects for new treatments for prostate cancer. *Int J Radiat Oncol Biol Phys* 2002; **52**: 3-5 [PMID: 11777616]

27 **Brenner DJ**. Hypofractionation for prostate cancer radiotherapy--what are the issues? *Int J Radiat Oncol Biol Phys* 2003; **57**: 912-914 [PMID: 14575821]

28 **Bentzen SM**, Saunders MI, Dische S, Bond SJ. Radiotherapy-related early morbidity in head and neck cancer: quantitative clinical radiobiology as deduced from the CHART trial. *Radiother Oncol* 2001; **60**: 123-135 [PMID: 11439207]

29 **Arcangeli G**, Fowler J, Gomellini S, Arcangeli S, Saracino B, Petrongari MG, Benassi M, Strigari L. Acute and late toxicity in a randomized trial of conventional versus hypofractionated three-dimensional conformal radiotherapy for prostate cancer. *Int J Radiat Oncol Biol Phys* 2011; **79**: 1013-1021 [PMID: 20447774 DOI: 10.1016/j.ijrobp.2009.12.045]

30 **Goldberg PA**, Nicholls RJ, Porter NH, Love S, Grimsey JE. Long-term results of a randomised trial of short-course low-dose adjuvant pre-operative radiotherapy for rectal cancer: reduction in local treatment failure. *Eur J Cancer* 1994; **30A**:

1602-1606 [PMID: 7530469]
31 **Kountouras J**, Zavos C. Recent advances in the management of radiation colitis. *World J Gastroenterol* 2008; **14**: 7289-7301 [PMID: 19109862]
32 **Dearnaley DP**, Khoo VS, Norman AR, Meyer L, Nahum A, Tait D, Yarnold J, Horwich A. Comparison of radiation side-effects of conformal and conventional radiotherapy in prostate cancer: a randomised trial. *Lancet* 1999; **353**: 267-272 [PMID: 9929018 DOI: 10.1016/S0140-6736(98)05180-0]
33 **O'Brien PC**. Radiation injury of the rectum. *Radiother Oncol* 2001; **60**: 1-14 [PMID: 11410298]
34 **Kasibhatla M**, Clough RW, Montana GS, Oleson JR, Light K, Steffey BA, Jones EL. Predictors of severe gastrointestinal toxicity after external beam radiotherapy and interstitial brachytherapy for advanced or recurrent gynecologic malignancies. *Int J Radiat Oncol Biol Phys* 2006; **65**: 398-403 [PMID: 16542793 DOI: 10.1016/j.ijrobp.2005.12.008]
35 **Waddell BE**, Rodriguez-Bigas MA, Lee RJ, Weber TK, Petrelli NJ. Prevention of chronic radiation enteritis. *J Am Coll Surg* 1999; **189**: 611-624 [PMID: 10589598]
36 **Hauer-Jensen M**. Late radiation injury of the small intestine. Clinical, pathophysiologic and radiobiologic aspects. A review. *Acta Oncol* 1990; **29**: 401-415 [PMID: 2202341]
37 **Morgan SC**, Waldron TS, Eapen L, Mayhew LA, Winquist E, Lukka H. Adjuvant radiotherapy following radical prostatectomy for pathologic T3 or margin-positive prostate cancer: a systematic review and meta-analysis. *Radiother Oncol* 2008; **88**: 1-9 [PMID: 18501455 DOI: 10.1016/j.radonc.2008.04.013]
38 **Zurlo A**, Collette L, van Tienhoven G, Blank L, Warde P, Dubois J, Jeanneret W, Storme G, Bernier J, Kuten A, Pierart M, Bolla M. Acute toxicity of conventional radiation therapy for high-risk prostate cancer in EORTC trial 22863. *Eur Urol* 2002; **42**: 125-132 [PMID: 12160582]
39 **Gérard JP**, Conroy T, Bonnetain F, Bouché O, Chapet O, Closon-Dejardin MT, Untereiner M, Leduc B, Francois E, Maurel J, Seitz JF, Buecher B, Mackiewicz R, Ducreux M, Bedenne L. Preoperative radiotherapy with or without concurrent fluorouracil and leucovorin in T3-4 rectal cancers: results of FFCD 9203. *J Clin Oncol* 2006; **24**: 4620-4625 [PMID: 17008704 DOI: 10.1200/JCO.2006.06.7629]
40 **Wilkes JD**. Prevention and treatment of oral mucositis following cancer chemotherapy. *Semin Oncol* 1998; **25**: 538-551 [PMID: 9783593]
41 **Parulekar W**, Mackenzie R, Bjarnason G, Jordan RC. Scoring oral mucositis. *Oral Oncol* 1998; **34**: 63-71 [PMID: 9659522]
42 **Jham BC**, Reis PM, Miranda EL, Lopes RC, Carvalho AL, Scheper MA, Freire AR. Oral health status of 207 head and neck cancer patients before, during and after radiotherapy. *Clin Oral Investig* 2008; **12**: 19-24 [PMID: 17876612 DOI: 10.1007/s00784-007-0149-5]
43 **Werner-Wasik M**, Scott C, Graham ML, Smith C, Byhardt RW, Roach M, Andras EJ. Interfraction interval does not affect survival of patients with non-small cell lung cancer treated with chemotherapy and/or hyperfractionated radiotherapy: a multivariate analysis of 1076 RTOG patients. *Int J Radiat Oncol Biol Phys* 1999; **44**: 327-331 [PMID: 10760427]
44 **Rakovitch E**, Fyles AW, Pintilie M, Leung PM. Role of mitomycin C in the development of late bowel toxicity following chemoradiation for locally advanced carcinoma of the cervix. *Int J Radiat Oncol Biol Phys* 1997; **38**: 979-987 [PMID: 9276362]
45 **Yamada T**. Textbook of Gastroenterology. In: Cohen SBS, editor. Radiation injury in the gastointestinal tract. 4 ed. Lippincott: Williams & Wilkins, 2003: 2760-2771
46 **Heimburger DK**, Shewach DS, Lawrence TS. The effect of fluorodeoxyuridine on sublethal damage repair in human colon cancer cells. *Int J Radiat Oncol Biol Phys* 1991; **21**: 983-987 [PMID: 1833363]
47 **Bruso CE**, Shewach DS, Lawrence TS. Fluorodeoxyuridine-induced radiosensitization and inhibition of DNA double strand break repair in human colon cancer cells. *Int J Radiat Oncol Biol Phys* 1990; **19**: 1411-1417 [PMID: 2148170]
48 **Andreyev J**. Gastrointestinal complications of pelvic radiotherapy: are they of any importance? *Gut* 2005; **54**: 1051-1054 [PMID: 16009675 DOI: 10.1136/gut.2004.062596]
49 **Pico JL**, Avila-Garavito A, Naccache P. Mucositis: Its Occurrence, Consequences, and Treatment in the Oncology Setting. *Oncologist* 1998; **3**: 446-451 [PMID: 10388137]
50 **Ahn SJ**, Kahn D, Zhou S, Yu X, Hollis D, Shafman TD, Marks LB. Dosimetric and clinical predictors for radiation-induced esophageal injury. *Int J Radiat Oncol Biol Phys* 2005; **61**: 335-347 [PMID: 15667951 DOI: 10.1016/j.ijrobp.2004.06.014]
51 **Piquet MA**, Ozsahin M, Larpin I, Zouhair A, Coti P, Monney M, Monnier P, Mirimanoff RO, Roulet M. Early nutritional intervention in oropharyngeal cancer patients undergoing radiotherapy. *Support Care Cancer* 2002; **10**: 502-504 [PMID: 12353130 DOI: 10.1007/s00520-002-0364-1]
52 **Raykher A**, Russo L, Schattner M, Schwartz L, Scott B, Shike M. Enteral nutrition support of head and neck cancer patients. *Nutr Clin Pract* 2007; **22**: 68-73 [PMID: 17242458]
53 **Chowhan NM**. Injurious effects of radiation on the esophagus. *Am J Gastroenterol* 1990; **85**: 115-120 [PMID: 2405641]
54 **Singh AK**, Lockett MA, Bradley JD. Predictors of radiation-induced esophageal toxicity in patients with non-small-cell lung cancer treated with three-dimensional conformal radiotherapy. *Int J Radiat Oncol Biol Phys* 2003; **55**: 337-341 [PMID: 12527046]
55 **Patel AB**, Edelman MJ, Kwok Y, Krasna MJ, Suntharalingam M. Predictors of acute esophagitis in patients with non-small-cell lung carcinoma treated with concurrent chemotherapy and hyperfractionated radiotherapy followed by surgery. *Int J Radiat Oncol Biol Phys* 2004; **60**: 1106-1112 [PMID: 15519781 DOI: 10.1016/j.ijrobp.2004.04.051]
56 **Qiao WB**, Zhao YH, Zhao YB, Wang RZ. Clinical and dosimetric factors of radiation-induced esophageal injury: radiation-induced esophageal toxicity. *World J Gastroenterol* 2005; **11**: 2626-2629 [PMID: 15849822]
57 **Werner-Wasik M**, Yorke E, Deasy J, Nam J, Marks LB. Radiation dose-volume effects in the esophagus. *Int J Radiat Oncol Biol Phys* 2010; **76**: S86-S93 [PMID: 20171523 DOI: 10.1016/j.ijrobp.2009.05.070]
58 **Henriksson R**, Bergström P, Franzén L, Lewin F, Wagenius G. Aspects on reducing gastrointestinal adverse effects associated with radiotherapy. *Acta Oncol* 1999; **38**: 159-164 [PMID: 10227436]
59 **Coia LR**, Myerson RJ, Tepper JE. Late effects of radiation therapy on the gastrointestinal tract. *Int J Radiat Oncol Biol Phys* 1995; **31**: 1213-1236 [PMID: 7713784]
60 **Emami B**, Lyman J, Brown A, Coia L, Goitein M, Munzenrider JE, Shank B, Solin LJ, Wesson M. Tolerance of normal tissue to therapeutic irradiation. *Int J Radiat Oncol Biol Phys* 1991; **21**: 109-122 [PMID: 2032882]
61 **Kavanagh BD**, Pan CC, Dawson LA, Das SK, Li XA, Ten Haken RK, Miften M. Radiation dose-volume effects in the stomach and small bowel. *Int J Radiat Oncol Biol Phys* 2010; **76**: S101-S107 [PMID: 20171503 DOI: 10.1016/j.ijrobp.2009.05.071]
62 **Lawrence TS**, Robertson JM, Anscher MS, Jirtle RL, Ensminger WD, Fajardo LF. Hepatic toxicity resulting from cancer treatment. *Int J Radiat Oncol Biol Phys* 1995; **31**: 1237-1248 [PMID: 7713785]
63 **Dawson LA**, Ten Haken RK. Partial volume tolerance of the liver to radiation. *Semin Radiat Oncol* 2005; **15**: 279-283 [PMID: 16183482 DOI: 10.1016/j.semradonc.2005.04.005]
64 **Yamasaki SA**, Marn CS, Francis IR, Robertson JM, Lawrence TS. High-dose localized radiation therapy for treatment of hepatic malignant tumors: CT findings and their relation to radiation hepatitis. *AJR Am J Roentgenol* 1995; **165**: 79-84 [PMID: 7785638]
65 **Cheng JC**, Liu HS, Wu JK, Chung HW, Jan GJ. Inclusion of biological factors in parallel-architecture normal-tissue

complication probability model for radiation-induced liver disease. *Int J Radiat Oncol Biol Phys* 2005; **62**: 1150-1156 [PMID: 15990021 DOI: 10.1016/j.ijrobp.2004.12.031]
66 **Dawson LA**, Normolle D, Balter JM, McGinn CJ, Lawrence TS, Ten Haken RK. Analysis of radiation-induced liver disease using the Lyman NTCP model. *Int J Radiat Oncol Biol Phys* 2002; **53**: 810-821 [PMID: 12095546]
67 **Pan CC**, Kavanagh BD, Dawson LA, Li XA, Das SK, Miften M, Ten Haken RK. Radiation-associated liver injury. *Int J Radiat Oncol Biol Phys* 2010; **76**: S94-100 [PMID: 20171524 DOI: 10.1016/j.ijrobp.2009.06.092]
68 **Perez CA**, Grigsby PW, Lockett MA, Chao KS, Williamson J. Radiation therapy morbidity in carcinoma of the uterine cervix: dosimetric and clinical correlation. *Int J Radiat Oncol Biol Phys* 1999; **44**: 855-866 [PMID: 10386643]
69 **Miller AR**, Martenson JA, Nelson H, Schleck CD, Ilstrup DM, Gunderson LL, Donohue JH. The incidence and clinical consequences of treatment-related bowel injury. *Int J Radiat Oncol Biol Phys* 1999; **43**: 817-825 [PMID: 10098437]
70 **Martin E**, Pointreau Y, Roche-Forestier S, Barillot I. [Normal tissue tolerance to external beam radiation therapy: small bowel]. *Cancer Radiother* 2010; **14**: 350-353 [PMID: 20598616 DOI: 10.1016/j.canrad.2010.03.013]
71 **Keys HM**, Bundy BN, Stehman FB, Muderspach LI, Chafe WE, Suggs CL, Walker JL, Gersell D. Cisplatin, radiation, and adjuvant hysterectomy compared with radiation and adjuvant hysterectomy for bulky stage IB cervical carcinoma. *N Engl J Med* 1999; **340**: 1154-1161 [PMID: 10202166 DOI: 10.1056/NEJM199904153401503]
72 **Classen J**, Belka C, Paulsen F, Budach W, Hoffmann W, Bamberg M. Radiation-induced gastrointestinal toxicity. Pathophysiology, approaches to treatment and prophylaxis. *Strahlenther Onkol* 1998; **174** Suppl 3: 82-84 [PMID: 9830465]
73 **Hauer-Jensen M**, Wang J, Boerma M, Fu Q, Denham JW. Radiation damage to the gastrointestinal tract: mechanisms, diagnosis, and management. *Curr Opin Support Palliat Care* 2007; **1**: 23-29 [PMID: 18660720 DOI: 10.1097/SPC.0b013e3281108014]
74 **Yeoh E**, Horowitz M, Russo A, Muecke T, Robb T, Maddox A, Chatterton B. Effect of pelvic irradiation on gastrointestinal function: a prospective longitudinal study. *Am J Med* 1993; **95**: 397-406 [PMID: 8213872]
75 **Sher ME**, Bauer J. Radiation-induced enteropathy. *Am J Gastroenterol* 1990; **85**: 121-128 [PMID: 2301333]
76 **Hauer-Jensen M**, Wang J, Denham JW. Bowel injury: current and evolving management strategies. *Semin Radiat Oncol* 2003; **13**: 357-371 [PMID: 12903023]
77 **Ooi BS**, Tjandra JJ, Green MD. Morbidities of adjuvant chemotherapy and radiotherapy for resectable rectal cancer: an overview. *Dis Colon Rectum* 1999; **42**: 403-418 [PMID: 10223765]
78 **Birgisson H**, Påhlman L, Gunnarsson U, Glimelius B. Late adverse effects of radiation therapy for rectal cancer - a systematic overview. *Acta Oncol* 2007; **46**: 504-516 [PMID: 17497318 DOI: 10.1080/02841860701348670]
79 **Holm T**, Singnomklao T, Rutqvist LE, Cedermark B. Adjuvant preoperative radiotherapy in patients with rectal carcinoma. Adverse effects during long term follow-up of two randomized trials. *Cancer* 1996; **78**: 968-976 [PMID: 8780533 DOI: 10.1002/(SICI)1097-0142(19960901)78:]
80 **Galland RB**, Spencer J. The natural history of clinically established radiation enteritis. *Lancet* 1985; **1**: 1257-1258 [PMID: 2860452]
81 **Nussbaum ML**, Campana TJ, Weese JL. Radiation-induced intestinal injury. *Clin Plast Surg* 1993; **20**: 573-580 [PMID: 8324995]
82 **Schultheiss TE**, Lee WR, Hunt MA, Hanlon AL, Peter RS, Hanks GE. Late GI and GU complications in the treatment of prostate cancer. *Int J Radiat Oncol Biol Phys* 1997; **37**: 3-11 [PMID: 9054871]
83 **Babb RR**. Radiation proctitis: a review. *Am J Gastroenterol* 1996; **91**: 1309-1311 [PMID: 8677984]
84 **Hauer-Jensen M**, Fink LM, Wang J. Radiation injury and the protein C pathway. *Crit Care Med* 2004; **32**: S325-S330 [PMID: 15118539]
85 **Abayomi J**, Kirwan J, Hackett A. The prevalence of chronic radiation enteritis following radiotherapy for cervical or endometrial cancer and its impact on quality of life. *Eur J Oncol Nurs* 2009; **13**: 262-267 [PMID: 19640788 DOI: 10.1016/j.ejon.2009.02.007]
86 **Gami B**, Harrington K, Blake P, Dearnaley D, Tait D, Davies J, Norman AR, Andreyev HJ. How patients manage gastrointestinal symptoms after pelvic radiotherapy. *Aliment Pharmacol Ther* 2003; **18**: 987-994 [PMID: 14616164]
87 **Michalski JM**, Gay H, Jackson A, Tucker SL, Deasy JO. Radiation dose-volume effects in radiation-induced rectal injury. *Int J Radiat Oncol Biol Phys* 2010; **76**: S123-S129 [PMID: 20171506 DOI: 10.1016/j.ijrobp.2009.03.078]
88 **Bosset JF**, Collette L, Calais G, Mineur L, Maingon P, Radosevic-Jelic L, Daban A, Bardet E, Beny A, Ollier JC. Chemotherapy with preoperative radiotherapy in rectal cancer. *N Engl J Med* 2006; **355**: 1114-1123 [PMID: 16971718 DOI: 10.1056/NEJMoa060829]
89 **Eifel PJ**, Levenback C, Wharton JT, Oswald MJ. Time course and incidence of late complications in patients treated with radiation therapy for FIGO stage IB carcinoma of the uterine cervix. *Int J Radiat Oncol Biol Phys* 1995; **32**: 1289-1300 [PMID: 7635768]
90 **Denton AS**, Bond SJ, Matthews S, Bentzen SM, Maher EJ. National audit of the management and outcome of carcinoma of the cervix treated with radiotherapy in 1993. *Clin Oncol (R Coll Radiol)* 2000; **12**: 347-353 [PMID: 11202086]
91 **Denham JW**, O'Brien PC, Dunstan RH, Johansen J, See A, Hamilton CS, Bydder S, Wright S. Is there more than one late radiation proctitis syndrome? *Radiother Oncol* 1999; **51**: 43-53 [PMID: 10386716]
92 **Turini M**, Redaelli A, Gramegna P, Radice D. Quality of life and economic considerations in the management of prostate cancer. *Pharmacoeconomics* 2003; **21**: 527-541 [PMID: 12751912]
93 **Bloch S**, Love A, Macvean M, Duchesne G, Couper J, Kissane D. Psychological adjustment of men with prostate cancer: a review of the literature. *Biopsychosoc Med* 2007; **1**: 2 [PMID: 17371571 DOI: 10.1186/1751-0759-1-2]
94 **Henderson A**, Andreyev HJ, Stephens R, Dearnaley D. Patient and physician reporting of symptoms and health-related quality of life in trials of treatment for early prostate cancer: considerations for future studies. *Clin Oncol (R Coll Radiol)* 2006; **18**: 735-743 [PMID: 17168208]
95 **Lev EL**, Eller LS, Gejerman G, Lane P, Owen SV, White M, Nganga N. Quality of life of men treated with brachytherapies for prostate cancer. *Health Qual Life Outcomes* 2004; **2**: 28 [PMID: 15198803 DOI: 10.1186/1477-7525-2-28]
96 **Gelblum DY**, Potters L. Rectal complications associated with transperineal interstitial brachytherapy for prostate cancer. *Int J Radiat Oncol Biol Phys* 2000; **48**: 119-124 [PMID: 10924980]
97 **Theodorescu D**, Gillenwater JY, Koutrouvelis PG. Prostatourethral-rectal fistula after prostate brachytherapy. *Cancer* 2000; **89**: 2085-2091 [PMID: 11066049 DOI: 10.1002/1097-0142(20001115)89:]
98 **Shakespeare D**, Mitchell DM, Carey BM, Finan P, Henry AM, Ash D, Bottomley DM, Al-Qaisieh B. Recto-urethral fistula following brachytherapy for localized prostate cancer. *Colorectal Dis* 2007; **9**: 328-331 [PMID: 17432984 DOI: 10.1111/j.1463-1318.2006.01119.x]
99 **Moon K**, Stukenborg GJ, Keim J, Theodorescu D. Cancer incidence after localized therapy for prostate cancer. *Cancer* 2006; **107**: 991-998 [PMID: 16878323 DOI: 10.1002/cncr.22083]
100 **Travis LB**, Curtis RE, Storm H, Hall P, Holowaty E, Van

Leeuwen FE, Kohler BA, Pukkala E, Lynch CF, Andersson M, Bergfeldt K, Clarke EA, Wiklund T, Stoter G, Gospodarowicz M, Sturgeon J, Fraumeni JF, Boice JD. Risk of second malignant neoplasms among long-term survivors of testicular cancer. *J Natl Cancer Inst* 1997; **89**: 1429-1439 [PMID: 9326912]

101 **Liauw SL**, Sylvester JE, Morris CG, Blasko JC, Grimm PD. Second malignancies after prostate brachytherapy: incidence of bladder and colorectal cancers in patients with 15 years of potential follow-up. *Int J Radiat Oncol Biol Phys* 2006; **66**: 669-673 [PMID: 16887293 DOI: 10.1016/j.ijrobp.2006.05.016]

102 **Bentzen SM**, Overgaard M, Thames HD. Fractionation sensitivity of a functional endpoint: impaired shoulder movement after post-mastectomy radiotherapy. *Int J Radiat Oncol Biol Phys* 1989; **17**: 531-537 [PMID: 2506157]

103 **Merrick GS**, Butler WM, Galbreath RW, Stipetich RL, Abel LJ, Lief JH. Erectile function after permanent prostate brachytherapy. *Int J Radiat Oncol Biol Phys* 2002; **52**: 893-902 [PMID: 11958881]

104 **Honoré HB**, Bentzen SM, Møller K, Grau C. Sensori-neural hearing loss after radiotherapy for nasopharyngeal carcinoma: individualized risk estimation. *Radiother Oncol* 2002; **65**: 9-16 [PMID: 12413669]

105 **Chon BH**, Loeffler JS. The effect of nonmalignant systemic disease on tolerance to radiation therapy. *Oncologist* 2002; **7**: 136-143 [PMID: 11961197]

106 **Isselbacher KBEH**. Principles of Internal Medicine. New York: McGraw-Hill Company, 1994: 1922-1974

107 **Rubin EFJ**. Pathology. Philadelphia: J.B. Lippincott Company, 1994: 651-686

108 **Herold DM**, Hanlon AL, Hanks GE. Diabetes mellitus: a predictor for late radiation morbidity. *Int J Radiat Oncol Biol Phys* 1999; **43**: 475-479 [PMID: 10078625]

109 **Matthews RH**. Collagen vascular disease and irradiation. *Int J Radiat Oncol Biol Phys* 1989; **17**: 1123-1124 [PMID: 2808050]

110 **Hareyama M**, Nagakura H, Tamakawa M, Hyodo K, Asakura K, Horikoshi T, Oouchi A, Shido M, Morita K. Severe reaction after chemoradiotherapy of nasopharyngeal carcinoma with collagen disease. *Int J Radiat Oncol Biol Phys* 1995; **33**: 971 [PMID: 7591915]

111 **Abu-Shakra M**, Lee P. Exaggerated fibrosis in patients with systemic sclerosis (scleroderma) following radiation therapy. *J Rheumatol* 1993; **20**: 1601-1603 [PMID: 8164225]

112 **Tiersten A**, Saltz LB. Influence of inflammatory bowel disease on the ability of patients to tolerate systemic fluorouracil-based chemotherapy. *J Clin Oncol* 1996; **14**: 2043-2046 [PMID: 8683234]

113 **Grann A**, Wallner K. Prostate brachytherapy in patients with inflammatory bowel disease. *Int J Radiat Oncol Biol Phys* 1998; **40**: 135-138 [PMID: 9422569]

114 **Willett CG**, Ooi CJ, Zietman AL, Menon V, Goldberg S, Sands BE, Podolsky DK. Acute and late toxicity of patients with inflammatory bowel disease undergoing irradiation for abdominal and pelvic neoplasms. *Int J Radiat Oncol Biol Phys* 2000; **46**: 995-998 [PMID: 10705022]

115 **Green S**, Stock RG, Greenstein AJ. Rectal cancer and inflammatory bowel disease: natural history and implications for radiation therapy. *Int J Radiat Oncol Biol Phys* 1999; **44**: 835-840 [PMID: 10386640]

116 **Ali RA**, Dooley C, Comber H, Newell J, Egan LJ. Clinical features, treatment, and survival of patients with colorectal cancer with or without inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2011; **9**: 584-9.e1-584-9.e2 [PMID: 21565283 DOI: 10.1016/j.cgh.2011.04.016]

117 **Cohn SM**, Schloemann S, Tessner T, Seibert K, Stenson WF. Crypt stem cell survival in the mouse intestinal epithelium is regulated by prostaglandins synthesized through cyclooxygenase-1. *J Clin Invest* 1997; **99**: 1367-1379 [PMID: 9077547 DOI: 10.1172/JCI119296]

118 **Du XX**, Doerschuk CM, Orazi A, Williams DA. A bone marrow stromal-derived growth factor, interleukin-11, stimulates recovery of small intestinal mucosal cells after cytoablative therapy. *Blood* 1994; **83**: 33-37 [PMID: 8274749]

119 **Houchen CW**, George RJ, Sturmoski MA, Cohn SM. FGF-2 enhances intestinal stem cell survival and its expression is induced after radiation injury. *Am J Physiol* 1999; **276**: G249-G258 [PMID: 9887002]

120 **Houchen CW**, Stenson WF, Cohn SM. Disruption of cyclooxygenase-1 gene results in an impaired response to radiation injury. *Am J Physiol Gastrointest Liver Physiol* 2000; **279**: G858-G865 [PMID: 11052981]

121 **Neta R**. Modulation of radiation damage by cytokines. *Stem Cells* 1997; **15** Suppl 2: 87-94 [PMID: 9368290 DOI: 10.1002/stem.5530150713]

122 **Neta R**, Stiefel SM, Ali N. In lethally irradiated mice interleukin-12 protects bone marrow but sensitizes intestinal tract to damage from ionizing radiation. *Ann N Y Acad Sci* 1995; **762**: 274-280; discussion 280-281 [PMID: 7545367]

123 **Neta R**, Stiefel SM, Finkelman F, Herrmann S, Ali N. IL-12 protects bone marrow from and sensitizes intestinal tract to ionizing radiation. *J Immunol* 1994; **153**: 4230-4237 [PMID: 7930625]

124 **Robertson JM**, Clarke DH, Pevzner MM, Matter RC. Breast conservation therapy. Severe breast fibrosis after radiation therapy in patients with collagen vascular disease. *Cancer* 1991; **68**: 502-508 [PMID: 1648431]

125 **Nisce LZ**, Safai B. Radiation therapy of Kaposi's sarcoma in AIDS. Memorial Sloan-Kettering experience. *Front Radiat Ther Oncol* 1985; **19**: 133-137 [PMID: 3920122]

126 **Cooper JS**, Fried PR. Defining the role of radiation therapy in the management of epidemic Kaposi's sarcoma. *Int J Radiat Oncol Biol Phys* 1987; **13**: 35-39 [PMID: 2433259]

127 **Formenti SC**, Chak L, Gill P, Buess EM, Hill CK. Increased radiosensitivity of normal tissue fibroblasts in patients with acquired immunodeficiency syndrome (AIDS) and with Kaposi's sarcoma. *Int J Radiat Biol* 1995; **68**: 411-412 [PMID: 7594966]

128 **Baeyens A**, Slabbert JP, Willem P, Jozela S, Van Der Merwe D, Vral A. Chromosomal radiosensitivity of HIV positive individuals. *Int J Radiat Biol* 2010; **86**: 584-592 [PMID: 20545573 DOI: 10.3109/09553001003734576]

129 **Ebell MH**, Siwek J, Weiss BD, Woolf SH, Susman J, Ewigman B, Bowman M. Strength of recommendation taxonomy (SORT): a patient-centered approach to grading evidence in the medical literature. *J Am Board Fam Pract* 2004; **17**: 59-67 [PMID: 15014055]

130 **Housri N**, Yarchoan R, Kaushal A. Radiotherapy for patients with the human immunodeficiency virus: are special precautions necessary? *Cancer* 2010; **116**: 273-283 [PMID: 20014399 DOI: 10.1002/cncr.24878]

131 **Coleman CN**. Molecular biology in radiation oncology. Radiation oncology perspective of BRCA1 and BRCA2. *Acta Oncol* 1999; **38** Suppl 13: 55-59 [PMID: 10612497]

132 **Trenz K**, Rothfuss A, Schütz P, Speit G. Mutagen sensitivity of peripheral blood from women carrying a BRCA1 or BRCA2 mutation. *Mutat Res* 2002; **500**: 89-96 [PMID: 11890937]

133 **Andreassen CN**. Can risk of radiotherapy-induced normal tissue complications be predicted from genetic profiles? *Acta Oncol* 2005; **44**: 801-815 [PMID: 16332587 DOI: 10.1080/02841860500374513]

134 **West C**, Rosenstein BS, Alsner J, Azria D, Barnett G, Begg A, Bentzen S, Burnet N, Chang-Claude J, Chuang E, Coles C, De Ruyck K, De Ruysscher D, Dunning A, Elliott R, Fachal L, Hall J, Haustermans K, Herskind C, Hoelscher T, Imai T, Iwakawa M, Jones D, Kulich C, Langendijk JH, O'Neils P, Ozsahin M, Parliament M, Polanski A, Rosenstein B, Seminara D, Symonds P, Talbot C, Thierens H, Vega A, West C, Yarnold J. Establishment of a Radiogenomics Consortium. *Int J Radiat Oncol Biol Phys* 2010; **76**: 1295-1296 [PMID: 20338472 DOI: 10.1016/j.ijrobp.2009.12.017]

135 **Turesson I**, Bernefors R, Book M, Flogegård M, Hermansson I, Johansson KA, Lindh A, Sigurdardottir S, Thunberg U, Nyman J. Normal tissue response to low doses of radiotherapy assessed by molecular markers--a study of skin in patients treated for prostate cancer. *Acta Oncol* 2001; **40**: 941-951 [PMID: 11845959]

136 **Ho AY**, Atencio DP, Peters S, Stock RG, Formenti SC, Cesaretti JA, Green S, Haffty B, Drumea K, Leitzin L, Kuten A, Azria D, Ozsahin M, Overgaard J, Andreassen CN, Trop CS, Park J, Rosenstein BS. Genetic predictors of adverse radiotherapy effects: the Gene-PARE project. *Int J Radiat Oncol Biol Phys* 2006; **65**: 646-655 [PMID: 16751059 DOI: 10.1016/j.ijrobp.2006.03.006]

137 **Safwat A**, Bentzen SM, Turesson I, Hendry JH. Deterministic rather than stochastic factors explain most of the variation in the expression of skin telangiectasia after radiotherapy. *Int J Radiat Oncol Biol Phys* 2002; **52**: 198-204 [PMID: 11777639]

138 **Tucker SL**, Turesson I, Thames HD. Evidence for individual differences in the radiosensitivity of human skin. *Eur J Cancer* 1992; **28A**: 1783-1791 [PMID: 1389511]

139 **Tucker SL**, Geara FB, Peters LJ, Brock WA. How much could the radiotherapy dose be altered for individual patients based on a predictive assay of normal-tissue radiosensitivity? *Radiother Oncol* 1996; **38**: 103-113 [PMID: 8966222]

140 **Taylor AM**, Harnden DG, Arlett CF, Harcourt SA, Lehmann AR, Stevens S, Bridges BA. Ataxia telangiectasia: a human mutation with abnormal radiation sensitivity. *Nature* 1975; **258**: 427-429 [PMID: 1196376]

141 **Cesaretti JA**, Stock RG, Atencio DP, Peters SA, Peters CA, Burri RJ, Stone NN, Rosenstein BS. A genetically determined dose-volume histogram predicts for rectal bleeding among patients treated with prostate brachytherapy. *Int J Radiat Oncol Biol Phys* 2007; **68**: 1410-1416 [PMID: 17490827 DOI: 10.1016/j.ijrobp.2007.02.052]

142 **O'Driscoll M**, Jeggo PA. The role of double-strand break repair - insights from human genetics. *Nat Rev Genet* 2006; **7**: 45-54 [PMID: 16369571 DOI: 10.1038/nrg1746]

143 **Petrini JH**, Stracker TH. The cellular response to DNA double-strand breaks: defining the sensors and mediators. *Trends Cell Biol* 2003; **13**: 458-462 [PMID: 12946624]

144 **Taylor RC**, Cullen SP, Martin SJ. Apoptosis: controlled demolition at the cellular level. *Nat Rev Mol Cell Biol* 2008; **9**: 231-241 [PMID: 18073771 DOI: 10.1038/nrm2312]

145 **Branzei D**, Foiani M. Regulation of DNA repair throughout the cell cycle. *Nat Rev Mol Cell Biol* 2008; **9**: 297-308 [PMID: 18285803 DOI: 10.1038/nrm2351]

146 **Mikkelsen RB**, Wardman P. Biological chemistry of reactive oxygen and nitrogen and radiation-induced signal transduction mechanisms. *Oncogene* 2003; **22**: 5734-5754 [PMID: 12947383 DOI: 10.1038/sj.onc.1206663]

147 **Olsson AK**, Dimberg A, Kreuger J, Claesson-Welsh L. VEGF receptor signalling - in control of vascular function. *Nat Rev Mol Cell Biol* 2006; **7**: 359-371 [PMID: 16633338 DOI: 10.1038/nrm1911]

148 **Weis SM**. Vascular permeability in cardiovascular disease and cancer. *Curr Opin Hematol* 2008; **15**: 243-249 [PMID: 18391792 DOI: 10.1097/MOH.0b013e3282f97d86]

149 **Andreassen CN**, Alsner J, Overgaard M, Overgaard J. Prediction of normal tissue radiosensitivity from polymorphisms in candidate genes. *Radiother Oncol* 2003; **69**: 127-135 [PMID: 14643949]

150 **De Ruyck K**, Van Eijkeren M, Claes K, Morthier R, De Paepe A, Vral A, De Ridder L, Thierens H. Radiation-induced damage to normal tissues after radiotherapy in patients treated for gynecologic tumors: association with single nucleotide polymorphisms in XRCC1, XRCC3, and OGG1 genes and in vitro chromosomal radiosensitivity in lymphocytes. *Int J Radiat Oncol Biol Phys* 2005; **62**: 1140-1149 [PMID: 15990020 DOI: 10.1016/j.ijrobp.2004.12.027]

151 **De Ruyck K**, Van Eijkeren M, Claes K, Bacher K, Vral A, De Neve W, Thierens H. TGFbeta1 polymorphisms and late clinical radiosensitivity in patients treated for gynecologic tumors. *Int J Radiat Oncol Biol Phys* 2006; **65**: 1240-1248 [PMID: 16798416 DOI: 10.1016/j.ijrobp.2006.03.047]

152 **Damaraju S**, Murray D, Dufour J, Carandang D, Myrehaug S, Fallone G, Field C, Greiner R, Hanson J, Cass CE, Parliament M. Association of DNA repair and steroid metabolism gene polymorphisms with clinical late toxicity in patients treated with conformal radiotherapy for prostate cancer. *Clin Cancer Res* 2006; **12**: 2545-2554 [PMID: 16638864 DOI: 10.1158/1078-0432.CCR-05-2703]

153 **Merritt AJ**, Potten CS, Kemp CJ, Hickman JA, Balmain A, Lane DP, Hall PA. The role of p53 in spontaneous and radiation-induced apoptosis in the gastrointestinal tract of normal and p53-deficient mice. *Cancer Res* 1994; **54**: 614-617 [PMID: 8306319]

154 **Potten CS**, Merritt A, Hickman J, Hall P, Faranda A. Characterization of radiation-induced apoptosis in the small intestine and its biological implications. *Int J Radiat Biol* 1994; **65**: 71-78 [PMID: 7905913]

155 **Rieder F**, Brenmoehl J, Leeb S, Schölmerich J, Rogler G. Wound healing and fibrosis in intestinal disease. *Gut* 2007; **56**: 130-139 [PMID: 17172588 DOI: 10.1136/gut.2006.090456]

156 **Rogler G**, Brand K, Vogl D, Page S, Hofmeister R, Andus T, Knuechel R, Baeuerle PA, Schölmerich J, Gross V. Nuclear factor kappaB is activated in macrophages and epithelial cells of inflamed intestinal mucosa. *Gastroenterology* 1998; **115**: 357-369 [PMID: 9679041]

157 **Hausmann M**, Spöttl T, Andus T, Rothe G, Falk W, Schölmerich J, Herfarth H, Rogler G. Subtractive screening reveals up-regulation of NADPH oxidase expression in Crohn's disease intestinal macrophages. *Clin Exp Immunol* 2001; **125**: 48-55 [PMID: 11472425]

158 **Rogler G**, Gelbmann CM, Vogl D, Brunner M, Schölmerich J, Falk W, Andus T, Brand K. Differential activation of cytokine secretion in primary human colonic fibroblast/myofibroblast cultures. *Scand J Gastroenterol* 2001; **36**: 389-398 [PMID: 11336164]

159 **Hovdenak N**, Fajardo LF, Hauer-Jensen M. Acute radiation proctitis: a sequential clinicopathologic study during pelvic radiotherapy. *Int J Radiat Oncol Biol Phys* 2000; **48**: 1111-1117 [PMID: 11072170]

160 **Stone HB**, Coleman CN, Anscher MS, McBride WH. Effects of radiation on normal tissue: consequences and mechanisms. *Lancet Oncol* 2003; **4**: 529-536 [PMID: 12965273]

161 **Denham JW**, Hauer-Jensen M. The radiotherapeutic injury--a complex 'wound'. *Radiother Oncol* 2002; **63**: 129-145 [PMID: 12063002]

162 **François S**, Mouiseddine M, Mathieu N, Semont A, Monti P, Dudoignon N, Saché A, Boutarfa A, Thierry D, Gourmelon P, Chapel A. Human mesenchymal stem cells favour healing of the cutaneous radiation syndrome in a xenogenic transplant model. *Ann Hematol* 2007; **86**: 1-8 [PMID: 17043780 DOI: 10.1007/s00277-006-0166-5]

163 **François S**, Bensidhoum M, Mouiseddine M, Mazurier C, Allenet B, Semont A, Frick J, Saché A, Bouchet S, Thierry D, Gourmelon P, Gorin NC, Chapel A. Local irradiation not only induces homing of human mesenchymal stem cells at exposed sites but promotes their widespread engraftment to multiple organs: a study of their quantitative distribution after irradiation damage. *Stem Cells* 2006; **24**: 1020-1029 [PMID: 16339642 DOI: 10.1634/stemcells.2005-0260]

164 **Haydont V**, Vozenin-Brotons MC. Maintenance of radiation-induced intestinal fibrosis: cellular and molecular features. *World J Gastroenterol* 2007; **13**: 2675-2683 [PMID: 17569135]

165 **Molla M**, Panes J. Radiation-induced intestinal inflammation. *World J Gastroenterol* 2007; **13**: 3043-3046 [PMID: 17589918]

166 **Earnest DLTJ**. Radiation enteritis and colitis. Gastrointestinal disease. Philadelphia: WB Saunders, 1989: 1369-1382

167 **Bentzen SM**. Preventing or reducing late side effects of radiation therapy: radiobiology meets molecular pathology. *Nat Rev Cancer* 2006; **6**: 702-713 [PMID: 16929324 DOI: 10.1038/nrc1950]

168 **Milliat F**, François A, Tamarat R, Benderitter M. [Role of endothelium in radiation-induced normal tissue damages]. *Ann Cardiol Angeiol (Paris)* 2008; **57**: 139-148 [PMID: 18579118 DOI: 10.1016/j.ancard.2008.02.015]

169 **Paris F**, Fuks Z, Kang A, Capodieci P, Juan G, Ehleiter D, Haimovitz-Friedman A, Cordon-Cardo C, Kolesnick R. Endothelial apoptosis as the primary lesion initiating intestinal radiation damage in mice. *Science* 2001; **293**: 293-297 [PMID: 11452123 DOI: 10.1126/science.1060191]

170 **Schuller BW**, Binns PJ, Riley KJ, Ma L, Hawthorne MF, Coderre JA. Selective irradiation of the vascular endothelium has no effect on the survival of murine intestinal crypt stem cells. *Proc Natl Acad Sci USA* 2006; **103**: 3787-3792 [PMID: 16505359 DOI: 10.1073/pnas.0600133103]

171 **Hahnfeldt P**, Panigrahy D, Folkman J, Hlatky L. Tumor development under angiogenic signaling: a dynamical theory of tumor growth, treatment response, and postvascular dormancy. *Cancer Res* 1999; **59**: 4770-4775 [PMID: 10519381]

172 **Bussink J**, Kaanders JH, van der Kogel AJ. Tumor hypoxia at the micro-regional level: clinical relevance and predictive value of exogenous and endogenous hypoxic cell markers. *Radiother Oncol* 2003; **67**: 3-15 [PMID: 12758235]

173 **Citrin D**, Ménard C, Camphausen K. Combining radiotherapy and angiogenesis inhibitors: clinical trial design. *Int J Radiat Oncol Biol Phys* 2006; **64**: 15-25 [PMID: 16377411 DOI: 10.1016/j.ijrobp.2005.03.065]

174 **Teicher BA**, Holden SA, Ara G, Dupuis NP, Liu F, Yuan J, Ikebe M, Kakeji Y. Influence of an anti-angiogenic treatment on 9L gliosarcoma: oxygenation and response to cytotoxic therapy. *Int J Cancer* 1995; **61**: 732-737 [PMID: 7768649]

175 **Lee CG**, Heijn M, di Tomaso E, Griffon-Etienne G, Ancukiewicz M, Koike C, Park KR, Ferrara N, Jain RK, Suit HD, Boucher Y. Anti-Vascular endothelial growth factor treatment augments tumor radiation response under normoxic or hypoxic conditions. *Cancer Res* 2000; **60**: 5565-5570 [PMID: 11034104]

176 **Griffin RJ**, Williams BW, Wild R, Cherrington JM, Park H, Song CW. Simultaneous inhibition of the receptor kinase activity of vascular endothelial, fibroblast, and platelet-derived growth factors suppresses tumor growth and enhances tumor radiation response. *Cancer Res* 2002; **62**: 1702-1706 [PMID: 11912143]

177 **Teicher BA**, Holden SA, Ara G, Sotomayor EA, Huang ZD, Chen YN, Brem H. Potentiation of cytotoxic cancer therapies by TNP-470 alone and with other anti-angiogenic agents. *Int J Cancer* 1994; **57**: 920-925 [PMID: 7515861]

178 **Mazeron R**, Anderson B, Supiot S, Paris F, Deutsch E. Current state of knowledge regarding the use of antiangiogenic agents with radiation therapy. *Cancer Treat Rev* 2011; **37**: 476-486 [PMID: 21546163 DOI: 10.1016/j.ctrv.2011.03.004]

179 **Prabhakar R**, Rath GK. A simple plan evaluation index based on the dose to critical structures in radiotherapy. *J Med Phys* 2011; **36**: 192-197 [PMID: 22228927 DOI: 10.4103/0971-6203.89965]

180 **Kehwar TS**. Analytical approach to estimate normal tissue complication probability using best fit of normal tissue tolerance doses into the NTCP equation of the linear quadratic model. *J Cancer Res Ther* 2005; **1**: 168-179 [PMID: 17998649]

181 **Choy H**, Akerley W, Safran H, Graziano S, Chung C, Williams T, Cole B, Kennedy T. Multiinstitutional phase II trial of paclitaxel, carboplatin, and concurrent radiation therapy for locally advanced non-small-cell lung cancer. *J Clin Oncol* 1998; **16**: 3316-3322 [PMID: 9779707]

182 **Antonadou D**, Coliarakis N, Synodinou M, Athanassiou H, Kouveli A, Verigos C, Georgakopoulos G, Panoussaki K, Karageorgis P, Throuvalas N. Randomized phase III trial of radiation treatment +/- amifostine in patients with advanced-stage lung cancer. *Int J Radiat Oncol Biol Phys* 2001; **51**: 915-922 [PMID: 11704311]

183 **Cosset JM**, Henry-Amar M, Burgers JM, Noordijk EM, Van der Werf-Messing B, Meerwaldt JH, van der Schueren E. Late radiation injuries of the gastrointestinal tract in the H2 and H5 EORTC Hodgkin's disease trials: emphasis on the role of exploratory laparotomy and fractionation. *Radiother Oncol* 1988; **13**: 61-68 [PMID: 3141982]

184 **Ogata K**, Hizawa K, Yoshida M, Kitamuro T, Akagi G, Kagawa K, Fukuda F. Hepatic injury following irradiation--a morphologic study. *Tokushima J Exp Med* 1963; **43**: 240-251 [PMID: 14049847]

185 **Jackson A**, Ten Haken RK, Robertson JM, Kessler ML, Kutcher GJ, Lawrence TS. Analysis of clinical complication data for radiation hepatitis using a parallel architecture model. *Int J Radiat Oncol Biol Phys* 1995; **31**: 883-891 [PMID: 7860402]

186 **Letschert JG**, Lebesque JV, Aleman BM, Bosset JF, Horiot JC, Bartelink H, Cionini L, Hamers JP, Leer JW, van Glabbeke M. The volume effect in radiation-related late small bowel complications: results of a clinical study of the EORTC Radiotherapy Cooperative Group in patients treated for rectal carcinoma. *Radiother Oncol* 1994; **32**: 116-123 [PMID: 7972904]

187 **Yeoh EE**, Botten R, Russo A, McGowan R, Fraser R, Roos D, Penniment M, Borg M, Sun W. Chronic effects of therapeutic irradiation for localized prostatic carcinoma on anorectal function. *Int J Radiat Oncol Biol Phys* 2000; **47**: 915-924 [PMID: 10863060]

P- Reviewers Matsuzaki Y, Ladas SD, Supiot S
S- Editor Gou SX **L- Editor** A **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i2.199

World J Gastroenterol 2013 January 14; 19(2): 199-208
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

REVIEW

Gastrointestinal radiation injury: Prevention and treatment

Abobakr K Shadad, Frank J Sullivan, Joseph D Martin, Laurence J Egan

Abobakr K Shadad, Laurence J Egan, Department of Gastroenterology, University Hospital Galway, University Hospital Galway, 34562 Galway, Ireland
Abobakr K Shadad, Laurence J Egan, Department of Pharmacology and Therapeutics, School of Medicine, University Hospital Galway, 34562 Galway, Ireland
Frank J Sullivan, Joseph D Martin, Department of Radiation Oncology, National University of Ireland Galway, University Hospital Galway, 34562 Galway, Ireland
Author contributions: Shadad AK wrote the review; Sullivan FJ, Martin JD and Egan LJ revised the contents.
Correspondence to: Laurence J Egan, Professor, Department of Pharmacology and Therapeutics, School of Medicine, University Hospital Galway, 34562 Galway,
Ireland. laurence.egan@nuigalway.ie
Telephone: +353-91-495370 Fax: +353-91-495572
Received: December 4, 2011 Revised: March 31, 2012
Accepted: April 2, 2012
Published online: January 14, 2013

Abstract

With the recent advances in detection and treatment of cancer, there is an increasing emphasis on the efficacy and safety aspects of cancer therapy. Radiation therapy is a common treatment for a wide variety of cancers, either alone or in combination with other treatments. Ionising radiation injury to the gastrointestinal tract is a frequent side effect of radiation therapy and a considerable proportion of patients suffer acute or chronic gastrointestinal symptoms as a result. These side effects often cause morbidity and may in some cases lower the efficacy of radiotherapy treatment. Radiation injury to the gastrointestinal tract can be minimised by either of two strategies: technical strategies which aim to physically shift radiation dose away from the normal intestinal tissues, and biological strategies which aim to modulate the normal tissue response to ionising radiation or to increase its resistance to it. Although considerable improvement in the safety of radiotherapy treatment has been achieved through the use of modern optimised planning and delivery techniques, biological techniques may offer additional further promise. Different agents have been used to prevent or minimize the severity of gastrointestinal injury induced by ionising radiation exposure, including biological, chemical and pharmacological agents. In this review we aim to discuss various technical strategies to prevent gastrointestinal injury during cancer radiotherapy, examine the different therapeutic options for acute and chronic gastrointestinal radiation injury and outline some examples of research directions and considerations for prevention at a pre-clinical level.

© 2013 Baishideng. All rights reserved.

Key words: Radiation enteritis; Radiation proctitis; Prevention; Treatment; Gastrointestinal radiation injury

Shadad AK, Sullivan FJ, Martin JD, Egan LJ. Gastrointestinal radiation injury: Prevention and treatment. *World J Gastroenterol* 2013; 19(2): 199-208 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i2/199.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i2.199

INTRODUCTION

External beam radiotherapy is a common treatment modality for wide varieties of cancers. Approximately 70% of all cancer patients receive radiotherapy during the course of their disease, while radiotherapy plays a central role in 25% of all cancer cures[1-2]. Radiotherapy is a cost effective treatment, accounting for only 5% of the total cancer care expenditure[3]. This estimate has increased over the last decade with the wide use of modern technological innovations in simulation, delineation, dose calculation and radiation treatment delivery[4].

Gastrointestinal radiation injury is an important problem for two main reasons. First, it causes morbidity associated with a significant economic burden[5] and a significant reduction in patient's quality of life[6,7]. Second, it limits the dose of radiation that can be used to control cancer, as radiotherapy-related side effects often limit the tolerability for patients. Clinical manifestations of gastrointestinal

radiation injury can present acutely during or soon following radiotherapy due to acute mucosal injury and inflammation or they can present insidiously within few months or years after radiotherapy due to a chronic process of transmural fibrosis and vascular sclerosis.

Radiation toxicity to the gastrointestinal tract can be reduced by either of two strategies: technical strategies, which aim to physically shift the radiation dose away from the normal tissues or through biological strategies which aim to modulate the cellular and tissue response to ionising radiation.

TECHNICAL STRATEGIES FOR PREVENTION

Attention to detail regarding both radiotherapy planning and radiation delivery methods can minimise the risk of intestinal toxicity during pelvic radiotherapy. Multiple field planning and delivery can reduce the radiation dose to normal intestine as well as minimise the volume of small intestine exposed to radiation in the pelvis[8].

Physical manoeuvres and tissue expander techniques

The impact of different physical measures such as patient position during pelvic radiotherapy have been evaluated in a randomized trial of supine and prone positioning in patients undergoing conformal radiotherapy for prostate cancer. There were significant improvements for small bowel, rectal wall and bladder wall doses in the supine position[9].

Another study utilised combined manoeuvres to displace the small intestine from the pelvis during radiotherapy by bladder distension, lower abdominal wall compression with the patient in prone position using two-field and four-field planning radiographs of contrast-enhanced small bowel[10]. The results demonstrated in 50% of patients the four-field volume of pelvic small bowel was significantly less in the prone position than in the supine position. Similarly, other techniques such as the use of a "belly board" while the patient is in the prone position where the opening in the table allows the abdomen to fall below the level of the table, displaces the small bowel by the effect of the gravity during radiotherapy. These technique were found to reduce the volume of the small intestine within the pelvis by a mean of 66%[11].

Pre-treatment small bowel contrast studies can assess the location and mobility of the small bowel and hence, determine the optimum treatment position that could minimise the volume of small intestine within the pelvis[12]. Tissue expander techniques have been used to minimise the volume of exposed small bowel within the irradiation field during abdominal and pelvic external beam radiotherapy. Studies have shown a reduction of 50% in the risk of chronic intestinal complications with the use of intra-pelvic tissue expander[13]. Other techniques used to minimise the radiation dose to the rectum include transperineal injection of human collagen to increase the distance between the prostate gland and anterior rectal wall. The mean reduction in dose to the anterior rectal wall was 50% with no rectal toxicity reported[14].

Prophylactic surgical techniques

Various prophylactic surgical therapies were also employed to reduce the small intestinal exposure during pelvic radiotherapy such as insertion of biodegradable mesh slings, intra-pelvic breast prostheses or omentoplasty during operative resection whenever postoperative radiotherapy may be indicated[15-20]. Mesh slings were found to reduce the volume of small intestine exposed by 50%[21,22] while other techniques such as pelvic reconstruction, omentoplasty and transposition of the large bowel were found to reduce the volume of bowel at risk by 60%[22,23]. Prostate gland immobilization and rectal wall sparing has been suggested by the use of endorectal balloons during prostate cancer radiotherapy. Endorectal balloons are reported to be well tolerated by patients and showed a significant rectal wall sparing effect during three-dimensional conformal radiotherapy and intensity-modulated radiotherapy for prostate cancer[24,25].

Optimised planning and delivery techniques

A significant improvement in the safety of radiotherapy treatment has been achieved through the use of optimised planning and delivery techniques which aim to reduce the field size, focus the radiation beam into the lesion and minimise the volume of exposed surrounding tissues in the radiation field[10]. Multiple field arrangements may also reduce normal tissue toxicity during pelvic radiotherapy.

The dose-volume histogram is a plot of a cumulative dose-volume frequency distribution, that graphically summarizes the simulated radiation distribution within a volume of interest of a patient which would result from a proposed radiation treatment plan[26]. It provides a graphic display of a simulated radiation treatment plan and generates valuable information on the dose distribution within the volume of interest[27]. The dose-volume histogram is used to predict the development of radiation toxicity and to identify low and high risk patient groups[28]. Careful review of the dose-volume histogram of both the intended targets as well as the structures of avoidance constitutes a standard part of the assessment of the radiation treatment plans in modern radiation therapy.

Three-dimensional conformal techniques which comprise computerised tomography scanning simulation techniques and intensity modulated radiation therapy are associated with a reduced risk of normal tissue toxicity and allow a higher radiation doses to be used compared to conventional two dimensional methods[29-32]. Computerised tomography scan simulation techniques were found to reduce the volume of the bowel unintentionally irradiated by 5% compared to conventional radiotherapy[33].

Intensity modulated radiation therapy allows clear definition of both target lesions and surrounding normal tissues and hence the ability to apply two different inten-

sities of radiation (high and low) within the same treatment field[34,35]. It represents the ultimate combination of treatment planning and delivery which provides the most flexible delivery of radiation to complex targets while minimising radiation doses to surrounding critical structures. It does so by utilizing sophisticated planning algorithms such as inverse planning and multiple small beams of varying intensity, to optimally deliver the treatment. Intensity modulated radiation therapy was associated with a significant reduction in toxicity following pelvic radiotherapy in prostate cancer patients[36]. It was also associated with a reduction of 15%-18% in intestinal volume unintentionally irradiated compared to conventional two dimensional radiotherapy, and a 10%-13% reduction in intestinal volume unintentionally irradiated compared to three-dimensional computerised tomography simulation techniques[33]. Furthermore, intensity modulated radiation therapy has been found to reduce acute and late toxicity and maintains good long term quality of life even when high radiation doses are administered to prostate cancer patients[37-39]. In 208 head and neck cancer patients treated with intensity modulated radiation therapy it was found that intensity modulated radiation therapy was associated with reduced late toxicities without jeopardising local cancer control and overall survival[40]. The long-term quality of life has been compared among survivors of head and neck cancer patients whom were treated with either intensity modulated radiation therapy or three-dimensional conformal radiotherapy. The study results have shown that the early improvement in quality of life associated with intensity modulated radiation therapy was maintained and become more magnified over time[41].

Brachytherapy is another advanced technique in which the source of radiation is implanted within the malignant tissues (interstitial brachytherapy) or within a cavity in its immediate vicinity (intra-cavitary brachytherapy). The sources can be permanently inserted and emits their radiation over a prolonged period of time (low dose rate) or temporary and emit over a short period (high dose rate). In this fashion, a high radiation dose is limited to the target lesion sparing the surrounding normal tissues. Brachytherapy alone or in combination with external beam radiotherapy can reduce normal tissue toxicity without compromising treatment efficacy in prostate cancer[42,43]. Brachytherapy can be an option for patients with history of inflammatory bowel disease and it is well tolerated in low risk prostate carcinoma[44].

Combining target definition on a daily basis and adjusting the treatments to account for target motion is referred to as image guided radiotherapy techniques[45]. Stereotactic radiation therapy can focus an extremely narrow ionizing radiation beam on a small target from different directions using an immobilisation system. The high accuracy of this technique has enabled targeting a small lesions such as brain metastatic lesions[46]. Stereotactic radiation therapy uses a small number of high doses of radiation to target small lesions. It has been used in the treatment of early-stage non-small-cell lung cancer, prostate cancer, renal-cell carcinoma, and liver cancer, and in the treatment of oligometastases in the lung, liver, and spine[47]. Stereotactic radiation therapy is associated with high local control rates of cancer[48] and it was evaluated in the treatment of prostate cancer using an accelerated form of hypofractitionation through fewer but larger treatment fractions. The early results of this new technique suggest that it may induce an initial prostate specific antigen response similar to that seen with conventional fractionation radiotherapy but with fewer acute side effects. Disease control and chronic toxicity have not yet been fully evaluated[49]. The use of higher (often > 10 Gy) daily radiation doses in stereotactic radiation therapy needs to be carefully considered in view of the potential for more severe side effects[50]. The impact of the image guided radiotherapy techniques has been shown in a large study with 331 patients treated with high-dose intensity modulated radiation therapy with fiducial marker-based position verification for prostate cancer which allowed daily correction of the prostate position using the fiducial markers. The results showed that high dose was well tolerated with lower rate of acute toxicity, which provide possibilities for further dose escalation[37].

High-energy proton beams stop abruptly in the tissue at the end of their range and deposit most of their energy there, in this fashion, they provide dose distributions that are superior to X-rays when used in comparable beam configurations[51] In addition, they are more densely ionising along their treatment path, and therefore a higher quantum of energy to kill the cancer cell and high linear energy transfer. With proton beam radiation therapy a smaller volume of normal tissues is irradiated at high dose levels for most anatomic sites than is feasible with any photon technique[52]. Clinical studies have shown that proton beam therapy in patients with hepatocellular carcinoma has been shown to be effective, safe and well tolerable[53]. Proton beam therapy allows large tumour volumes to be irradiated to high doses without significant dose exposure to surrounding normal tissue which make it a promising modality for the treatment of large-volume tumours[54]. However, they have yet to show significant advantages in comparison with the conventional radiation therapy.

MEDICAL THERAPY FOR GASTROINTESTINAL RADIATION INJURY

Although technical strategies have achieved a significant degree of normal tissue protection during radiotherapy delivery, biological strategies may offer additional future promise. A wide variety of pharmacological agents, nutritional supplements, biological response modifiers, and dietary measures have been investigated for potential benefit to prevent or minimize the severity of intestinal tissue injury induced by ionising radiation exposure.

SUPPORTIVE TREATMENT

Patient selection for radiotherapy

Patient risk profile for developing radiation toxicity merits a careful consideration while assessing individual patient for radiotherapy treatment. Co-morbid diseases such as vascular disease, hypertension, diabetes mellitus and atherosclerosis[55], inflammatory bowel disease[56-58], collagen vascular disease[59] and human immunodeficiency virus (HIV) infection[60,61] have been linked to an increased risk of toxicity following external beam radiotherapy.

Nutritional status and effect of diet during radiotherapy

Patient general health and nutritional status during radiotherapy may affect outcomes. Measures to improve patient nutrition such as dietary counselling, oral supplements and intensive nutrition intervention have been associated with an improved outcome in terms of body weight, morbidity, and quality of life during and after radiotherapy[62,63]. McGough *et al*[64] reviewed the efficacy of nutritional intervention on bowel symptoms during pelvic radiotherapy in data from 2646 patients. They found no evidence base for nutritional interventions identified to mitigate bowel symptoms following radiotherapy. However, the role of probiotic supplements, low fat diet and elemental diet was recommended for further evaluation. Subsequent studies on elemental diet identified the difficulty of patients tolerance to large volumes of elemental diet regimen during radiotherapy period[65,66]. The effect of high-potency probiotic preparation VSL3 has been investigated in double-blind, placebo-controlled trial of 490 pelvic radiotherapy patients. The results showed more diarrhoea and daily bowel movements in the placebo group compared with VSL3 recipients[67].

Biological, chemical and pharmacological therapy

Biological, chemical or pharmacological interventions to ameliorate the effect of radiation injury on gastrointestinal tract can be categorized according to the time of administration with respect to radiation exposure. Agents administered prior to radiotherapy for normal tissue prophylaxis are described as radioprotectors. Agents administered during the course of radiotherapy to minimize the injury are called mitigators, while agents administered to ameliorate an established injury are called treatment[68].

The complexity of the pathophysiology of intestinal radiation injury along with the increasing prevalence of the problem has attracted significant research interest in exploring the effectiveness of many agents. Treatment of delayed radiation injury is challenging as it is often refractory to different therapeutic modalities[69].

At the pre-clinical level, there has been a significant interest to test the efficacy of different agents to modulate the biological process implicated in radiation induced tissue injury and normal tissue damage (Table 1).

At the clinical level, there are no formal trials to reliably assess the effectiveness of most of the suggested therapies; hence most of the evidence is obtained from small studies which are often from a single centre. In addition, the results reported in many studies are often directly related to the radiation toxicity scale in use and its sensitivity, specificity as well as to methods of interpretation and grading of symptoms reported by patients.

Topical therapy

Topical therapy for radiation proctitis has been tried with different agents. Local instillation of formalin has been reported to be effective in the treatment of severe haemorrhagic radiation proctitis[70]. Sodium butyrate enemas were reported to improve the acute symptoms of radiation proctitis with no impact on the incidence and severity of late proctitis[71]. Steroid enemas and other topical steroid preparations, commonly prescribed for other anorectal inflammatory conditions have also been in use for symptom relief in radiation proctitis, but without conclusive evidence of efficacy.

Hyperbaric oxygen was suggested to exert its therapeutic role in chronic radiation proctitis through induction of neovascularization that reverses tissue hypoxia[72,73] with trophic effect on vasculogenic stem cells[74]. Treatment of chronic radiation proctitis with hyperbaric oxygen has shown promising results in a large randomized double-blind trial with 120 patients with long term follow up. Hyperbaric oxygen therapy significantly improved the healing responses in patients with refractory radiation proctitis with enhanced bowel-specific quality of life[75].

A recent systematic review demonstrated the therapeutic benefit of hyperbaric oxygen based on the evidence and expert consensus opinion. The review demonstrated its efficacy in patients with radiation damage to the anus and rectum and refractory chronic radiation injury[73].

Anti-inflammatory agents

Anti-inflammatory agents were suggested to reduce the severity of acute intestinal inflammation following irradiation. 5 aminosalicylic acid (5-ASA) has been studied, but most have reported no benefit or even worse symptoms with the use of 5-ASA compared to placebo[76-79]. Sulphasalazine showed clinical improvement in symptoms in a case series of four patients[80]. Balsalazide has been suggested to improve proctitis and toxicity grade in patients undergoing prostate cancer radiotherapy, in a study of 27 patients[81]. Taken together, the clinical evidence on therapeutic role of 5-ASA are not sufficient to recommend routine use in radiation injury.

Antioxidants

Antioxidants were suggested to have cytoprotective effects by reducing cellular oxidative stress following radiation injury to intestinal tissue. A sustained therapeutic benefit after 4 wk therapy with vitamin E and C has been reported in a study involving twenty patients with chronic radiation proctitis[82]. More studies will be required to assess the therapeutic role of different antioxidant agents.

Table 1 Examples of putative intestinal radioprotectants tested in pre-clinical studies

Biological agent	Suggested effect/mechanism	Suggested radioprotection
Glutamine, arginine enriched diet[103]	Enhanced mucosal healing	Protective effect on rat intestine compared to control
Vitamin- E[104]	Reduction of oxidative stress	Protective effect on rat intestine compared to control
Captopril[105]	Inhibition of pro-inflammatory enzyme angiotensin-1-converting enzyme	Protective effect on mouse intestine compared to control
Rofecoxib[106]	Selective Inhibition of cyclooxygenases -2 enzyme	Protective effect on rat intestine compared to control
Clopidogrel[107]	Inhibition of platelets aggregation with reduced vascular sclerosis	Protective effect on rat intestine
Thalidomide[108]	Protection to microvascular bed	Attenuated injury to rat endothelial cells of micro vascular bed compared to control
Simvastatin[109]	Attenuate endothelial cell injury	Attenuated delayed intestinal injury in rats
Glucagon-like peptide-2 (GLP-2)[110]	Increased mucosal mass	Intestinal trophic and protective effect to rat intestine
Octreotide[111,112]	Modulation the inflammatory effects mediated by over expression of NFκB	Ameliorated inflammation and injury in rat intestine
Prostaglandin E-2[113]	Pro-proliferative and anti-apoptotic effect on intestinal epithelium	Increased crypts survival
Anti-Transforming growth factors beta receptor[114]	Biological inhibition of extracellular remodeling	Reduced intestinal injury and fibrosis compared to IgG treated control mice
Toll like receptor 5 agonist derived from Salmonella flagellin[115]	Activation of intestinal immune response *via* NFκB signalling pathway	Protective effect to mice intestine
Intestinal sterilisation[116] Germ-free raised mice	Reduced number of translocated bacteria, sepsis and inflammation	Reduced intestinal cell apoptosis
Probiotic bacteria[117] (*Lactobacillus species*)	Restored intestinal microbiota imbalance	Reduces intestinal damage, sepsis and death in rodents

Amifostine

Amifostine is a scavenger of reactive oxygen species. Its protective effects have been related to its ability to minimise the injurious effects of free radicals on intestinal cells[83]. Amifostine has been investigated for both systemic and topical routes of administration during radiotherapy. Intravenous amifostine administered daily before radiotherapy has been shown to reduce the incidence of radiation proctitis during pelvic radiotherapy[84,85]. Similarly, rectal suspension of amifostine in two doses (1 g in 18 patients and 2 g in 12 patients) administrated daily before radiation therapy for prostate cancer has resulted in significant improvement in acute and late bowel quality of life toxicity parameters, more noticeable with higher doses[86]. Despite the results of these studies and that of many others, the updated clinical practice guidelines for the prevention and treatment of mucositis published in 2007[87] concluded that data on amifostine are mostly obtained from small, single-centre studies with conflicting results. The group concluded that intravenous amifostine daily administration prior to radiotherapy may prevent radiation proctitis in patients who are receiving standard-dose radiotherapy for rectal cancer (Level Ⅲ evidence, grade B recommendation)[87].

In previous studies, amifostine has been shown to reduce the incidence of xerostomia in patients during head and neck radiotherapy[88]. The American Society for Clinical Oncology recommended its use in 2002 published guidelines[89]. However, the recommendation has been subsequently withdrawn in the guidelines update in 2008 due to an insufficient data[90].

Sucralfate

Protection of mucosal cells has been suggested with sucralfate (aluminium sucrose octasulfate). A beneficial effect of sucralfate has been previously reported in two double-blind placebo-controlled studies in patients who received pelvic radiotherapy. It reported that the frequency of defecation and stool consistency were improved by sucralfate[91,92]. Sucralfate enemas have been evaluated in 26 patients with radiation-induced proctitis with persistent rectal bleeding and the results showed a reduction in the severity of rectal bleeding in all patients with sucralfate enemas after four weeks treatment[93]. However, in another study the therapeutic effect of sucralfate rectal enemas were evaluated compared to placebo, both administered during the course of prostate cancer radiotherapy. The results showed no difference in the rate of rectal bleeding between sucralfate and placebo treated group after a median follow-up of five years[94]. The therapeutic benefits of oral sucralfate have also been assessed in a prospective randomised placebo-controlled study on fifty one patients receiving pelvic radiotherapy. Results from 44 study subjects showed significantly increased diarrhoea in the sucralfate group which led to cessation of the trial. The study concluded that sucralfate cannot be recommended for prophylaxis of acute radiation to the rectum and may even worsen the symptoms[95]. Similarly, the lack of effect of micronized sucralfate mouthwash has been reported in a randomized, controlled trial in radiation-induced oral mucositis patients[96]. Despite extensive investigation, clinical data on sucralfate have shown variable results, which do not support its protective role

for routine clinical use.

ENDOSCOPIC MANAGEMENT, BIOPSY AND LASER CAUTERY

A variety of endoscopic therapies have been tried for the treatment of chronic radiation injury such as the heater probe, bipolar electrocoagulation and argon plasma coagulation with newer methods of endoscopic ablation such as radiofrequency ablation and cryotherapy[97]. Endoscopic treatment has been used primarily aimed to treat bleeding rectal telengectasia. Argon plasma coagulation therapy has been shown to be effective and safe treatment for treatment of rectal bleeding resulting from chronic radiation injury[98,99]. Caution should be applied however when considering the use of these invasive techniques following high dose treatment delivery. For instance the use of rectal wall biopsy and laser cautery has been associated with the development of recto-urinary fistulae[100-102] in men treated with brachytherapy for prostate cancer.

CONCLUSION

Prevention of gastrointestinal toxicity starts with a thorough assessment of patient risk profile before radiotherapy. There is no prophylactic or therapeutic agent available to radiotherapy patients proven to mitigate the acute and chronic symptoms of gastrointestinal radiation injury or to allow safe radiation dose escalation for better control of cancer. Many therapeutic agents are in use, but often with little evidence base. The recent advances in radiotherapy planning and delivery techniques provide a considerable degree of protection to the gastrointestinal during external beam radiotherapy. Newer techniques such as image guided radiotherapy techniques, stereotactic therapy and proton beam therapy may confer additional protection. On the other hand, a better understanding of the pathophysiology of intestinal radiation injury will allow the development of more effective biological strategies that could increase the end organ resistance to radiation toxicity. In this regard, modulation of various cellular and cytokine pathways that have been implicated in the development of the acute pathological process of radiation injury can give future promise. In addition to targeting different mechanisms mediating chronic inflammatory and fibrotic process that underlie the delayed pathological changes in the gastrointestinal tract, such approaches may provide new therapeutics insights to this problem.

ACKNOWLEDGMENTS

The Health Research Board, Ireland.

REFERENCES

1 **DeVita VT**, Hellman SRS. Cancer: Principles and and Practice of Oncolology. Philadelphia: Lippincott Williams and Wilkins, 2005
2 **Wang J**, Boerma M, Fu Q, Hauer-Jensen M. Significance of endothelial dysfunction in the pathogenesis of early and delayed radiation enteropathy. *World J Gastroenterol* 2007; **13**: 3047-3055 [PMID: 17589919]
3 **Ringborg U**, Bergqvist D, Brorsson B, Cavallin-Ståhl E, Ceberg J, Einhorn N, Frödin JE, Järhult J, Lamnevik G, Lindholm C, Littbrand B, Norlund A, Nylén U, Rosén M, Svensson H, Möller TR. The Swedish Council on Technology Assessment in Health Care (SBU) systematic overview of radiotherapy for cancer including a prospective survey of radiotherapy practice in Sweden 2001--summary and conclusions. *Acta Oncol* 2003; **42**: 357-365 [PMID: 14596499]
4 **Van de Werf E**, Verstraete J, Lievens Y. The cost of radiotherapy in a decade of technology evolution. *Radiother Oncol* 2012; **102**: 148-153 [PMID: 21872955]
5 **Sher DJ**. Cost-effectiveness studies in radiation therapy. *Expert Rev Pharmacoecon Outcomes Res* 2010; **10**: 567-582 [PMID: 20950072 DOI: 10.1586/erp.10.51]
6 **Bacon CG**, Giovannucci E, Testa M, Kawachi I. The impact of cancer treatment on quality of life outcomes for patients with localized prostate cancer. *J Urol* 2001; **166**: 1804-1810 [PMID: 11586228]
7 **Bacon CG**, Giovannucci E, Testa M, Glass TA, Kawachi I. The association of treatment-related symptoms with quality-of-life outcomes for localized prostate carcinoma patients. *Cancer* 2002; **94**: 862-871 [PMID: 11857323 DOI: 10.1002/cncr.10248]
8 **Waddell BE**, Rodriguez-Bigas MA, Lee RJ, Weber TK, Petrelli NJ. Prevention of chronic radiation enteritis. *J Am Coll Surg* 1999; **189**: 611-624 [PMID: 10589598 DOI: S1072-7515(99)00199-4]
9 **Bayley AJ**, Catton CN, Haycocks T, Kelly V, Alasti H, Bristow R, Catton P, Crook J, Gospodarowicz MK, McLean M, Milosevic M, Warde P. A randomized trial of supine vs. prone positioning in patients undergoing escalated dose conformal radiotherapy for prostate cancer. *Radiother Oncol* 2004; **70**: 37-44 [PMID: 15036850 DOI: 10.1016/j.radonc.2003.08.007]
10 **Gallagher MJ**, Brereton HD, Rostock RA, Zero JM, Zekoski DA, Poyss LF, Richter MP, Kligerman MM. A prospective study of treatment techniques to minimize the volume of pelvic small bowel with reduction of acute and late effects associated with pelvic irradiation. *Int J Radiat Oncol Biol Phys* 1986; **12**: 1565-1573 [PMID: 3759581]
11 **Shanahan TG**, Mehta MP, Bertelrud KL, Buchler DA, Frank LE, Gehring MA, Kubsad SS, Utrie PC, Kinsella TJ. Minimization of small bowel volume within treatment fields utilizing customized "belly boards". *Int J Radiat Oncol Biol Phys* 1990; **19**: 469-476 [PMID: 2394624]
12 **Green N**, Iba G, Smith WR. Measures to minimize small intestine injury in the irradiated pelvis. *Cancer* 1975; **35**: 1633-1640 [PMID: 1148997]
13 **Herbert SH**, Solin LJ, Hoffman JP, Schultz DJ, Curran WJ, Lanciano RM, Rosenblum N, Hogan M, Eisenberg B, Hanks GE. Volumetric analysis of small bowel displacement from radiation portals with the use of a pelvic tissue expander. *Int J Radiat Oncol Biol Phys* 1993; **25**: 885-893 [PMID: 8478241]
14 **Noyes WR**, Hosford CC, Schultz SE. Human collagen injections to reduce rectal dose during radiotherapy. *Int J Radiat Oncol Biol Phys* 2012; **82**: 1918-1922 [PMID: 21514738]
15 **Meric F**, Hirschl RB, Mahboubi S, Womer RB, Goldwein J, Ross AJ, Schnaufer L. Prevention of radiation enteritis in children, using a pelvic mesh sling. *J Pediatr Surg* 1994; **29**: 917-921 [PMID: 7931970]
16 **Tuech JJ**, Chaudron V, Thoma V, Ollier JC, Tassetti V, Duval D, Rodier JF. Prevention of radiation enteritis by intrapelvic breast prosthesis. *Eur J Surg Oncol* 2004; **30**: 900-904 [PMID: 15336738 DOI: 10.1016/j.ejso.2004.06.012]
17 **Waddell BE**, Lee RJ, Rodriguez-Bigas MA, Weber TK, Pe-

trelli NJ. Absorbable mesh sling prevents radiation-induced bowel injury during "sandwich" chemoradiation for rectal cancer. *Arch Surg* 2000; **135**: 1212-1217 [PMID: 11030884]
18 **Haider N**, O'Sullivan C, Corbally MT, Fitzgerald RJ. Abdominopelvic mesh compartmentalization reduces the complications of radiotherapy in children: a preliminary report. *Eur J Pediatr Surg* 2006; **16**: 348-351 [PMID: 17160781 DOI: 10.1055/s-2006-924521]
19 **Soper JT**, Clarke-Pearson DL, Creasman WT. Absorbable synthetic mesh (910-polyglactin) intestinal sling to reduce radiation-induced small bowel injury in patients with pelvic malignancies. *Gynecol Oncol* 1988; **29**: 283-289 [PMID: 3345950]
20 **Sezeur A**, Martella L, Abbou C, Gallot D, Schlienger M, Vibert JF, Touboul E, Martel P, Malafosse M. Small intestine protection from radiation by means of a removable adapted prosthesis. *Am J Surg* 1999; **178**: 22-25; discussion 25-26 [PMID: 10456697]
21 **Dasmahapatra KS**, Swaminathan AP. The use of a biodegradable mesh to prevent radiation-associated small-bowel injury. *Arch Surg* 1991; **126**: 366-369 [PMID: 1847798]
22 **Logmans A**, van Lent M, van Geel AN, Olofsen-Van Acht M, Koper PC, Wiggers T, Trimbos JB. The pedicled omentoplasty, a simple and effective surgical technique to acquire a safe pelvic radiation field; theoretical and practical aspects. *Radiother Oncol* 1994; **33**: 269-271 [PMID: 7716269]
23 **Chen JS**, ChangChien CR, Wang JY, Fan HA. Pelvic peritoneal reconstruction to prevent radiation enteritis in rectal carcinoma. *Dis Colon Rectum* 1992; **35**: 897-901 [PMID: 1387358]
24 **van Lin EN**, Hoffmann AL, van Kollenburg P, Leer JW, Visser AG. Rectal wall sparing effect of three different endorectal balloons in 3D conformal and IMRT prostate radiotherapy. *Int J Radiat Oncol Biol Phys* 2005; **63**: 565-576 [PMID: 16168848]
25 **Woel R**, Beard C, Chen MH, Hurwitz M, Loffredo M, McMahon E, Ching J, Lopes L, D'Amico AV. Acute gastrointestinal, genitourinary, and dermatological toxicity during dose-escalated 3D-conformal radiation therapy (3DCRT) using an intrarectal balloon for prostate gland localization and immobilization. *Int J Radiat Oncol Biol Phys* 2005; **62**: 392-396 [PMID: 15890580]
26 **Drzymala RE**, Mohan R, Brewster L, Chu J, Goitein M, Harms W, Urie M. Dose-volume histograms. *Int J Radiat Oncol Biol Phys* 1991; **21**: 71-78 [PMID: 2032898 DOI: 0360-3016(91)90168-4]
27 **Niemierko A**, Goitein M. Dose-volume distributions: a new approach to dose-volume histograms in three-dimensional treatment planning. *Med Phys* 1994; **21**: 3-11 [PMID: 8164585]
28 **Greco C**, Mazzetta C, Cattani F, Tosi G, Castiglioni S, Fodor A, Orecchia R. Finding dose-volume constraints to reduce late rectal toxicity following 3D-conformal radiotherapy (3D-CRT) of prostate cancer. *Radiother Oncol* 2003; **69**: 215-222 [PMID: 14643961]
29 **Meyer J**, Czito B, Yin FF, Willett C. Advanced radiation therapy technologies in the treatment of rectal and anal cancer: intensity-modulated photon therapy and proton therapy. *Clin Colorectal Cancer* 2007; **6**: 348-356 [PMID: 17311699]
30 **Meyer JJ**, Willett CG, Czito BG. Emerging role of intensity-modulated radiation therapy in anorectal cancer. *Expert Rev Anticancer Ther* 2008; **8**: 585-593 [PMID: 18402525 DOI: 10.1586/14737140.8.4.585]
31 **Willett CG**. Technical advances in the treatment of patients with rectal cancer. *Int J Radiat Oncol Biol Phys* 1999; **45**: 1107-1108 [PMID: 10613301]
32 **Melian E**, Mageras GS, Fuks Z, Leibel SA, Niehaus A, Lorant H, Zelefsky M, Baldwin B, Kutcher GJ. Variation in prostate position quantitation and implications for three-dimensional conformal treatment planning. *Int J Radiat Oncol Biol Phys* 1997; **38**: 73-81 [PMID: 9212007]
33 **Nutting CM**, Convery DJ, Cosgrove VP, Rowbottom C, Padhani AR, Webb S, Dearnaley DP. Reduction of small and large bowel irradiation using an optimized intensity-modulated pelvic radiotherapy technique in patients with prostate cancer. *Int J Radiat Oncol Biol Phys* 2000; **48**: 649-656 [PMID: 11020560]
34 **Ling CC**, Burman C, Chui CS, Kutcher GJ, Leibel SA, LoSasso T, Mohan R, Bortfeld T, Reinstein L, Spirou S, Wang XH, Wu Q, Zelefsky M, Fuks Z. Conformal radiation treatment of prostate cancer using inversely-planned intensity-modulated photon beams produced with dynamic multileaf collimation. *Int J Radiat Oncol Biol Phys* 1996; **35**: 721-730 [PMID: 8690638]
35 **Fraass BA**, Kessler ML, McShan DL, Marsh LH, Watson BA, Dusseau WJ, Eisbruch A, Sandler HM, Lichter AS. Optimization and clinical use of multisegment intensity-modulated radiation therapy for high-dose conformal therapy. *Semin Radiat Oncol* 1999; **9**: 60-77 [PMID: 10196399]
36 **Zelefsky MJ**, Fuks Z, Hunt M, Lee HJ, Lombardi D, Ling CC, Reuter VE, Venkatraman ES, Leibel SA. High dose radiation delivered by intensity modulated conformal radiotherapy improves the outcome of localized prostate cancer. *J Urol* 2001; **166**: 876-881 [PMID: 11490237]
37 **Lips IM**, Dehnad H, van Gils CH, Boeken Kruger AE, van der Heide UA, van Vulpen M. High-dose intensity-modulated radiotherapy for prostate cancer using daily fiducial marker-based position verification: acute and late toxicity in 331 patients. *Radiat Oncol* 2008; **3**: 15 [PMID: 18495016]
38 **Lips IM**, van Gils CH, van der Heide UA, Kruger AE, van Vulpen M. Health-related quality of life 3 years after high-dose intensity-modulated radiotherapy with gold fiducial marker-based position verification. *BJU Int* 2009; **103**: 762-767 [PMID: 18990145]
39 **Marchand V**, Bourdin S, Charbonnel C, Rio E, Munos C, Campion L, Bonnaud-Antignac A, Lisbona A, Mahé MA, Supiot S. No impairment of quality of life 18 months after high-dose intensity-modulated radiotherapy for localized prostate cancer: a prospective study. *Int J Radiat Oncol Biol Phys* 2010; **77**: 1053-1059 [PMID: 19880259]
40 **Toledano I**, Graff P, Serre A, Boisselier P, Bensadoun RJ, Ortholan C, Pommier P, Racadot S, Calais G, Alfonsi M, Favrel V, Giraud P, Lapeyre M. Intensity-modulated radiotherapy in head and neck cancer: results of the prospective study GORTEC 2004-03. *Radiother Oncol* 2012; **103**: 57-62 [PMID: 22296746]
41 **Chen AM**, Farwell DG, Luu Q, Vazquez EG, Lau DH, Purdy JA. Intensity-Modulated Radiotherapy is Associated with Improved Global Quality of Life Among Long-Term Survivors of Head-and-Neck Cancer. *Int J Radiat Oncol Biol Phys* 2012; Epub ahead of print [PMID: 22300572]
42 **Chen AB**, D'Amico AV, Neville BA, Earle CC. Patient and treatment factors associated with complications after prostate brachytherapy. *J Clin Oncol* 2006; **24**: 5298-5304 [PMID: 17114664]
43 **Lee WR**, Bae K, Lawton C, Gillin M, Morton G, Firat S, Baikadi M, Kuettel M, Greven K, Sandler H. Late toxicity and biochemical recurrence after external-beam radiotherapy combined with permanent-source prostate brachytherapy: analysis of Radiation Therapy Oncology Group study 0019. *Cancer* 2007; **109**: 1506-1512 [PMID: 17340591 DOI: 10.1002/cncr.22560]
44 **Peters CA**, Cesaretti JA, Stone NN, Stock RG. Low-dose rate prostate brachytherapy is well tolerated in patients with a history of inflammatory bowel disease. *Int J Radiat Oncol Biol Phys* 2006; **66**: 424-429 [PMID: 16887295]
45 **Chung HT**, Xia P, Chan LW, Park-Somers E, Roach M. Does image-guided radiotherapy improve toxicity profile in whole pelvic-treated high-risk prostate cancer? Comparison between IG-IMRT and IMRT. *Int J Radiat Oncol Biol Phys* 2009; **73**: 53-60 [PMID: 18501530]
46 **Ikushima H**. Radiation therapy: state of the art and the fu-

ture. *J Med Invest* 2010; **57**: 1-11 [PMID: 20299738]
47 **Lo SS**, Fakiris AJ, Chang EL, Mayr NA, Wang JZ, Papiez L, Teh BS, McGarry RC, Cardenes HR, Timmerman RD. Stereotactic body radiation therapy: a novel treatment modality. *Nat Rev Clin Oncol* 2010; **7**: 44-54 [PMID: 19997074]
48 **Dilling TJ**, Hoffe SE. Stereotactic body radiation therapy: transcending the conventional to improve outcomes. *Cancer Control* 2008; **15**: 104-111 [PMID: 18376377]
49 **Buyyounouski MK**, Price RA, Harris EE, Miller R, Tomé W, Schefter T, Parsai EI, Konski AA, Wallner PE. Stereotactic body radiotherapy for primary management of early-stage, low- to intermediate-risk prostate cancer: report of the American Society for Therapeutic Radiology and Oncology Emerging Technology Committee. *Int J Radiat Oncol Biol Phys* 2010; **76**: 1297-1304 [PMID: 20338473 DOI: S0360-3016(09)03543-3]
50 **Kavanagh BD**, Pan CC, Dawson LA, Das SK, Li XA, Ten Haken RK, Miften M. Radiation dose-volume effects in the stomach and small bowel. *Int J Radiat Oncol Biol Phys* 2010; **76**: S101-S107 [PMID: 20171503]
51 **Loeffler JS**, Smith AR, Suit HD. The potential role of proton beams in radiation oncology. *Semin Oncol* 1997; **24**: 686-695 [PMID: 9422264]
52 **Suit H**, Goldberg S, Niemierko A, Trofimov A, Adams J, Paganetti H, Chen GT, Bortfeld T, Rosenthal S, Loeffler J, Delaney T. Proton beams to replace photon beams in radical dose treatments. *Acta Oncol* 2003; **42**: 800-808 [PMID: 14968940]
53 **Chiba T**, Tokuuye K, Matsuzaki Y, Sugahara S, Chuganji Y, Kagei K, Shoda J, Hata M, Abei M, Igaki H, Tanaka N, Akine Y. Proton beam therapy for hepatocellular carcinoma: a retrospective review of 162 patients. *Clin Cancer Res* 2005; **11**: 3799-3805 [PMID: 15897579]
54 **Sugahara S**, Oshiro Y, Nakayama H, Fukuda K, Mizumoto M, Abei M, Shoda J, Matsuzaki Y, Thono E, Tokita M, Tsuboi K, Tokuuye K. Proton beam therapy for large hepatocellular carcinoma. *Int J Radiat Oncol Biol Phys* 2010; **76**: 460-466 [PMID: 19427743]
55 **Yeoh E**, Horowitz M, Russo A, Muecke T, Robb T, Maddox A, Chatterton B. Effect of pelvic irradiation on gastrointestinal function: a prospective longitudinal study. *Am J Med* 1993; **95**: 397-406 [PMID: 8213872]
56 **Matthews RH**. Collagen vascular disease and irradiation. *Int J Radiat Oncol Biol Phys* 1989; **17**: 1123-1124 [PMID: 2808050]
57 **Hareyama M**, Nagakura H, Tamakawa M, Hyodo K, Asakura K, Horikoshi T, Oouchi A, Shido M, Morita K. Severe reaction after chemoradiotherapy of nasopharyngeal carcinoma with collagen disease. *Int J Radiat Oncol Biol Phys* 1995; **33**: 971 [PMID: 7591915]
58 **Abu-Shakra M**, Lee P. Exaggerated fibrosis in patients with systemic sclerosis (scleroderma) following radiation therapy. *J Rheumatol* 1993; **20**: 1601-1603 [PMID: 8164225]
59 **Chon BH**, Loeffler JS. The effect of nonmalignant systemic disease on tolerance to radiation therapy. *Oncologist* 2002; **7**: 136-143 [PMID: 11961197]
60 **Nisce LZ**, Safai B. Radiation therapy of Kaposi's sarcoma in AIDS. Memorial Sloan-Kettering experience. *Front Radiat Ther Oncol* 1985; **19**: 133-137 [PMID: 3920122]
61 **Cooper JS**, Fried PR. Defining the role of radiation therapy in the management of epidemic Kaposi's sarcoma. *Int J Radiat Oncol Biol Phys* 1987; **13**: 35-39 [PMID: 2433259]
62 **Ravasco P**, Monteiro-Grillo I, Marques Vidal P, Camilo ME. Impact of nutrition on outcome: a prospective randomized controlled trial in patients with head and neck cancer undergoing radiotherapy. *Head Neck* 2005; **27**: 659-668 [PMID: 15920748 DOI: 10.1002/hed.20221]
63 **Isenring EA**, Bauer JD, Capra S. Nutrition support using the American Dietetic Association medical nutrition therapy protocol for radiation oncology patients improves dietary intake compared with standard practice. *J Am Diet Assoc* 2007; **107**: 404-412 [PMID: 17324657]
64 **McGough C**, Baldwin C, Frost G, Andreyev HJ. Role of nutritional intervention in patients treated with radiotherapy for pelvic malignancy. *Br J Cancer* 2004; **90**: 2278-2287 [PMID: 15162154 DOI: 10.1038/sj.bjc.6601868]
65 **McGough C**, Baldwin C, Norman A, Frost G, Blake P, Tait D, Khoo V, Harrington K, Andreyev HJ. Is supplementation with elemental diet feasible in patients undergoing pelvic radiotherapy? *Clin Nutr* 2006; **25**: 109-116 [PMID: 16289498]
66 **McGough C**, Wedlake L, Baldwin C, Hackett C, Norman AR, Blake P, Harrington K, Tait D, Khoo V, Frost G, Andreyev HJ. Clinical trial: normal diet vs. partial replacement with oral E028 formula for the prevention of gastrointestinal toxicity in cancer patients undergoing pelvic radiotherapy. *Aliment Pharmacol Ther* 2008; **27**: 1132-1139 [PMID: 18315590]
67 **Delia P**, Sansotta G, Donato V, Frosina P, Messina G, De Renzis C, Famularo G. Use of probiotics for prevention of radiation-induced diarrhea. *World J Gastroenterol* 2007; **13**: 912-915 [PMID: 17352022]
68 **Citrin D**, Cotrim AP, Hyodo F, Baum BJ, Krishna MC, Mitchell JB. Radioprotectors and mitigators of radiation-induced normal tissue injury. *Oncologist* 2010; **15**: 360-371 [PMID: 20413641]
69 **Denton A**, Forbes A, Andreyev J, Maher EJ. Non surgical interventions for late radiation proctitis in patients who have received radical radiotherapy to the pelvis. *Cochrane Database Syst Rev* 2002; CD003455 [PMID: 11869662]
70 **Chattopadhyay G**, Ray D, Chakravartty S, Mandal S. Formalin instillation for uncontrolled radiation induced haemorrhagic proctitis. *Trop Gastroenterol* 2010; **31**: 291-294 [PMID: 21568145]
71 **Hille A**, Herrmann MK, Kertesz T, Christiansen H, Hermann RM, Pradier O, Schmidberger H, Hess CF. Sodium butyrate enemas in the treatment of acute radiation-induced proctitis in patients with prostate cancer and the impact on late proctitis. A prospective evaluation. *Strahlenther Onkol* 2008; **184**: 686-692 [PMID: 19107351 DOI: 10.1007/s00066-008-1896-1]
72 **Fok TC**, Jan A, Peel SA, Evans AW, Clokie CM, Sándor GK. Hyperbaric oxygen results in increased vascular endothelial growth factor (VEGF) protein expression in rabbit calvarial critical-sized defects. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2008; **105**: 417-422 [PMID: 18206401]
73 **Craighead P**, Shea-Budgell MA, Nation J, Esmail R, Evans AW, Parliament M, Oliver TK, Hagen NA. Hyperbaric oxygen therapy for late radiation tissue injury in gynecologic malignancies. *Curr Oncol* 2011; **18**: 220-227 [PMID: 21980249]
74 **Milovanova TN**, Bhopale VM, Sorokina EM, Moore JS, Hunt TK, Hauer-Jensen M, Velazquez OC, Thom SR. Hyperbaric oxygen stimulates vasculogenic stem cell growth and differentiation in vivo. *J Appl Physiol* 2009; **106**: 711-728 [PMID: 19023021]
75 **Clarke RE**, Tenorio LM, Hussey JR, Toklu AS, Cone DL, Hinojosa JG, Desai SP, Dominguez Parra L, Rodrigues SD, Long RJ, Walker MB. Hyperbaric oxygen treatment of chronic refractory radiation proctitis: a randomized and controlled double-blind crossover trial with long-term follow-up. *Int J Radiat Oncol Biol Phys* 2008; **72**: 134-143 [PMID: 18342453]
76 **Baughan CA**, Canney PA, Buchanan RB, Pickering RM. A randomized trial to assess the efficacy of 5-aminosalicylic acid for the prevention of radiation enteritis. *Clin Oncol* (*R Coll Radiol*) 1993; **5**: 19-24 [PMID: 8424910]
77 **Resbeut M**, Marteau P, Cowen D, Richaud P, Bourdin S, Dubois JB, Mere P, N'Guyen TD. A randomized double blind placebo controlled multicenter study of mesalazine for the prevention of acute radiation enteritis. *Radiother Oncol* 1997; **44**: 59-63 [PMID: 9288859]
78 **Martenson JA**, Hyland G, Moertel CG, Mailliard JA, O'Fallon JR, Collins RT, Morton RF, Tewfik HH, Moore RL, Frank AR, Urias RE, Deming RL. Olsalazine is contraindicated during pelvic radiation therapy: results of a double-blind,

randomized clinical trial. *Int J Radiat Oncol Biol Phys* 1996; **35**: 299-303 [PMID: 8635937]
79 **Sanguineti G**, Franzone P, Marcenaro M, Foppiano F, Vitale V. Sucralfate versus mesalazine versus hydrocortisone in the prevention of acute radiation proctitis during conformal radiotherapy for prostate carcinoma. A randomized study. *Strahlenther Onkol* 2003; **179**: 464-470 [PMID: 12835883 DOI: 10.1007/s00066-003-1082-4]
80 **Goldstein F**, Khoury J, Thornton JJ. Treatment of chronic radiation enteritis and colitis with salicylazosulfapyridine and systemic corticosteroids. A pilot study. *Am J Gastroenterol* 1976; **65**: 201-208 [PMID: 7136]
81 **Jahraus CD**, Bettenhausen D, Malik U, Sellitti M, St Clair WH. Prevention of acute radiation-induced proctosigmoiditis by balsalazide: a randomized, double-blind, placebo controlled trial in prostate cancer patients. *Int J Radiat Oncol Biol Phys* 2005; **63**: 1483-1487 [PMID: 16099600]
82 **Kennedy M**, Bruninga K, Mutlu EA, Losurdo J, Choudhary S, Keshavarzian A. Successful and sustained treatment of chronic radiation proctitis with antioxidant vitamins E and C. *Am J Gastroenterol* 2001; **96**: 1080-1084 [PMID: 11316150]
83 **Francois A**, Milliat F, Vozenin-Brotons MC. Bowel injury associated with pelvic radiotherapy. *Radiat Phys Chem* 2005; **72**: 399-407 [DOI: 10.1016/j.radphyschem.2004.04.140]
84 **Liu T**, Liu Y, He S, Zhang Z, Kligerman MM. Use of radiation with or without WR-2721 in advanced rectal cancer. *Cancer* 1992; **69**: 2820-2825 [PMID: 1315211]
85 **Athanassiou H**, Antonadou D, Coliarakis N, Kouveli A, Synodinou M, Paraskevaidis M, Sarris G, Georgakopoulos GR, Panousaki K, Karageorgis P, Throuvalas N. Protective effect of amifostine during fractionated radiotherapy in patients with pelvic carcinomas: results of a randomized trial. *Int J Radiat Oncol Biol Phys* 2003; **56**: 1154-1160 [PMID: 12829154]
86 **Simone NL**, Ménard C, Soule BP, Albert PS, Guion P, Smith S, Godette D, Crouse NS, Sciuto LC, Cooley-Zgela T, Camphausen K, Coleman CN, Singh AK. Intrarectal amifostine during external beam radiation therapy for prostate cancer produces significant improvements in Quality of Life measured by EPIC score. *Int J Radiat Oncol Biol Phys* 2008; **70**: 90-95 [PMID: 17855015]
87 **Keefe DM**, Schubert MM, Elting LS, Sonis ST, Epstein JB, Raber-Durlacher JE, Migliorati CA, McGuire DB, Hutchins RD, Peterson DE. Updated clinical practice guidelines for the prevention and treatment of mucositis. *Cancer* 2007; **109**: 820-831 [PMID: 17236223 DOI: 10.1002/cncr.22484]
88 **Antonadou D**, Pepelassi M, Synodinou M, Puglisi M, Throuvalas N. Prophylactic use of amifostine to prevent radiochemotherapy-induced mucositis and xerostomia in head-and-neck cancer. *Int J Radiat Oncol Biol Phys* 2002; **52**: 739-747 [PMID: 11849797]
89 **Schuchter LM**, Hensley ML, Meropol NJ, Winer EP. 2002 update of recommendations for the use of chemotherapy and radiotherapy protectants: clinical practice guidelines of the American Society of Clinical Oncology. *J Clin Oncol* 2002; **20**: 2895-2903 [PMID: 12065567]
90 **Hensley ML**, Hagerty KL, Kewalramani T, Green DM, Meropol NJ, Wasserman TH, Cohen GI, Emami B, Gradishar WJ, Mitchell RB, Thigpen JT, Trotti A, von Hoff D, Schuchter LM. American Society of Clinical Oncology 2008 clinical practice guideline update: use of chemotherapy and radiation therapy protectants. *J Clin Oncol* 2009; **27**: 127-145 [PMID: 19018081]
91 **Henriksson R**, Franzén L, Littbrand B. Effects of sucralfate on acute and late bowel discomfort following radiotherapy of pelvic cancer. *J Clin Oncol* 1992; **10**: 969-975 [PMID: 1588377]
92 **Valls A**, Pestchen I, Prats C, Pera J, Aragón G, Vidarte M, Algara M. [Multicenter double-blind clinical trial comparing sucralfate vs placebo in the prevention of diarrhea secondary to pelvic irradiation]. *Med Clin* (*Barc*) 1999; **113**: 681-684 [PMID: 10650568]
93 **Kochhar R**, Sriram PV, Sharma SC, Goel RC, Patel F. Natural history of late radiation proctosigmoiditis treated with topical sucralfate suspension. *Dig Dis Sci* 1999; **44**: 973-978 [PMID: 10235606]
94 **O'Brien PC**, Franklin CI, Poulsen MG, Joseph DJ, Spry NS, Denham JW. Acute symptoms, not rectally administered sucralfate, predict for late radiation proctitis: longer term follow-up of a phase III trial--Trans-Tasman Radiation Oncology Group. *Int J Radiat Oncol Biol Phys* 2002; **54**: 442-449 [PMID: 12243820]
95 **Hovdenak N**, Sørbye H, Dahl O. Sucralfate does not ameliorate acute radiation proctitis: randomised study and meta-analysis. *Clin Oncol* (*R Coll Radiol*) 2005; **17**: 485-491 [PMID: 16149294]
96 **Dodd MJ**, Miaskowski C, Greenspan D, MacPhail L, Shih AS, Shiba G, Facione N, Paul SM. Radiation-induced mucositis: a randomized clinical trial of micronized sucralfate versus salt & soda mouthwashes. *Cancer Invest* 2003; **21**: 21-33 [PMID: 12643006]
97 **Rustagi T**, Mashimo H. Endoscopic management of chronic radiation proctitis. *World J Gastroenterol* 2011; **17**: 4554-4562 [PMID: 22147960 DOI: 10.3748/wjg.v17.i41.4554]
98 **de la Serna Higuera C**, Martín Arribas M, Rodríguez Gómez S, Pérez Villoria A, Martínez Moreno J, Betancourt González A. Efficacy and safety of argon plasma coagulation for the treatment of hemorrhagic radiation proctitis. *Rev Esp Enferm Dig* 2004; **96**: 758-764 [PMID: 15584849]
99 **Latorre Sánchez M**, Sempere García-Argüelles J, Barceló Cerdá S, Huguet Malaves JM, Canelles Gamir P, Quiles Teodoro F, Medina Chuliá E. [Evaluation of the endoscopic response to argon plasma coagulation in patients with chronic radiation proctopathy]. *Rev Esp Enferm Dig* 2008; **100**: 619-624 [PMID: 19119787]
100 **Gelblum DY**, Potters L. Rectal complications associated with transperineal interstitial brachytherapy for prostate cancer. *Int J Radiat Oncol Biol Phys* 2000; **48**: 119-124 [PMID: 10924980]
101 **Theodorescu D**, Gillenwater JY, Koutrouvelis PG. Prostatourethral-rectal fistula after prostate brachytherapy. *Cancer* 2000; **89**: 2085-2091 [PMID: 11066049 DOI: 10.1002/1097-0142(20001115)89:]
102 **Shakespeare D**, Mitchell DM, Carey BM, Finan P, Henry AM, Ash D, Bottomley DM, Al-Qaisieh B. Recto-urethral fistula following brachytherapy for localized prostate cancer. *Colorectal Dis* 2007; **9**: 328-331 [PMID: 17432984]
103 **Ersin S**, Tuncyurek P, Esassolak M, Alkanat M, Buke C, Yilmaz M, Telefoncu A, Kose T. The prophylactic and therapeutic effects of glutamine- and arginine-enriched diets on radiation-induced enteritis in rats. *J Surg Res* 2000; **89**: 121-125 [PMID: 10729239 DOI: 10.1006/jsre.1999.5808]
104 **Empey LR**, Papp JD, Jewell LD, Fedorak RN. Mucosal protective effects of vitamin E and misoprostol during acute radiation-induced enteritis in rats. *Dig Dis Sci* 1992; **37**: 205-214 [PMID: 1735337]
105 **Yoon SC**, Park JM, Jang HS, Shinn KS, Bahk YW. Radioprotective effect of captopril on the mouse jejunal mucosa. *Int J Radiat Oncol Biol Phys* 1994; **30**: 873-878 [PMID: 7960990]
106 **Keskek M**, Gocmen E, Kilic M, Gencturk S, Can B, Cengiz M, Okten RM, Koc M. Increased expression of cyclooxygenase-2 (COX-2) in radiation-induced small bowel injury in rats. *J Surg Res* 2006; **135**: 76-84 [PMID: 16780881]
107 **Wang J**, Albertson CM, Zheng H, Fink LM, Herbert JM, Hauer-Jensen M. Short-term inhibition of ADP-induced platelet aggregation by clopidogrel ameliorates radiation-induced toxicity in rat small intestine. *Thromb Haemost* 2002; **87**: 122-128 [PMID: 11848440]
108 **Kim KT**, Chae HS, Kim JS, Kim HK, Cho YS, Choi W, Choi KY, Rho SY, Kang SJ. Thalidomide effect in endothelial cell

of acute radiation proctitis. *World J Gastroenterol* 2008; **14**: 4779-4783 [PMID: 18720539]
109 **Wang J**, Boerma M, Fu Q, Kulkarni A, Fink LM, Hauer-Jensen M. Simvastatin ameliorates radiation enteropathy development after localized, fractionated irradiation by a protein C-independent mechanism. *Int J Radiat Oncol Biol Phys* 2007; **68**: 1483-1490 [PMID: 17674978]
110 **Torres S**, Thim L, Milliat F, Vozenin-Brotons MC, Olsen UB, Ahnfelt-Rønne I, Bourhis J, Benderitter M, François A. Glucagon-like peptide-2 improves both acute and late experimental radiation enteritis in the rat. *Int J Radiat Oncol Biol Phys* 2007; **69**: 1563-1571 [PMID: 18035212]
111 **Wang J**, Zheng H, Hauer-Jensen M. Influence of Short-Term Octreotide Administration on Chronic Tissue Injury, Transforming Growth Factor beta (TGF-beta) Overexpression, and Collagen Accumulation in Irradiated Rat Intestine. *J Pharmacol Exp Ther* 2001; **297**: 35-42 [PMID: 11259525]
112 **Olgaç V**, Erbil Y, Barbaros U, Oztezcan S, Giriş M, Kaya H, Bilge H, Güler S, Toker G. The efficacy of octreotide in pancreatic and intestinal changes: radiation-induced enteritis in animals. *Dig Dis Sci* 2006; **51**: 227-232 [PMID: 16416241 DOI: 10.1007/s10620-006-3113-3]
113 **Stenson WF**. Prostaglandins and epithelial response to injury. *Curr Opin Gastroenterol* 2007; **23**: 107-110 [PMID: 17268236 DOI: 10.1097/MOG.0b013e3280143cb6]
114 **Zheng H**, Wang J, Koteliansky VE, Gotwals PJ, Hauer-Jensen M. Recombinant soluble transforming growth factor beta type II receptor ameliorates radiation enteropathy in mice. *Gastroenterology* 2000; **119**: 1286-1296 [PMID: 11054386]
115 **Burdelya LG**, Krivokrysenko VI, Tallant TC, Strom E, Gleiberman AS, Gupta D, Kurnasov OV, Fort FL, Osterman AL, Didonato JA, Feinstein E, Gudkov AV. An agonist of toll-like receptor 5 has radioprotective activity in mouse and primate models. *Science* 2008; **320**: 226-230 [PMID: 18403709]
116 **Crawford PA**, Gordon JI. Microbial regulation of intestinal radiosensitivity. *Proc Natl Acad Sci USA* 2005; **102**: 13254-13259 [PMID: 16129828]
117 **Ciorba MA**, Stenson WF. Probiotic therapy in radiation-induced intestinal injury and repair. *Ann N Y Acad Sci* 2009; **1165**: 190-194 [PMID: 19538306]

P- Reviewers Matsuzaki Y, Ladas SD, Supiot S
S- Editor Gou SX **L- Editor** A **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i2.209

World J Gastroenterol 2013 January 14; 19(2): 209-218
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

ORIGINAL ARTICLE

Intestinal injury can be reduced by intra-arterial postischemic perfusion with hypertonic saline

Oleg Kornyushin, Michael Galagudza, Anna Kotslova, Gelfia Nutfullina, Nina Shved, Alexey Nevorotin, Valeriy Sedov, Timur Vlasov

Oleg Kornyushin, Anna Kotslova, Valeriy Sedov, Department of Surgery, I.P. Pavlov Federal Medical University, 197022 St. Petersburg, Russia
Michael Galagudza, Timur Vlasov, Department of Pathophysiology, I.P. Pavlov Federal Medical University, 197022 St. Petersburg, Russia
Michael Galagudza, Timur Vlasov, Institute of Experimental Medicine, V.A. Almazov Federal Heart, Blood and Endocrinology Centre, 197341 St. Petersburg, Russia
Gelfia Nutfullina, Nina Shved, Department of Pathology, I.P. Pavlov Federal Medical University, 197022 St. Petersburg, Russia
Alexey Nevorotin, Laboratory of Electron Microscopy, I.P. Pavlov Federal Medical University, 197022 St. Petersburg, Russia
Author contributions: Kornyushin O, Galagudza M and Kotslova A performed the experiments; Nutfullina G and Shved N performed histological analyses; Nevorotin A was involved in data analysis and manuscript editing; Sedov V and Vlasov T designed the study and wrote the manuscript.
Supported by Grant 2359.2012.7 of the President of the Russian Federation for the Support of Leading Scientific Groups
Correspondence to: Dr. Michael Galagudza, MD, Department of Pathophysiology, I.P. Pavlov Federal Medical University, 197022 St. Petersburg, Russia. galagoudza@mail.ru
Telephone: +7-812-4997035 Fax: +7-812-4997069
Received: August 29, 2012 Revised: September 26, 2012
Accepted: November 14, 2012
Published online: January 14, 2013

Abstract

AIM: To investigate the effect of local intestinal perfusion with hypertonic saline (HTS) on intestinal ischemia-reperfusion injury (IRI) in both *ex vivo* and *in vivo* rat models.

METHODS: All experiments were performed on male Wistar rats anesthetized with pentobarbital sodium given intraperitoneally at a dose of 60 mg/kg. *Ex vivo* vascularly perfused rat intestine was subjected to 60-min ischemia and either 30-min reperfusion with isotonic buffer (controls), or 5 min with HTS of 365 or 415 mOsm/L osmolarity ($HTS^{365mOsm}$ or $HTS^{415mOsm}$, respectively) followed by 25-min reperfusion with isotonic buffer. The vascular intestinal perfusate flow (IPF) rate was determined by collection of the effluent from the portal vein in a calibrated tube. Spontaneous intestinal contraction rate was monitored throughout. Irreversible intestinal injury or area of necrosis (AN) was evaluated histochemically using 2.3.5-triphenyltetrazolium chloride staining. *In vivo*, 30-min ischemia was followed by either 30-min blood perfusion or 5-min reperfusion with $HTS^{365mOsm}$ through the superior mesenteric artery (SMA) followed by 25-min blood perfusion. Arterial blood pressure (BP) was measured in the common carotid artery using a miniature pressure transducer. Histological injury was evaluated in both preparations using the Chui score.

RESULTS: *Ex vivo*, intestinal IRI resulted in a reduction in the IPF rate during reperfusion ($P < 0.05$ *vs* sham). The postischemic recovery of the IPF rate did not differ between the controls and the $HTS^{365mOsm}$ group. In the $HTS^{415mOsm}$ group, postischemic IPF rates were lower than in the controls and the $HTS^{365mOsm}$ group ($P < 0.05$). The intestinal contraction rate was similar at baseline in all groups. An increase in this parameter was observed during the first 10 min of reperfusion in the control group as compared to the sham-treated group, but no such increase was seen in the $HTS^{365mOsm}$ group. In controls, AN averaged 14.8% ± 5.07% of the total tissue volume. Administration of $HTS^{365mOsm}$ for 5 min after 60-min ischemia resulted in decrease in AN (5.1% ± 1.20% *vs* controls, $P < 0.01$). However, perfusion of the intestine with the HTS of greater osmolarity ($HTS^{415mOsm}$) failed to protect the intestine from irreversible injury. The Chiu score was lower in the $HTS^{365mOsm}$ group in comparison with controls (2.4 ± 0.54 *vs* 3.2 ± 0.44, $P = 0.042$), while intestinal perfusion with $HTS^{415mOsm}$ failed to improve the Chiu score. Intestinal reperfusion with $HTS^{365mOsm}$ in

the *in vivo* series secured rapid recovery of BP after its transient fall, whereas in the controls no recovery was seen. The Chiu score was lower in the $HTS^{365mOsm}$ group *vs* controls (3.1 ± 0.26 and 3.8 ± 0.22, $P = 0.0079$ respectively,), although the magnitude of the effect was lower than in the *ex vivo* series.

CONCLUSION: Brief intestinal postischemic perfusion with $HTS^{365mOsm}$ through the SMA followed by blood flow restoration is a protective procedure that could be used for the prevention of intestinal IRI.

© 2013 Baishideng. All rights reserved.

Key words: Intestinal ischemia-reperfusion injury; Superior mesenteric artery; Perfusion; Hypertonic saline; Acute mesenteric ischemia; Intestinal perfusate flow rate

Kornyushin O, Galagudza M, Kotslova A, Nutfullina G, Shved N, Nevorotin A, Sedov V, Vlasov T. Intestinal injury can be reduced by intra-arterial postischemic perfusion with hypertonic saline. *World J Gastroenterol* 2013; 19(2): 209-218 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i2/209.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i2.209

INTRODUCTION

Acute mesenteric ischemia (AMI) is a life-threatening vascular emergency with a mortality rate as high as 60%-80%[1]. It can be caused by local arterial obstruction, impairment of venous outflow, or a systemic hemodynamic disorder resulting in severe intestinal hypoxic injury. The rather non-specific clinical presentation of AMI can be an obstacle to its early recognition, thus delaying the proper treatment, namely revascularization[2]. However, if AMI can be diagnosed unambiguously prior to the development of intestinal infarction, either embolectomy or the intra-arterial infusion of thrombolytic agents can materially improve the outcome[3]. Unfortunately, even after timely revascularization, the positive effect can be compromised, at least in part, by the development of reperfusion injury, including edema, the no-reflow phenomenon, and the progressive death of mucosal cells[4]. In experimental models, intestinal ischemia-reperfusion injury (IRI) has been shown to be alleviated by a variety of pre- and postconditioning procedures[5,6], while various antioxidants, anesthetics, vasodilators, anti-inflammatory agents, and cytokine receptor blockers can also be helpful[7,8]. Furthermore, the systemic administration of hypertonic saline (HTS) has given clearly positive results[9,10]. The protective effect of HTS in these experiments is believed to be based on the following mechanisms: arteriolar vasodilation, osmotic stress-induced inhibition of inflammatory cells, and prevention of intestinal edema[10-12]. It is to be noted that the application of either HTS or some of the above-mentioned drugs may have certain limitations, such as an increased risk of electrolyte imbalance[12] and the development of adverse drug reactions, in either case as a result of systemic administration. It has been hypothesized, therefore, that perfusion of the intestine with HTS through the superior mesenteric artery (SMA) might prove to be a more advantageous strategy, given its greater efficacy in delivering the hypertonic solution to the target.

The present study was an experimental investigation of the effect of local intra-arterial HTS administration during the early reperfusion phase on the functional and structural manifestations of intestinal injury. To the best of our knowledge, this approach has not yet been attempted.

MATERIALS AND METHODS

Chemicals and materials

2.3.5-triphenyltetrazolium chloride (TTC) was obtained from Sigma-Aldrich (St. Louis, MO, United States). Pentobarbital sodium was purchased from Apoteket (Umea, Sweden). Bovine serum albumin was obtained from Biowest (Nuaille, France). Dextran 60 000 was obtained from Biochemist (Saransk, Russian Federation). Other chemicals used for the preparation of both Krebs-Henseleit buffer and HTS were purchased from Acros Organics (Geel, Belgium).

Animals

All experiments were performed on male Wistar rats weighting 250-300 g. The animals were fed regular chow, and water was available *ad libitum*. The procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals and were approved by the local Ethics Committee. The animals were anesthetized with pentobarbital sodium given intraperitoneally at a dose of 60 mg/kg. Core body temperature was maintained at 37.0 ± 0.5 ℃ by a feedback-controlled heating pad (TCAT-2LV controller, PHYSITEMP Instruments Inc., United States).

Ex vivo experiments

Isolated perfused small intestine preparation: The intestine was isolated and perfused according to a previously described technique[13]. Briefly, the abdominal cavity was opened by a midline incision. To prevent blood coagulation, 50 units of heparin sodium were injected into the inferior vena cava. The abdominal aorta was exposed from the level of the celiac trunk (CT) to the aortic bifurcation. The intestinal segment between the pyloric sphincter and the distal portion of the ileum was cut using an electrocautery unit. The branches of the SMA that supply the cecum and proximal part of the colon were exposed, ligated, and cut, followed by the removal of the proximal colon. The intestinal lumen was rinsed with warm (37 ℃) saline. The aorta was cannulated through its infrarenal segment with subsequent advancement of the cannula tip to the SMA level. The aorta was then ligated immediately above the CT, and perfusion of the small

intestine with oxygenated (95% O_2 and 5% CO_2) Krebs-Henseleit buffer (KHB) was initiated through the SMA and CT at a pressure of 80 mmHg. KHB solution was modified to contain high molecular weight compounds (1.0% bovine serum albumin and 3.0% dextran 60 000) plus (all in mmol/L) 118.5 NaCl, 25.0 $NaHCO_3$, 4.7 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 1.5 $CaCl_2$, 5.0 glucose, 2.0 lactate, 0.2 pyruvate. The portal vein was cannulated to ensure outflow of the perfusion fluid. After ligation of the splenic and gastric vessels, the exposed part of the intestine was placed into the water-jacketed bath filled with normal saline to maintain the temperature at 37 ℃ during the experiment. All surgical procedures described were performed within 50-60 min and were followed by a 20-min stabilization period.

Experimental protocol: After stabilization, ischemia of the intestine was induced by cessation of the perfusate flow for 60 min, followed by reperfusion. During the initial 5-min reperfusion, the perfusion was resumed with the modified KHB of either normal (physiological) or increased osmolarity (315 *vs* 365 or 415 mOsm/L, designated as $HTS^{365mOsm}$ or $HTS^{415mOsm}$, respectively), in each case delivered under a pressure of 80 mmHg, followed by perfusion with normotonic KHB for 25 min. The experiments were randomized and allocated to the following 4 groups**:** (1) Sham (n = 6): the intestine was continuously perfused for 110 min; (2) Controls (n = 6): 60-min intestinal ischemia, followed by 30-min reperfusion with normotonic KHB (osmolarity 315 mOsm/L); (3) $HTS^{365mOsm}$ (n = 5): 60-min intestinal ischemia, followed by 5-min reperfusion with HTS having an increased osmolarity of 365 mOsm/L (KHB supplemented with 50 mmol NaCl), followed by 25-min reperfusion with normotonic KHB; and (4) $HTS^{415mOsm}$ (n = 6): the same sequence of procedures as in the previous group, but using HTS with osmolarity increased to 415 mOsm/L.

Assessment of vascular intestinal perfusate flow rate: The vascular intestinal perfusate flow (IPF) rate was determined each 5 min during both the stabilization and reperfusion periods by collection of the effluent from the portal vein in a calibrated tube.

Determination of luminal intestinal flow: The outflow from the intestinal lumen was determined in all experiments during the stabilization period. Luminal flow exceeding 0.5 mL/min is indicative of pathological intestinal secretion, which is usually due to ischemic injury of the intestine during surgical manipulations. Thus, the experiments with luminal flow exceeding 0.5 mL/min were excluded from the final analysis.

Histochemical analysis: We used the technique of visualizing the area of necrosis (AN) with 2.3.5-triphenyltetrazolium chloride (TTC), a method generally considered to be a "gold standard" for AN delineation in the heart in other studies, including our own[14,15]. Briefly, immediately after the end of reperfusion into the SMA, 10 mL of 0.5% TTC solution were administered for 30 s. Fifteen minutes after that, 3 equally spaced segments of the intestine with a total length of 3 cm were excised and fixed in 4% buffered formaldehyde solution for 24 h at room temperature. The samples were cut into 7-8 transverse slices, each 1.5 mm thick, which were photographed for further analysis using Adobe Photoshop CS. TTC-positive tissue, brick red in color, was considered to be viable, in contrast to the necrotic tissue, which had a pale appearance. The AN volume in each sample was calculated by multiplying the TTC-negative area by the slice thickness. The AN volume of the whole intestinal segment was calculated by summing the AN volumes of the slices and was expressed as the percentage of AN in relation to the total volume of the intestinal tissue being analyzed.

Evaluation of spontaneous intestinal motility: According to the original technique described by Hoffman *et al*[16], this parameter is assessed by recording the movement of fecal pellets down the intestine. We used a different approach to register the spontaneous intestinal contraction rate. In brief, a pair of soft rubber rings, hand-crafted from a black tube with a diameter close to that of the intestine, were cut at one point and carefully placed around the intestine to signify equally spaced anatomical locations throughout the experiments. Sixty-second video recordings (5 frames per second) were taken every 10 min during the stabilization and reperfusion periods and were digitized with VirtualDub software (Avery Lee, United States). Based on the distances between the rings, the values were plotted against time, followed by a Fourier transform and probability plotting of the frequency distribution. The data were presented as the rates of low-frequency intestinal contractions (Hz).

In vivo experiments

For the investigation of the effect of HTS perfusion on the intestinal IRI, we developed a technique for perfusion through the SMA, which, in our opinion, offers some advantages over the systemic administration of HTS used before. The infrarenal aorta was occluded with a microclip and cannulated with an 18G polyethylene cannula (Figure 1A). The micro clip was then removed and placed on the aorta between the SMA and the CT, after which the cannula was advanced up to the SMA level, where its tip was fixed by ligation (Figure 1B). The next step included 2 manipulations: the occlusion of the distal part of the superior mesenteric vein (SMV) and the incision of the ileocecal SMV branch to secure the outflow of the perfusate from the intestinal circulation. The oxygenated HTS maintained at 37 ℃ was delivered through the SMA with an infusion pump (SPACE infusion system, B. Braun, Germany) at a constant rate of 15 mL/min. The flow rate was selected on the basis of our pilot experiments showing that the mean blood flow in the SMA of animals of comparable weight, having blood pressure (BP) in the range between 100 and 120 mmHg,

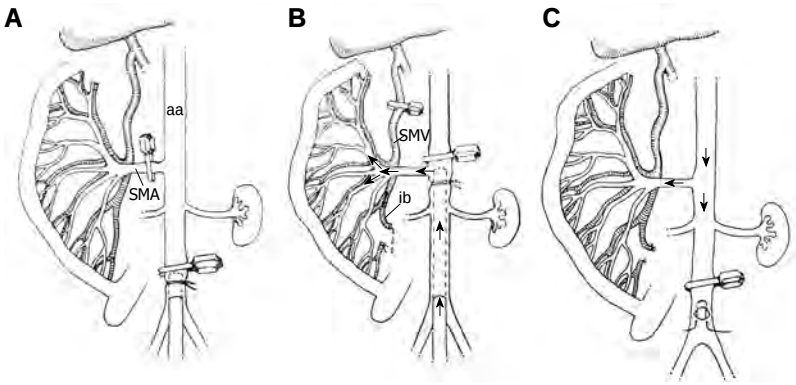


Figure 1 The sequential steps of the intestinal perfusion *in vivo*. A: The abdominal aorta (aa) is occluded by a clip, followed by cannulation of the vessel; the superior mesenteric artery (SMA) is clipped; B: The aortic clip is positioned above the SMA; the cannula is positioned at the level of SMA to allow its perfusion with hypertonic saline; the perfusate outflow is secured through the opened ileocecal branch (ib) of the superior mesenteric vein (SMV); the main trunk of SMV is clipped; C: The cannula is removed to allow blood flow through the SMA to resume simultaneously with the following procedures: the aortic clip is positioned as in Figure 1A; the clip occluding the SMV is removed, and bleeding from the ileocecal branch of the SMV is prevented by ligation.

was approximately 10% higher than 15 mL/min. Any appearance of blood in the perfusate was checked by visual inspection. At the end of the perfusion, the ileocecal SMV branch was ligated and the main trunk of the SMV was reopened. The aortic cannula was removed and the aortic incision closed with a suture (Figure 1C), so that blood flow was restored in both the aorta and the mesenteric vascular bed.

Experimental protocol: The animals were randomly allocated into 2 groups: (1) Controls (n = 6): intestinal ischemia by SMA occlusion for 30 min, followed by 30-min reperfusion with blood; and (2) HTS^{365mOsm} (n = 5): intestinal ischemia by SMA occlusion for 30 min, followed by 5-min reperfusion with HTS^{365mOsm}, followed by 25-min reperfusion with blood.

Blood pressure measurement: Arterial BP was measured in the common carotid artery using a miniature pressure transducer (Baxter, United States), to be monitored using PhysExp X4 software (Cardioprotect Ltd., St. Petersburg, Russian Federation). BP values were registered under the following conditions: at baseline, prior to SMA occlusion, 15 and 30 min after SMA occlusion, and at the 15th and 30th min of reperfusion.

Histological studies: At the end of reperfusion, intestinal samples were taken and routinely fixed in 10% buffered formaldehyde solution, embedded in paraffin, cut into 5 μm sections, and stained with hematoxylin and eosin. The slides were analyzed under a light microscope by the pathologist blinded to the treatment mode used in each group. The results were scored from 0 to 5 according to the scale of Chiu *et al*[17]. The method is based on the well established relationship between the grade of intestinal injury, if any, and its structural manifestation (grade 0: normal villi; grade 1: development of subepithelial space; grade 2: moderate lifting of subepithelial layer; grade 3: massive epithelial lifting with a few tips denuded; grade 4: denuded villi with lamina propria and dilated capillaries exposed; grade 5: digestion and disintegration of lamina propria with ulceration). In the present study, we rigorously followed this gradation as applied in similar trials by several groups[10,18,19].

Statistical analysis

Data were analyzed with SPSS 11.0 (SPSS Inc. Software, Chicago, IL, United States). All values are expressed as mean ± SD. Differences in the intestinal motility and IPF rate were tested by repeated-measures analysis of variance, followed by a Tukey post-hoc test. The non-parametric Mann-Whitney U test was used to determine the inter-group differences in AN value and histopathological score. $P \leqslant 0.05$ were considered significant.

RESULTS

Ex vivo experiments

Exclusions: Two animals were excluded from the final analysis (one in the control group and one in the HTS^{415mOsm} group) because the luminal flow exceeded 0.5 mL/min.

Intestinal perfusate flow rate: There were no inter-group differences in the IPF rate during the stabilization period (Figure 2). The IPF values remained unchanged over the entire 110-min perfusion period in the sham-treated group. In contrast, intestinal ischemia-reperfusion resulted in a reduction in the IPF rate during reperfusion (P < 0.05 *vs* sham). This reduction could have been due to IRI in the intestinal microcirculatory bed, resulting in no-reflow. The postischemic recovery of the IPF rate did not differ between the controls and the HTS^{365mOsm} group. In the HTS^{415mOsm} group, postischemic IPF rates were lower than in the controls and the HTS^{365mOsm} group (P < 0.05).

Area of necrosis: TTC-negative intestinal tissue was not present in the samples obtained from the sham-treated group. In the controls, AN averaged 14.8% ± 5.07% of the total tissue volume within the analyzed intestinal seg-

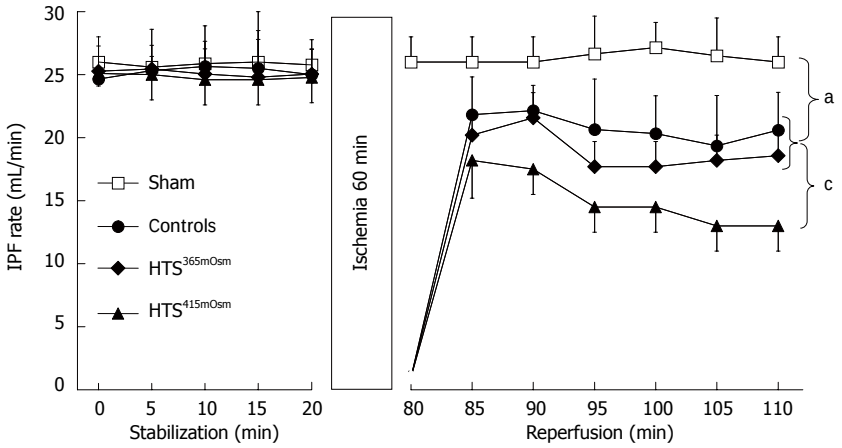


Figure 2 Intestinal perfusate flow rate in the *ex vivo* rat experiments with 60-min ischemia followed by varying conditions of reperfusion. The groups tested were: sham-operated (no ischemia at all, $n = 6$); controls (30 min reperfusion with normotonic buffer, $n = 5$); 2 groups with either HTS^{365mOsm} ($n = 5$) or HTS^{415mOsm} ($n = 5$), both subjected to 5-min reperfusion with the respective hypertonic solution followed by 25 min reperfusion with normotonic buffer. The pre-ischemic values of Intestinal perfusate flow (IPF) rate were similar in all groups. No changes were apparent in the shams over the whole period, whereas IPF values were lower in all the ischemia-reperfusion groups, although the groups showed different patterns. HTS^{415mOsm} resulted in the most prominent decrease in the IPF rate, with a further progressive fall to the end of the experiment. In contrast, IPF rate was less affected in the control and HTS^{365mOsm} groups, to a similar degree in both, while an appreciable degree of stabilization took place towards the end of the postischemic period. Postischemic IPF rate recovery did not differ between the controls and the HTS^{365mOsm} group, whereas postischemic IPF rate values were significantly lower in the HTS^{415mOsm} group, compared to both the controls and the HTS^{365mOsm} group. Data are mean ± SD. $^{a}P < 0.05$ *vs* sham; $^{c}P < 0.05$ *vs* controls.

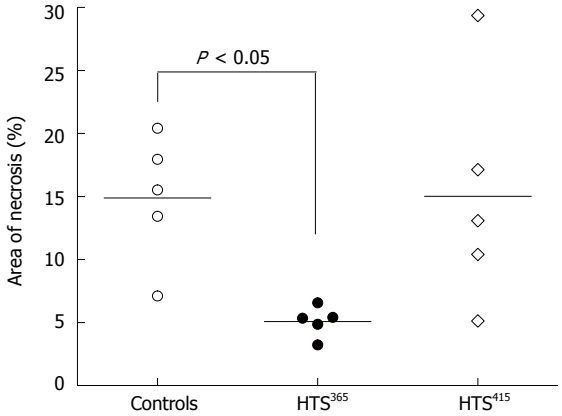


Figure 3 Area of necrosis within the isolated rat intestine after 60-min ischemia followed by the varying conditions of reperfusion. The area of necrosis (AN) is defined as the percentage of 2.3.5-triphenyltetrazolium chloride-negative in relation to the total intestinal tissue volume. Controls (reperfusion with normotonic saline) and the HTS^{415mOsm} group did not differ statistically regarding AN volume, whereas HTS^{365mOsm} administration resulted in a significantly lower value of AN. In both hypertonic saline (HTS) groups, the 5-min reperfusion with the appropriate hyperosmotic saline was followed by 25-min normotonic reperfusion. The data are presented as dot plots with median values.

ment (Figure 3). Administration of HTS^{365mOsm} for 5 min after 60-min ischemia resulted in decrease in AN (5.1% ± 1.20% *vs* controls, $P < 0.01$). However, perfusion of the intestine with the HTS of greater osmolarity (HTS^{415mOsm}) failed to protect the intestine from irreversible injury (AN = 15.0% ± 9.12% *vs* controls, $P > 0.05$).

Intestinal motility: The rate of intestinal contraction-relaxation pulses, which was used as a measure of intestinal motility in the present study, was similar at baseline in all groups (Figure 4). An increase in this parameter was observed during the first 10 min of reperfusion in the control group as compared to the sham-treated group, but no such increase was seen in the HTS^{365mOsm} group.

Histological data: Histological data from the intestinal samples obtained after 30-min reperfusion *ex vivo* are shown in Figure 5A. Sham-treated animals had normal or near-normal histology. The Chiu score was lower in the HTS^{365mOsm} group in comparison with controls (2.4 ± 0.54 *vs* 3.2 ± 0.44, $P = 0.042$, Figure 5A), while intestinal perfusion with HTS^{415mOsm} failed to improve the Chiu score (3.6 ± 0.54 *vs* controls, $P > 0.05$).

In vivo experiments

Exclusions: Although the experimental model suggested is highly invasive and complex, the extensive training prior to the beginning of the main experimental series has minimized the number of animals excluded due to technical reasons. Only one rat was excluded in the control group because of the severe bleeding and low BP.

Blood pressure values during intestinal perfusion *in vivo*: Baseline BP values did not differ between the controls and the HTS^{365mOsm} group (Table 1, Figure 6). SMA occlusion did not cause appreciable changes in BP level in either group. Aortic occlusion during the 5-min SMA perfusion period in the HTS^{365mOsm} group resulted in a trend to BP elevation. Reinstitution of blood flow in the aorta caused a temporary decrease in BP that lasted for only 2-3 min before it returned to the baseline values (Table 1). In contrast, SMA reperfusion in controls caused a decrease in BP level that persisted during the entire reperfusion period (Table 1, Figure 6A).

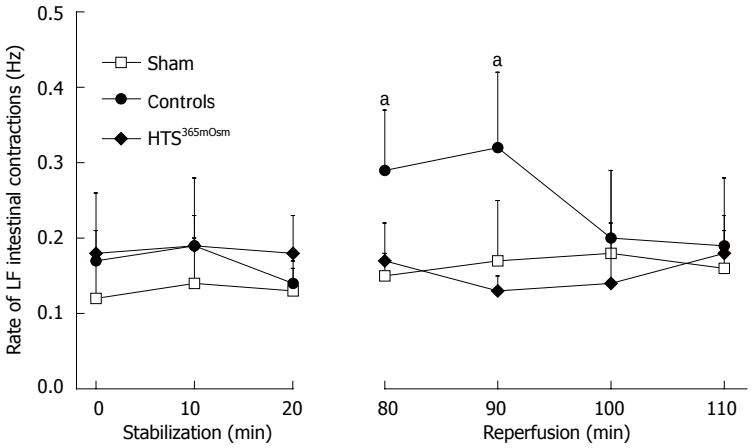


Figure 4 The rate of low frequency intestinal contractions in the *ex vivo* experiments. In shams, the intestine was continuously perfused for 110 min. In controls (60-min ischemia followed by 30-min reperfusion), intestinal contraction rate was significantly higher over the initial 10 min of reperfusion in comparison with the shams. Intestinal reperfusion with hyperosmotic saline in the HTS^{365mOsm} group prevented the increase in postischemic intestinal contraction rate. Data are mean ± SD. $^{a}P < 0.05$ *vs* sham. LF: Low frequency.

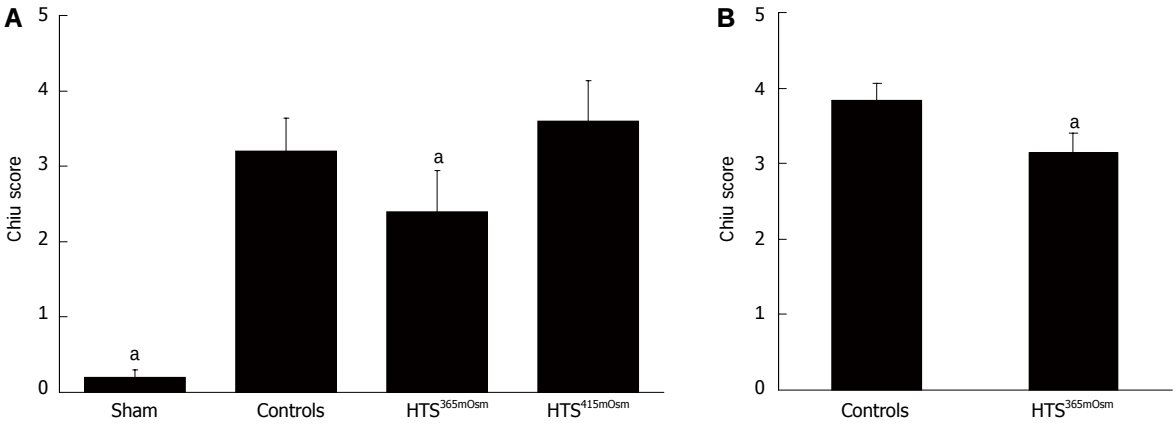


Figure 5 Histologically defined scores in the *ex vivo* (A) and *in vivo* (B) experiments. A, B: In the sham group (no ischemia at all, A) slight injury only is evident, probably due to the prolonged perfusion in the extracorporeal environment. Sixty-minute ischemia followed by 30-min normotonic reperfusion (controls) resulted in a significant injurious effect, which, however, was substantially less expressed in the HTS^{365mOsm} but not in the HTS^{415mOsm} group. The analogous difference between the controls and the HTS^{365mOsm} group is apparent in the *in vivo* experiments (B). The data are expressed as mean ± SD; $^{a}P < 0.05$ *vs* controls.

Table 1 Arterial blood pressure (mmHg) in the *in vivo* experiments (mean ± SD)

	Controls (n = 5)	HTS^{365mOsm} (n = 5)
Baseline	125 ± 8	132 ± 12
Before SMA occlusion	127 ± 10	128 ± 9
SMA occlusion		
15 min	118 ± 11	125 ± 7
30 min	121 ± 9	123 ± 6
SMA reperfusion		
15 min	68 ± 12	118 ± 13[a]
30 min	74 ± 8	126 ± 11[a]

SMA: Superior mesenteric artery. $^{a}P < 0.05$ *vs* controls.

Histology: Histological data from the intestinal samples obtained after 30-min reperfusion *in vivo* are shown in Figure 5B. The Chiu score was lower in the HTS^{365mOsm} group *vs* controls (3.1 ± 0.26 and 3.8 ± 0.22, $P = 0.0079$, respectively, Figure 5B), although the magnitude of the effect was lower than in the *ex vivo* series.

DISCUSSION

The results of this study indicate that the technique of isolated *in vivo* vascular perfusion of the intestine is reproducible and reliable. Using this approach, intestinal perfusion with HTS^{365mOsm} over the initial 5 min of reperfusion resulted in significant protection of the intestine from IRI, as demonstrated using morphological data (per both TTC and Chiu *et al*[17]). In order to evaluate both the experimental and also the possible clinical implications of this strategy, it seems appropriate to review in detail the relevant findings from systemic HTS administration. A salvation effect on the intestinal IRI, as well as limitation of remote organ injury, were observed in rats that were subjected to 60-min SMA occlusion followed by 6-h reperfusion, when intravenous administration of HTS was performed during the last 5 min of ischemia[10]. In parallel, an improvement in intestinal transit time, a reduction in mucosal injury, and limitation of intestinal myeloperoxidase activity were demonstrated using the same model; all effects were considered to be dependent on heme oxygenase-1 activity[18]. In addition, HTS infusion performed within the first 5 min of reperfusion after 120 min of SMA occlusion in pigs resulted in a better recovery of both mean arterial blood pressure and intestinal microvascular perfusion in comparison with conventional isotonic resuscitation[9]. In a somewhat different rat mod-

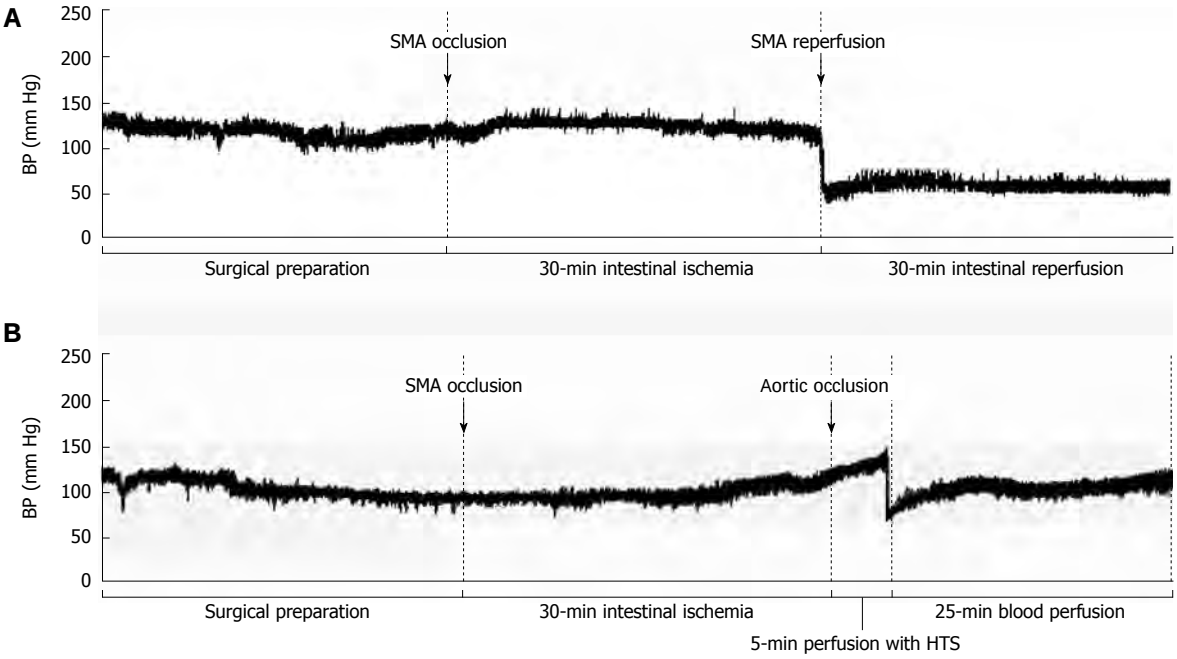


Figure 6 Representative samples of arterial blood pressure recordings in anesthetized rats subjected to intestinal ischemia-reperfusion *in vivo*. A: Thirty-minute superior mesenteric artery (SMA) occlusion followed by 30-min reperfusion (controls) resulted in a persistent blood pressure (BP) decrease over the entire reperfusion period; B: Thirty-minute SMA occlusion finalized by aortic clamping followed immediately by 5-min SMA perfusion with hypertonic saline (365 mOsm/L), with a subsequent 25-min reperfusion with blood, resulted in a transient drop with a rapid restoration of BP up to the initial values. HTS: Hypertonic saline.

el of intestinal IRI, this time induced by hemorrhagic shock followed by blood reinfusion, the administration of HTS for 25 min after the return of shed blood into circulation resulted in better post-resuscitation blood flow and improved endothelial function of the intestinal vasculature[12]. It also suppressed the apoptotic death of intestinal epithelial cells[20] and prevented intestinal mucosal injury[21]. As to the remote effects of systemic HTS treatment, a more complete and stable restoration of BP[12] as well as the prevention of systemic oxidative stress[21] have been observed. However, in general, systemic HTS administration has several intrinsic drawbacks, including an increased risk of hypernatremia, plasma hyperosmolarity, cardiac arrhythmias, and heart failure[12]. Apart from being free of the limitations inherent to systemic HTS administration, the technique of local HTS delivery seems to have 2 more additional advantages over a variety of protocols attempted previously[22,23]. First, the delivery of perfusate directly into the SMA, with outflow from the opened vein, completely prevents the otherwise inevitable generalized spread throughout the systemic circulation of the metabolites and bacterial toxins accumulated during ischemia. Second, the pharmacological agents recommended for strengthening the protective effect of HTS[24,25]-which could thus be potentially helpful to HTS administration *via* a local route-could be used at higher doses and therefore with higher efficiency, analogously with the strategy applied for regional perfusion in chemotherapy for certain neoplasms[26].

The question might arise as to whether the HTS osmolarity value used in the present study was optimal. While the osmolarity of HTS for hypertonic resuscitation averages approximately 7.5% NaCl, in our local delivery model we tested solutions with much lower osmolarities. Previous studies have tried values ranging from normal (300-310 mOsm/L) to nearly 400 mOsm/L for local organ anti-ischemic protection, particularly the heart; the optimal osmolarity was found to be 334 mOsm/L[27]. Interestingly, in *ex vivo* experiments, osmolarity values above 400 mOsm/L apparently produced a negative effect; that is, HTS delivery resulted in a significant deterioration in postischemic IPF rate recovery as compared to controls (Figure 2). Although different mechanisms might be involved in the observed effect, the most likely are endothelial injury and impaired microvascular function; however, this matter requires further investigation. Based on these findings, in this study a solution with a lower osmolarity ($HTS^{365mOsm}$) was preferred and was found to be effective for the preservation of intestinal viability, not only *ex vivo*, but also in the *in vivo* model. A higher HTS osmolarity value than that optimal for the heart could be justified by the higher hydrophilicity of the intestinal tissue, which would thus also require more potent anti-edema protection. It should be noted, however, that further experimentation will be needed in order to determine more precisely the optimal HTS parameter(s) for the anti-IRI protection of intestinal tissue.

Although not fully understood, the mechanism of the intestine protection with HTS may include such factors as dilatation of precapillary arterioles[12], inhibition of neutrophil-mediated reperfusion injury[11], or prevention of intestinal edema due to reperfusion-induced water influx into the cells[28]. At the molecular level, the major factor underlying the removal of excess water from the cells

has been shown to be the up-regulation of aquaporin 4 within the plasma membrane of the mucosal cells[29].

As mentioned before, protective effects of local HTS delivery on reperfusion injury have also been found in the heart, suggesting that common mechanisms may be involved. The concept of controlled cardiac reperfusion was originally proposed in the late 1980s to protect the myocardium from IRI by modifying the composition and/or delivery rate of the perfusion fluid[30]. One of the major modifications was the increased osmolarity of the perfusate, which was shown to decrease the incidence of reperfusion-induced arrhythmias, to attenuate tissue injury, and to reduce edema in the isolated hearts of pig and rabbit[27,31]. This promising approach is still awaiting implementation in clinical practice, initially in open heart surgery.

Local infusion of saline before reperfusion was also found to be protective in the brain. It was found that flushing the ischemic area of the brain with isotonic saline prior to reperfusion resulted in a reduced infarct volume and improved cerebral blood flow in the rat model[32]. Since normotonic saline was used in this study, the authors attributed its protective effect to the local clearance of proinflammatory cytokines and metabolic end-products.

In the present study, the spontaneous intestinal motility was assessed with a technique that has not yet been employed in the experiments simulating IRI. The observation of significant augmentation of the intestinal contraction rate over the first 10 min of intra-arterial perfusate reflow after 60-min *in vitro* ischemia (Figure 4) is in general agreement with data showing a transient increase in both the contraction rate and baseline tension observed upon reoxygenation of the jejunal rings after they were subjected to 60-min *in vitro* hypoxia[33]. In both situations, the increased motor activity of the intestine within the early postischemic period might be explained, in gross analogy with the postischemic heart response[34], by sudden oxygen-evoked re-energizing of the cells immediately after severe hypoxia. The subsequent excessive activation of contractile machinery might, in turn, contribute to the progression of mucosal injury, most likely due to the adverse effect of hypermotility. Its complete prevention with HTS perfusion (Figure 4) may point to the solution of the relevant clinical problem.

One obvious limitation of our *in vivo* model is the need to advance the perfusion cannula through the infrarenal aorta, rather than directly into the SMA. As a result, the kidneys and hindlimbs were exposed to an additional 5 min of ischemia. However, this limitation is imposed by the small size of the animal and the need to restore blood flow in the mesenteric vascular bed. In order to make the results comparable, control animals also received 5-min occlusion of the infrarenal aorta. Future studies are needed to verify the protective effect of local HTS infusion in large animal models. For instance, the study might be repeated in pigs using an endovascular approach with percutaneous puncture in the femoral artery and balloon occlusion of the SMA.

As to further extrapolations useful for clinical practice, the findings from the present study imply that intra-arterial HTS administration could contribute to the prevention, or at least amelioration, of 2 seemingly inevitable negative effects that would otherwise arise; that is, if "unprotected" reperfusion is applied. The first of these is reperfusion-induced intestinal damage, while the second includes whole-body disturbances, such as the systemic inflammatory response syndrome[4], hemodynamic impairments[35], and remote organ injuries[36], all of which are associated with the massive washout of metabolites and bacterial toxins from the ischemia-affected intestine into the systemic circulation. Our *in vivo* model could lead to the development of a novel therapy that combines local perfusion of the ischemic region with mechanical restoration of the blood flow and/or the administration of pharmacological agents. As to the clinical translation of the model, it might be speculated that perfusion of the SMA in patients with AMI could be performed after laparotomy, either *via* a catheter directly placed in the SMA or during percutaneous intervention. The perfusate outflow could be ensured through the punctured SMV. It should be noted that the suggested technique delayed restoration of blood flow to the intestine by 5 min; however, it is unlikely that perfusion with an oxygen- and glucose-enriched solution could cause any ischemic/hypoxic injury to the intestine during this period. It is also of importance to determine the optimal osmolarity value of the HTS, since the results of the present study have demonstrated the negative effect of HTS with excessive osmolarity, i.e., 415 mOsm/L.

In conclusion, the results of our experiments show that isolated intestinal perfusion with HTS at the beginning of the reperfusion period can appreciably protect the intestine from IRI. Despite the positive effect of intra-arterial HTS delivery, aimed in the present study at establishing the validity of this approach in principle, its further improvement by optimization of the particular parameters during all steps of the procedure will be required before clinical trials can be contemplated.

COMMENTS

Background

Acute mesenteric ischemia (AMI) is a life-threatening vascular emergency with high mortality rate. The major treatment approach for AMI is rapid intestinal revascularization. Unfortunately, even after timely revascularization, its positive effect can be compromised, at least in part, by the development of intestinal reperfusion injury.

Research frontiers

Systemic administration of hypertonic saline (HTS) was shown to be protective in the experimental models of AMI. In the present study, the authors investigated the effect of local intra-arterial HTS administration during the early reperfusion phase on the functional and structural manifestations of intestinal injury.

Innovations and breakthroughs

The authors showed for the first time that isolated intestinal perfusion with HTS at the beginning of the reperfusion period can appreciably protect the intestine from ischemia-reperfusion injury. In addition, the technique of isolated *in vivo* vascular perfusion of the intestine is suggested and experimentally validated.

Applications

This *in vivo* model could lead to the development of a novel therapy that combines local perfusion of the ischemic intestine with mechanical restoration of the blood flow and/or the administration of pharmacological agents.

Terminology

Intestinal ischemia-reperfusion injury (IRI) develops because of the temporal complete or partial cessation of blood supply to the intestine. HTS is crystalloid or colloid solution with osmolarity value exceeding physiological. Potential benefits of HTS in the settings of AMI include prevention of edema, improvement in the microcirculation, and washout of potentially toxic metabolites.

Peer review

This paper investigated the effect of local intestinal perfusion with HTS on intestinal IRI in both *ex vivo* and *in vivo* rat models, to find that brief intestinal postischemic perfusion with $HTS^{365mOsm}$ through the SMA followed by blood flow restoration was a protective procedure that could be used for the prevention of intestinal IRI. It is well written and organized.

REFERENCES

1 **Wyers MC**. Acute mesenteric ischemia: diagnostic approach and surgical treatment. *Semin Vasc Surg* 2010; **23**: 9-20 [PMID: 20298945 DOI: 10.1053/j.semvascsurg.2009.12.002]

2 **Berland T**, Oldenburg WA. Acute mesenteric ischemia. *Curr Gastroenterol Rep* 2008; **10**: 341-346 [PMID: 18625147]

3 **Arthurs ZM**, Titus J, Bannazadeh M, Eagleton MJ, Srivastava S, Sarac TP, Clair DG. A comparison of endovascular revascularization with traditional therapy for the treatment of acute mesenteric ischemia. *J Vasc Surg* 2011; **53**: 698-704; discussion 704-705 [PMID: 21236616 DOI: 10.1016/j.jvs.2010.09.049]

4 **Vollmar B**, Menger MD. Intestinal ischemia/reperfusion: microcirculatory pathology and functional consequences. *Langenbecks Arch Surg* 2011; **396**: 13-29 [PMID: 21088974 DOI: 10.1007/s00423-010-0727-x]

5 **Takeshita M**, Tani T, Harada S, Hayashi H, Itoh H, Tajima H, Ohnishi I, Takamura H, Fushida S, Kayahara M. Role of transcription factors in small intestinal ischemia-reperfusion injury and tolerance induced by ischemic preconditioning. *Transplant Proc* 2010; **42**: 3406-3413 [PMID: 21094787 DOI: 10.1016/j.transproceed.2010.06.038]

6 **Liu KX**, Li YS, Huang WQ, Chen SQ, Wang ZX, Liu JX, Xia Z. Immediate postconditioning during reperfusion attenuates intestinal injury. *Intensive Care Med* 2009; **35**: 933-942 [PMID: 19190893 DOI: 10.1007/s00134-009-1428-1]

7 **Mallick IH**, Yang W, Winslet MC, Seifalian AM. Ischemia-reperfusion injury of the intestine and protective strategies against injury. *Dig Dis Sci* 2004; **49**: 1359-1377 [PMID: 15481305 DOI: 10.1023/B:]

8 **Cámara CR**, Guzmán FJ, Barrera EA, Cabello AJ, Garcia A, Fernández NE, Caballero E, Ancer J. Ketamine anesthesia reduces intestinal ischemia/reperfusion injury in rats. *World J Gastroenterol* 2008; **14**: 5192-5196 [PMID: 18777596 DOI: 10.3748/wjg.14.5192]

9 **Jonas J**, Heimann A, Strecker U, Kempski O. Hypertonic/hyperoncotic resuscitation after intestinal superior mesenteric artery occlusion: early effects on circulation and intestinal reperfusion. *Shock* 2000; **14**: 24-29 [PMID: 10909889 DOI: 10.1097/00024382-200014010-00005]

10 **Gonzalez EA**, Kozar RA, Suliburk JW, Weisbrodt NW, Mercer DW, Moore FA. Conventional dose hypertonic saline provides optimal gut protection and limits remote organ injury after gut ischemia reperfusion. *J Trauma* 2006; **61**: 66-73; discussion 73-74 [PMID: 16832251 DOI: 10.1097/01.ta.0000224190.65542.e2]

11 **Shukla A**, Hashiguchi N, Chen Y, Coimbra R, Hoyt DB, Junger WG. Osmotic regulation of cell function and possible clinical applications. *Shock* 2004; **21**: 391-400 [PMID: 15087814 DOI: 10.1097/00024382-200405000-00001]

12 **Zakaria el R**, Tsakadze NL, Garrison RN. Hypertonic saline resuscitation improves intestinal microcirculation in a rat model of hemorrhagic shock. *Surgery* 2006; **140**: 579-587; discussion 587-588 [PMID: 17011905 DOI: 10.1016/j.surg.2006.05.015]

13 **Gardemann A**, Watanabe Y, Grosse V, Hesse S, Jungermann K. Increases in intestinal glucose absorption and hepatic glucose uptake elicited by luminal but not vascular glutamine in the jointly perfused small intestine and liver of the rat. *Biochem J* 1992; **283** (Pt 3): 759-765 [PMID: 1590766]

14 **Galagudza M**, Vaage J, Valen G. Isoflurane and other commonly used anaesthetics do not protect the isolated buffer perfused mouse heart from ischemia-reperfusion injury. *Clin Exp Pharmacol Physiol* 2006; **33**: 315-319 [PMID: 16620294 DOI: 10.1111/j.1440-1681.2006.04368.x]

15 **Csonka C**, Kupai K, Kocsis GF, Novák G, Fekete V, Bencsik P, Csont T, Ferdinandy P. Measurement of myocardial infarct size in preclinical studies. *J Pharmacol Toxicol Methods* 2010; **61**: 163-170 [PMID: 20188845 DOI: 10.1016/j.vascn.2010.02.014]

16 **Hoffman JM**, Brooks EM, Mawe GM. Gastrointestinal Motility Monitor (GIMM). *J Vis Exp* 2010; (**46**): 2435 [PMID: 21189461 DOI: 10.3791/2435]

17 **Chiu CJ**, McArdle AH, Brown R, Scott HJ, Gurd FN. Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic, and metabolic reappraisal. *Arch Surg* 1970; **101**: 478-483 [PMID: 5457245 DOI: 10.1001/archsurg.1970.01340280030009]

18 **Attuwaybi B**, Kozar RA, Gates KS, Moore-Olufemi S, Sato N, Weisbrodt NW, Moore FA. Hypertonic saline prevents inflammation, injury, and impaired intestinal transit after gut ischemia/reperfusion by inducing heme oxygenase 1 enzyme. *J Trauma* 2004; **56**: 749-758; discussion 758-759 [PMID: 15187737 DOI: 10.1097/01.TA.0000119686.33487.65]

19 **Guzmán-de la Garza FJ**, Cámara-Lemarroy CR, Alarcón-Galván G, Cordero-Pérez P, Muñoz-Espinosa LE, Fernández-Garza NE. Different patterns of intestinal response to injury after arterial, venous or arteriovenous occlusion in rats. *World J Gastroenterol* 2009; **15**: 3901-3907 [PMID: 19701970 DOI: 10.3748/wjg.15.3901]

20 **Lu YQ**, Gu LH, Huang WD, Mou HZ. Effect of hypertonic saline resuscitation on heme oxygenase-1 mRNA expression and apoptosis of the intestinal mucosa in a rat model of hemorrhagic shock. *Chin Med J (Engl)* 2010; **123**: 1453-1458 [PMID: 20819606]

21 **Powers KA**, Zurawska J, Szaszi K, Khadaroo RG, Kapus A, Rotstein OD. Hypertonic resuscitation of hemorrhagic shock prevents alveolar macrophage activation by preventing systemic oxidative stress due to gut ischemia/reperfusion. *Surgery* 2005; **137**: 66-74 [PMID: 15614283 DOI: 10.1016/j.surg.2004.05.051]

22 **Pang KS**, Yuen V, Fayz S, te Koppele JM, Mulder GJ. Absorption and metabolism of acetaminophen by the in situ perfused rat small intestine preparation. *Drug Metab Dispos* 1986; **14**: 102-111 [PMID: 2868852]

23 **Scholtka B**, Stümpel F, Jungermann K. Acute increase, stimulated by prostaglandin E2, in glucose absorption via the sodium dependent glucose transporter-1 in rat intestine. *Gut* 1999; **44**: 490-496 [PMID: 10075955 DOI: 10.1136/gut.44.4.490]

24 **Shih HC**, Huang MS, Lee CH. Estrogen augments the protection of hypertonic saline treatment from mesenteric ischemia-reperfusion injury. *Shock* 2011; **35**: 302-307 [PMID: 20926986 DOI: 10.1097/SHK.0b013e3181f8b420]

25 **Shih HC**, Huang MS, Lee CH. Magnolol attenuates the lung injury in hypertonic saline treatment from mesenteric ischemia reperfusion through diminishing iNOS. *J Surg Res* 2012; **175**: 305-311 [PMID: 21704335 DOI: 10.1016/j.jss.2011.04.063]

26 **Sanki A**, Kroon HM, Kam PC, Thompson JF. Isolated limb perfusion and isolated limb infusion for malignant lesions of the extremities. *Curr Probl Surg* 2011; **48**: 371-430 [PMID:

21549235 DOI: 10.1067/j.cpsurg.2011.02.002]
27 **Weng ZC**, Nicolosi AC, Detwiler PW, Hsu DT, Schierman SW, Goldstein AH, Spotnitz HM. Effects of crystalloid, blood, and University of Wisconsin perfusates on weight, water content, and left ventricular compliance in an edema-prone, isolated porcine heart model. *J Thorac Cardiovasc Surg* 1992; **103**: 504-513 [PMID: 1545549]
28 **Radhakrishnan RS**, Radhakrishnan HR, Xue H, Moore-Olufemi SD, Mathur AB, Weisbrodt NW, Moore FA, Allen SJ, Laine GA, Cox CS. Hypertonic saline reverses stiffness in a Sprague-Dawley rat model of acute intestinal edema, leading to improved intestinal function. *Crit Care Med* 2007; **35**: 538-543 [PMID: 17205008 DOI: 10.1097/01.CCM.0000254330.39804.9C]
29 **Radhakrishnan RS**, Shah SK, Lance SH, Radhakrishnan HR, Xue H, Radhakrishnan GL, Ramaswamy US, Walker PA, Uray KS, Laine GA, Stewart RH, Cox CS. Hypertonic saline alters hydraulic conductivity and up-regulates mucosal/submucosal aquaporin 4 in resuscitation-induced intestinal edema. *Crit Care Med* 2009; **37**: 2946-2952 [PMID: 19770732 DOI: 10.1097/CCM.0b013e3181ab878b]
30 **Okamoto F**, Allen BS, Buckberg GD, Young H, Bugyi H, Leaf J. Reperfusate composition: interaction of marked hyperglycemia and marked hyperosmolarity in allowing immediate contractile recovery after four hours of regional ischemia. *J Thorac Cardiovasc Surg* 1986; **92**: 583-593 [PMID: 3747586]
31 **Careaga G**, Argüero R, Chavez-Negrete A, Valero G, Portilla E, Garcia RM, Mendoza L, Angulo L, Miranda Y. Control of myocardial reperfusion injury with hypertonic-hyperosmotic solution in isolated rabbit heart. *Eur Surg Res* 1995; **27**: 269-276 [PMID: 7649214 DOI: 10.1159/000129409]
32 **Ding Y**, Li J, Rafols JA, Phillis JW, Diaz FG. Prereperfusion saline infusion into ischemic territory reduces inflammatory injury after transient middle cerebral artery occlusion in rats. *Stroke* 2002; **33**: 2492-2498 [PMID: 12364743 DOI: 10.1161/01.STR.0000028237.15541.CC]
33 **Bielefeldt K**, Conklin JL. Intestinal motility during hypoxia and reoxygenation in vitro. *Dig Dis Sci* 1997; **42**: 878-884 [PMID: 9149037]
34 **Abdallah Y**, Gkatzoflia A, Gligorievski D, Kasseckert S, Euler G, Schlüter KD, Schäfer M, Piper HM, Schäfer C. Insulin protects cardiomyocytes against reoxygenation-induced hypercontracture by a survival pathway targeting SR Ca2+ storage. *Cardiovasc Res* 2006; **70**: 346-353 [PMID: 16569400 DOI: 10.1016/j.cardiores.2006.02.020]
35 **Walensi M**, de Groot H, Schulz R, Hartmann M, Petrat F. Mesenteric ischemia-reperfusion injury: Clearly improved hemodynamics but only minor protection of the rat small intestine by (sub)therapeutic heparin sodium and enoxaparin doses. *J Surg Res* 2013; **179**: e57-e69 [PMID: 22494914 DOI: 10.1016/j.jss.2012.01.002]
36 **Varga J**, Tóth S, Staško P, Tóth S, Bilecová-Rabajdová M, Ostró A, Veselá J. Intestinal ischemia-reperfusion injury - the histopathological status of remote vital organs in acute and subacute phases. *Ann Transplant* 2012; **17**: 11-20 [PMID: 22466904]

P- Reviewer Augustin G **S- Editor** Xiong L
L- Editor A **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i2.219

World J Gastroenterol 2013 January 14; 19(2): 219-226
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

ORIGINAL ARTICLE

Characterization of focal liver masses using acoustic radiation force impulse elastography

Hana Park, Jun Yong Park, Do Young Kim, Sang Hoon Ahn, Chae Yoon Chon, Kwang-Hyub Han, Seung Up Kim

Hana Park, Division of Gastroenterology, Department of Internal Medicine, CHA Bundang Medical Center, CHA University, Gyeonggi-do 463-712, South Korea
Jun Yong Park, Do Young Kim, Chae Yoon Chon, Seung Up Kim, Department of Internal Medicine, Yonsei University College of Medicine, Seoul 120-752, South Korea
Sang Hoon Ahn, Kwang-Hyub Han, Department of Internal Medicine, Yonsei University College of Medicine, Seoul 120-752, South Korea
Author contributions: Park H designed the study, analyzed the data, performed the study, and wrote the paper; Park JY, Kim DY, Ahn SH, Chon CY collected the data; Han KH, Kim SU designed the study, collected the data, performed the study and wrote the paper.
Supported by A Grant of the Korea Healthcare Technology R and D Project, Ministry of Health and Welfare, South Korea, A102065
Correspondence to: Seung Up Kim, MD, Department of Internal Medicine, Yonsei University College of Medicine, 134 Shinchon-dong, Seodaemun-gu, Seoul 120-752, South Korea. ksukorea@yuhs.ac
Telephone: +82-2-3936884 Fax: +82-2-22281982
Received: September 6, 2012 Revised: October 24, 2012
Accepted: November 14, 2012
Published online: January 14, 2013

Abstract

AIM: To investigate the diagnostic performance of acoustic radiation force impulse (ARFI) elastography for characterizing focal liver mass by quantifying their stiffness.

METHODS: This prospective study included 62 patients with a focal liver mass that was well visualized on conventional ultrasonography performed in our institution from February 2011 to November 2011. Among them, 12 patients were excluded for ARFI measurement failure due to a lesion that was smaller than the region of the interest and at an inaccessible location (deeper than 8 cm) (n = 7) or poor compliance to hold their breath as required (n = 5). Finally, 50 patients with valid ARFI measurements were enrolled. If a patient had multiple liver masses, only one mass of interest was chosen. The masses were diagnosed by histological examination or clinical diagnostic criteria. During ultrasonographic evaluation, stiffness, expressed as velocity, was checked 10 times per focal liver mass and the surrounding liver parenchyma.

RESULTS: After further excluding three masses that were non-diagnostic on biopsy, a total of 47 focal mass lesions were tested, including 39 (83.0%) malignant masses [24 hepatocellular carcinomas (HCC), seven cholangiocellular carcinomas (CCC), and eight liver metastases] and eight (17.0%) benign masses (five hemangiomas and three focal nodular hyperplasias, FNH). Thirty-seven (74.0%) masses were confirmed by histological examination. The mean velocity was 2.48 m/s in HCCs, 1.65 m/s in CCCs, 2.35 m/s in metastases, 1.83 m/s in hemangiomas, and 0.97 m/s in FNHs. Although considerable overlap was still noted between malignant and benign masses, significant differences in ARFI values were observed between malignant and benign masses (mean 2.31 m/s *vs* 1.51 m/s, P = 0.047), as well as between HCCs and benign masses (mean 2.48 m/s *vs* 1.51 m/s, P = 0.006). The areas under the receiver operating characteristics curves (AUROC) for discriminating the malignant masses from benign masses was 0.724 (95%CI, 0.566-0.883, P = 0.048), and the AUROC for discriminating HCCs from benign masses was 0.813 (95%CI, 0.649-0.976, P = 0.008). To maximize the sum of sensitivity and specificity, an ARFI value of 1.82 m/s was selected as the cutoff value to differentiate malignant from benign liver masses. Furthermore, the cutoff value for distinguishing HCCs from benign masses was also determined to be 1.82 m/s. The diagnostic performance of the sum of the ARFI values for focal liver masses and the surrounding liver parenchyma to differentiate liver masses improved (AUROC = 0.853;

95%CI, 0.745-0.960; P = 0.002 in malignant liver masses *vs* benign ones and AUROC = 0.948; 95%CI, 0.896-0.992, P < 0.001 in HCCs *vs* benign masses).

CONCLUSION: ARFI elastography provides additional information for the differential diagnosis of liver masses. However, our results should be interpreted in clinical context, because considerable overlap in ARFI values existed among liver masses.

© 2013 Baishideng. All rights reserved.

Key words: Acoustic radiation force impulse; Focal liver mass; Hepatocellular carcinoma; Hemangioma; Focal nodular hyperplasia; Cholangiocellular carcinoma; Liver metastasis

Park H, Park JY, Kim DY, Ahn SH, Chon CY, Han KH, Kim SU. Characterization of focal liver masses using acoustic radiation force impulse elastography. *World J Gastroenterol* 2013; 19(2): 219-226 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i2/219.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i2.219

INTRODUCTION

Focal liver masses are being discovered at increasing rates due to the wide accessibility of modern high resolution imaging procedures. Conventional ultrasonography (US) is typically used as a first imaging modality to evaluate a focal liver mass. The differential diagnosis of a focal liver mass using imaging studies is based on the characteristics of the surrounding liver parenchyma and underlying clinical conditions such as cirrhosis and the characteristics of the mass itself. Although contrast-enhanced US, computed tomography (CT), and magnetic resonance imaging (MRI) can assess the morphology of a focal liver mass and its vasculature with a high level of diagnostic accuracy[1-5], patients are exposed to potential risks, including contrast medium-induced side-effects and irradiation hazards[6], particularly when repeated examinations are required.

Acoustic radiation force impulse (ARFI) elastography has been introduced as a new ultrasound imaging modality to evaluate tissue stiffness using the radiation forced-based imaging method. The tissue response to the radiation force is observed using conventional B-mode imaging pulses, and it is possible to display the quantitative shear wave velocity (m/s) of the ARFI image[7,8]. Because velocity is directly related to tissue stiffness, ARFI imaging can be applied to evaluate tissue elasticity. Generally, the stiffer the tissue, the faster the shear wave propagates[6,9-13]. Although several reports have indicated a good correlation of ARFI elastography with the other elastography systems such as transient elastography[14,15] and with histological fibrosis grade[14,16,17], ARFI elastography differs from other elastography systems which apply pressure manually to the surface of the organs or a mechanical vibration to induce an elastic shear wave with only M-mode US imaging. Instead, ARFI uses short-duration acoustic pulses generated from a probe under real-time B-mode imaging to produce localized displacements in tissue[18-20]. Furthermore, because ARFI elastography uses elastography with a flexible metering box of the region of the interest (ROI), it is the only elastography method suitable for quantifying focal liver mass stiffness.

Few reports have investigated the applicability of ARFI elastography to evaluate focal liver masses[6,20-25]. Thus, we prospectively recruited patients with a focal liver mass and investigated the diagnostic performance of ARFI elastography to discriminate malignant liver masses and hepatocellular carcinoma (HCC) from benign masses by quantifying their stiffness.

MATERIALS AND METHODS

Subjects

From February 2011 to November 2011, a total of 62 patients with a focal liver mass that was well visualized on conventional US were prospectively recruited for this study. The subjects were referred to our institute for further evaluation of a focal liver mass from primary or secondary clinics, or had been diagnosed with a focal liver mass during a surveillance examination at our institute. Of these, 12 patients were excluded for the following reasons: (1) ARFI measurement failure due to a lesion that was smaller than the ROI and at an inaccessible location (deeper than 8 cm) (n = 7) or (2) poor compliance or inability to hold their breath as required (n = 5). Finally, 50 patients with valid ARFI measurements were enrolled. If a patient had multiple liver masses, only one mass of interest was chosen.

This study was approved by the institutional review board at Severance Hospital in Seoul, South Korea, and conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Written informed consent was obtained from all participants.

Histological confirmation of the liver masses

We performed a targeted biopsy on masses when a confirmatory histological diagnosis was needed or the radiological diagnosis was not confirmative. Among the 50 masses in our study, 37 (74.0%) received targeted biopsy for histological confirmation. Masses that had any component of cholangiocellular carcinomas (CCC) or combined HCC (CHCC) were classified as CCC and the others as HCC.

Clinical diagnosis of the liver masses

A clinical diagnosis of HCC was made according to the American Association for the Study of Liver Disease recommendation[26]. Briefly, patients were diagnosed with HCC if they had a tumor with a maximum diameter > 2 cm and exhibited typical features of HCC on dynamic CT, defined as enhancement in the arterial phase, early washout on the portal phase, and an α-fetoprotein level



Figure 1 Measurement of acoustic radiation force impulse value within the region of interest of a focal liver mass. The shear wave velocity measured when the region of interest box was placed within the mass.

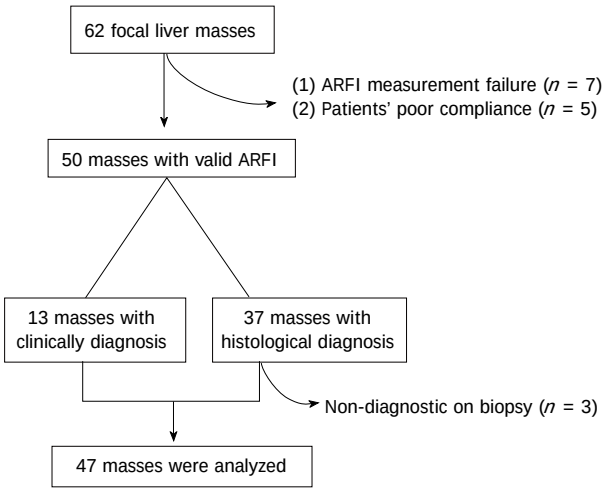


Figure 2 Recruitment algorithm.

> 200 ng/mL. Overall, nine patients satisfied these criteria sufficiently to be diagnosed with HCC without a histological examination.

The presence of a hemangioma was diagnosed clinically in four patients, based on a combination of typical findings, determined using CT or MRI, and a lack of growth for at least 12 mo[22]. A hemangioma appears as a mass with hypoattenuation on unenhanced CT, very high signal intensity on T2-weighted images, peripheral nodular enhancement in the arterial phase, and progressive filling-in of enhancement with no washout in later phases[20].

ARFI elastography

ARFI elastography was performed with an Acuson S2000 ultrasound system (Siemens, Erlanger, Germany), using a 4-1 MHz curved array probe. ARFI elastography was performed by a single physician in all patients (2 years of experience with US and more than 100 examinations with ARFI elastography) who was blinded with regard to the clinical and biochemical data. For patients who underwent liver biopsy, ARFI elastography was performed just before the biopsy, on the same day. For patients with a solitary liver mass that was clinically diagnosed, ARFI elastography was performed at the time of enrollment in this study.

Details of the technical background and examination procedure have been described previously[27]. Briefly, a B-mode US image of the lesion was identified utilizing a ROI, characterized by a box with a fixed dimension of 1 cm × 0.5 cm and a maximum depth of 8 cm. The ROI was entirely located in the lesion, and the ROI location was changed to cover large masses as much as possible without including any vascular or biliary structures (Figure 1). The potential presence of degeneration, such as necrotic, cystic, or calcified portions, was not included in the ROI. To evaluate background liver status of the focal mass, measurements were also taken in the surrounding liver parenchyma with the ROI within 2-3 cm from the target mass, taking care not to comprise any vascular or biliary structure. To ensure quality of the ARFI measurement, 10 measurements were performed for each mass and surrounding liver parenchyma.

Statistical analysis

Continuous variables are expressed as medians and ranges. The χ^2 test or Fisher's exact test and the Mann-Whitney test were used to compare categorical and continuous variables, respectively. Receiver operating characteristics (ROC) curves and areas under the ROC curves (AUROC) were used to estimate diagnostic performance. The cutoff ARFI value for maximal diagnostic accuracy was selected by considering the highest sum of sensitivity and specificity. A P-value < 0.05 was considered statistically significant. The statistical analysis was performed using SPSS software ver. 18.0.0 (Chicago, IL, United States).

RESULTS

Final diagnosis of focal liver masses

We further excluded three masses that were non-diagnostic on biopsy due to a lack of sufficient tissue or ineffective targeting of the mass. Thus, 47 masses (34 histologically confirmed and 13 clinically diagnosed) were evaluated (Figure 2).

Overall, 15 HCCs, eight metastases (three from colorectal cancers, two from gallbladder cancer, one from pancreatic cancer, one from a gastrointestinal stromal tumor, and one from cervical cancer), seven CCCs, three focal nodular hyperplasias (FNH), and one hemangioma were diagnosed. Figure 3 describes pre-biopsy and post-biopsy diagnoses: 21 (61.8%) masses were consistent with the pre-biopsy diagnosis, and 10 masses diagnosed as HCC or CHCC at the time of pre-biopsy were finally confirmed as six HCCs and four CCCs through the biopsy. The histological diagnosis of the other three masses changed (two FNHs to one hemangioma and one HCC; one poorly-differentiated malignancy or metastatic mass to CCC).

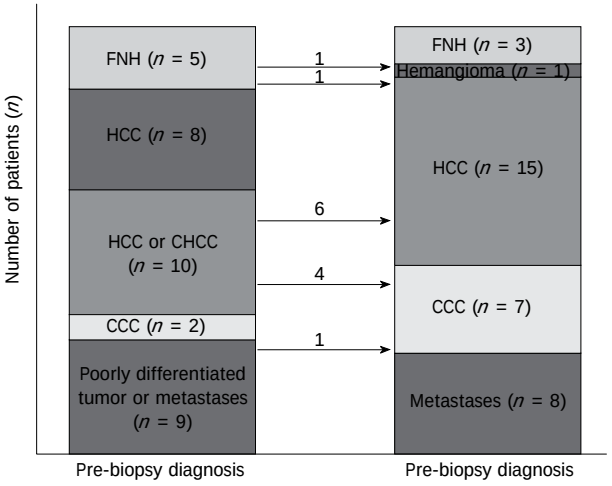


Figure 3 The flow of histological diagnosis. The values on arrows indicate the number of patients who obtained diagnoses differing from those pre-biopsy diagnoses after liver biopsy.

Characteristics and ARFI values of focal liver masses

The depth, location (right *vs* left lobe), and size of each focal liver mass are described in Table 1. ARFI values of HCCs and metastases were higher (2.48 and 2.35 m/s) than those of hemangiomas (1.83 m/s), CCCs (1.65 m/s), and FNH (0.97 m/s). ARFI values for CCCs, hemangiomas, and metastases were significantly higher than those for their surrounding liver parenchyma (mean 1.65 *vs* 1.07 m/s in CCCs; 1.83 m/s *vs* 1.10 m/s in hemangiomas; 2.35 m/s *vs* 1.45 m/s in metastases; all $P < 0.05$), whereas ARFI values for HCCs were similar to those of surrounding liver parenchyma (2.48 m/s *vs* 2.14 m/s, $P = 0.134$).

When HCCs, CCCs, and metastases were categorized into a malignant liver mass group ($n = 39$) and the others were stratified into a benign mass group ($n = 8$), a significant difference appeared in the between the malignant and benign liver masses (2.31 ± 1.05 m/s *vs* 1.51 ± 0.69 m/s, $P = 0.047$) (Figure 4A). However, considerable overlap in ARFI values was noted between malignant and benign masses. Additionally, a significant difference in the ARFI values was observed between the HCCs and the benign masses (2.48 ± 0.84 m/s *vs* 1.51 ± 0.69 m/s, $P = 0.006$) (Figure 4A). To address this overlap, we attempted further analysis taking into consideration the ARFI values of surrounding liver parenchyma as well as those of focal liver masses. When ARFI values of focal liver masses and their surrounding liver parenchyma were combined, the differences in ARFI values for each tumor type became more prominent (4.29 ± 1.22 m/s *vs* 2.92 ± 0.35 m/s for malignant masses *vs* benign masses, $P < 0.001$; 4.62 ± 0.96 m/s *vs* 2.92 ± 0.35 m/s for HCCs *vs* benign masses, $P < 0.001$) (Figure 4B).

Discrimination of malignant liver masses and HCCs from benign masses

The AUROC for discriminating the malignant masses from benign masses was 0.724 (95%CI, 0.566-0.883, $P = 0.048$), and the AUROC for discriminating HCCs from benign masses was 0.813 (95%CI, 0.649-0.976, $P = 0.008$) (Table 2). To maximize the sum of sensitivity and specificity, an ARFI value of 1.82 m/s was selected as the cutoff value to differentiate malignant from benign liver masses. Furthermore, the cutoff value for distinguishing HCCs from benign masses was also determined to be 1.82 m/s (Table 2). Additionally, the diagnostic performance of the sum of the ARFI values for focal liver masses and the surrounding liver parenchyma to differentiate liver masses improved (AUROC = 0.853; 95%CI, 0.745-0.960; $P = 0.002$ in malignant liver masses *vs* benign ones and AUROC = 0.948; 95%CI, 0.896-0.992, $P < 0.001$ in HCCs *vs* benign masses) (Table 2).

DISCUSSION

ARFI elastography has been proposed as a new method for assessing liver stiffness[7-9]. Although ARFI elastography uses shear wave velocity (m/s) to assess liver stiffness, which is similar to FibroScan®, it exhibits several unique properties. First, ARFI elastography allows for the evaluation of deep tissue by generating a shear wave without the need for exertional compression[7,28]. Second, ARFI elastography has the distinct advantage of being integrated into a conventional US system and can provide additional real-time information during a conventional US study[14,15]. Third, ARFI elastography can be performed regardless of the presence of impediments such as ascites, although the reproducibility in this setting should be further investigated[19,29]. Finally, ARFI elastography offers a flexible metering box at variable depths, allowing the examination of specific liver areas[21,30]. Due to these characteristics, the clinical applicability of ARFI elastography has expanded to characterize and distinguish focal liver masses beyond a simple assessment of liver fibrosis[6,20-25].

According to previous studies[22,24,25], malignant liver masses are stiffer than benign masses, as reflected by higher ARFI values. Consistent with this finding, the ARFI values of malignant liver masses (2.31 m/s) or HCCs (2.48 m/s) in our cohort were significantly higher than those of benign masses (1.51 m/s) (all $P < 0.05$). However, when we consider that two types of malignant liver masses (HCCs and CCCs) with different ARFI values were stratified into one malignant mass group in our study, the accuracy of ARFI elastography for identifying malignant liver masses would change according to the proportion of HCCs and CCCs. Thus, an exact comparison between the ARFI values of HCCs and CCCs as well as those of benign masses should be performed in future, larger-scale studies. Additionally, the respective ARFI values of HCCs and metastatic masses in our study were 2.48 m/s and 2.35 m/s, which were similar to data from previous studies (2.45 to 2.63 m/s in HCC and 2.18 to 2.88 m/s in metastasis)[22,23].

ARFI values for hemangiomas vary among reports[6,20,22,23]. The ARFI values of hemangiomas reported

Table 1 Characteristics and acoustic radiation force impulse values of focal liver masses

Variables	Total masses (n = 47)	HCC (n = 24)	CCC (n = 7)	Metastases (n = 8)	Hemangioma (n = 5)	FNH (n = 3)
Histologic confirmation	34 (72.3)	15 (62.5)	7 (100)	8 (100)	1 (20.0)	3 (100)
Size, cm	5.0 (1.4-20.5)	4.9 (1.4-18.3)	9.4 (7.4-18.8)	8.0 (1.6-20.5)	1.9 (1.5-4.7)	2.7 (2.0-3.0)
Depth, cm	5.6 (2.7-7.8)	5.7 (2.7-7.8)	6.4 (4.8-7.2)	4.9 (3.5-7.6)	5.3 (4.5-6.5)	5.6 (2.8-7.8)
Right/left lobe	34 (72.3)/13 (27.7)	19 (79.2)/5 (20.8)	5 (71.4)/2 (28.6)	5 (62.5)/3 (37.5)	4 (80.0)/1 (20.0)	1 (33.3)/2 (66.7)
ARFI value, m/s						
Masses	2.23 ± 0.98	2.48 ± 0.84	1.65 ± 1.43	2.35 ± 1.18	1.83 ± 0.62	0.97 ± 0.48
Surrounding parenchyma	1.83 ± 0.73	2.14 ± 0.59	1.07 ± 0.49	1.45 ± 0.51	1.10 ± 0.14	1.63 ± 0.40
P value	0.029	0.134	0.015	0.043	0.013	0.581

Data was expressed as median (range) or n (%). HCC: Hepatocellular carcinoma; CCC: Cholangiocellular carcinoma; FNH: Focal nodular hyperplasia; ARFI: Acoustic radiation force impulse.

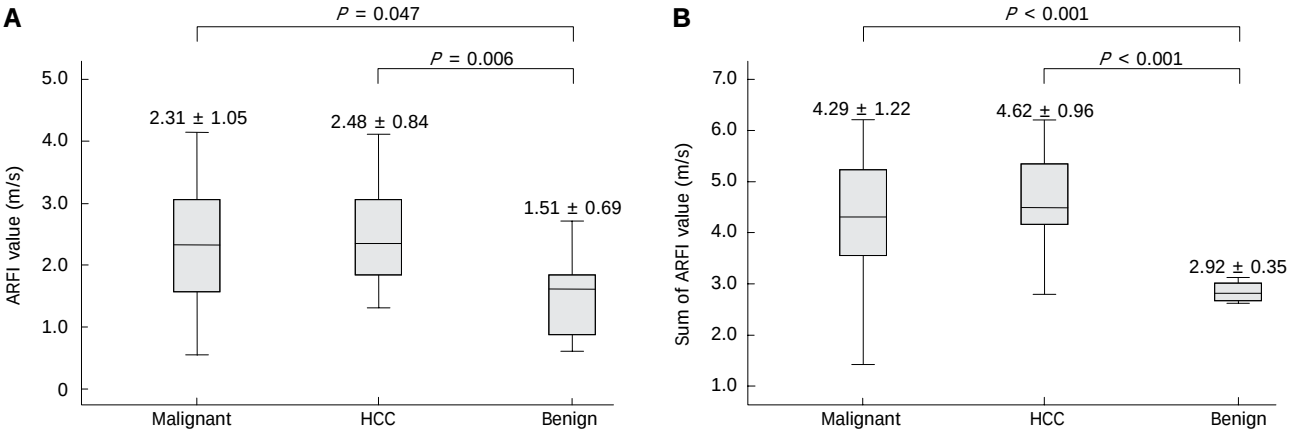


Figure 4 Acoustic radiation force impulse values of malignant masses, hepatocellular carcinoma, and benign masses. A: Acoustic radiation force impulse (AFRI) value of a focal liver mass; B: Sum of the ARFI values of liver mass and the surrounding liver parenchyma. HCC: Hepatocellular carcinoma.

Table 2 Optimal cutoff acoustic radiation force impulse values and corresponding diagnostic indices for discriminating malignant masses and hepatocellular carcinoma from benign liver masses

	Malignant *vs* benign		HCC *vs* benign	
	Masses	Masses with surrounding liver parenchyma	Masses	Masses with surrounding liver parenchyma
Cutoff ARFI value, m/s	1.82[1]	3.72[2]	1.82[1]	3.79[2]
AUROC (95%CI)	0.724 (0.566-0.883)	0.853 (0.745-0.960)	0.813 (0.649-0.976)	0.948 (0.896-0.992)
Sensitivity, %	71.8	71.8	79.2	87.5
Specificity, %	75.0	100	75.0	100
Positive predictive value, %	93.3	100	90.5	100
Negative predictive value, %	35.3	42.1	54.5	72.9

[1]ARFI value of a focal liver mass; [2]Sum of ARFI values of focal liver mass and surrounding liver parenchyma. ARFI: Acoustic radiation force impulse; HCC: Hepatocellular carcinoma; AUROC: Area under the receiver operating characteristics; CI: Confidence intervals.

in two previous studies (1.51 and 1.75 m/s, respectively) were comparable to our data (1.83 m/s)[20,22,23], whereas the ARFI value for a hemangioma in another study was rather higher (2.36 m/s)[22,23]. The variability in hemangioma ARFI values has been explained based on the amount of fibrotic septa that divide the dilated vascular spaces[22]. That is, hemangiomas are composed of multiple vascular channels filled with blood so they would not be expected to be stiff, and therefore would have low ARFI values; in contrast, those including pathological patterns such as sclerosis, thrombosis of the vessels, or calcification, would be stiff and have high ARFI values[20]. Because relatively few hemangioma cases were analyzed in these studies, this discrepancy will need to be investigated *via* further study including a larger number of cases.

Some researchers have attempted to suggest a cutoff ARFI value for distinguishing malignant liver masses from benign masses. We obtained a high PPV (malignant masses *vs* benign masses, PPV 93.3%; HCC *vs* benign masses, 90.5%) using the cutoff ARFI value of 1.82 m/s, which maximized the sum of the sensitivity and specificity for distinguishing masses. Our cutoff value of 1.82 m/s for identifying malignant liver masses was slightly lower than those in previous studies (2.0 to 2.5 m/s)[22,24,25],

which can be partially explained by the different methods used to categorize the masses. In one study, which reported a rather high cutoff value of 2.5 m/s, only metastases that had a high ARFI value of 4.18 m/s were categorized into a malignant group and compared with benign masses[24], whereas another study that reported 2.22 m/s as a cutoff value for discriminating malignant masses from benign masses included FNHs, adenomas, and focal fatty change lesions in the benign mass group as well as hemangiomas[25]. Thus, in this regard, further study is needed to prevent potential bias due to heterogeneity of the masses and to confirm the clinically applicable cutoff values for ARFI elastography. Although our cutoff value may be useful to physicians who encounter focal liver masses during routine US evaluation, it should be interpreted cautiously in the clinical context for several reasons. First, although our cutoff value would correctly characterize HCC and metastasis, it would mischaracterize CCC with a relatively low ARFI value and hemangioma with a relatively high ARFI value. Second, there was considerable overlap of ARFI values between malignant and benign masses.

Underlying fibrosis of the liver is a consideration for the differential diagnosis of a focal liver mass. Several previous studies have proposed that HCCs generally appear softer (lower ARFI values) than the surrounding liver[21], whereas metastases and hemangiomas generally appear harder than that of the surrounding liver despite some controversies among studies[21]. Similarly, we found that CCCs, hemangiomas, and metastases had higher ARFI values than those of surrounding liver parenchyma, whereas ARFI values of HCCs were statistically equivalent to those of the surrounding liver parenchyma. Because hemangiomas and metastases were evaluated in patients without chronic liver disease in most studies[6,25], ARFI values seemed consistently higher than those of a background liver. In contrast, because HCCs were evaluated in patients with chronic liver disease and diverse degrees of background liver fibrosis, the comparative results between ARFI values of HCCs and those of background liver differed among studies based on the characteristics of each study cohort[6,21,25]. That is, simultaneously measuring ARFI values of focal liver mass and the surrounding liver should focus on assessing the respective characteristics of the hepatic mass and surrounding fibrosis to prevent a misdiagnosis of the hepatic mass using the correlation of ARFI values between the liver mass and background liver parenchyma. However, when we used the sum of ARFI values of focal liver mass and the surrounding liver parenchyma, the diagnostic performance in terms of distinguishing liver mass improved. Thus, these controversial findings concerning the simultaneous measurement of liver masses and their surrounding liver parenchyma should be further investigated in future larger-scale studies.

Although most cases (72.3%) were histologically confirmed in our study, the relative small sample size of our cohort and inclusion of patients with high ALT, which has the potential of overestimating influences on ARFI values[30,31], are potential limitations. Although ARFI elastography can freely locate the ROI box in a specific area within a mass and measure its stiffness, morphological characteristics of liver masses including heterogeneous components such as HCC with hemorrhage and fatty metamorphosis[6,20,32] and lesion shapes were not considered in our study, which is a limitation of this study. Further investigation of how to evaluate such heterogeneous or morphologically varying liver masses using ARFI elastography and their influences on ARFI values of liver masses should be conducted.

In our study, we demonstrated the potential clinical utility of ARFI elastography for characterization of focal liver masses. Although this study had limitations and should be interpreted cautiously, our findings provide a useful reference for the differential diagnosis of a focal liver mass and will provide additional information to clinicians who are confronted with a need for an immediate diagnosis of a focal liver mass during a routine US examination before a further diagnostic imaging study such as contrast-enhanced US, CT or MRI. However, further studies with larger numbers of cases are warranted to assess the utility of ARFI elastography in the clinic.

ACKNOWLEDGMENTS

The authors are grateful to Dong-Su Jang (Medical Illustrator, Medical Research Support Section, Yonsei University College of Medicine, Seoul, South Korea) for his help with the figures.

COMMENTS

Background

Acoustic radiation force impulse (ARFI) elastography can quantify tissue elasticity. Compared to previous elastography techniques, one of the distinguishing advantages of ARFI elastography is that it offers a flexible metering box at variable depths, which allows an examination of specific liver areas. With using this characteristic of ARFI elastography, authors investigated its applicability to evaluate focal liver masses.

Research frontiers

According to previous studies, malignant liver masses are stiffer than benign masses, as reflected by higher ARFI values. However, previous studies have showed somewhat controversial results with various median ARFI values for each focal liver mass and they have had limitations, such as most subjects not being supported by adequate histological confirmation.

Innovations and breakthroughs

Authors prospectively investigated 50 focal liver masses found during routine ultrasonography (US) study, and measured the stiffness of each focal liver mass using ARFI elastography. Focal liver masses included hepatocellular carcinomas (HCCs), cholangiocellular carcinomas, metastases, hemangiomas, and focal nodular hyperplasia. The advantage of our study over other previous studies is that majority of our subjects (72.3%) are confirmed with histological examinations.

Applications

Using ARFI elastography, physicians who are confronted with a need for an immediate diagnosis of a focal liver mass during a routine US examination can get additional information for the differential diagnosis of liver masses in clinical practice. Further future studies with larger numbers of cases are warranted to assess the utility of ARFI elastography.

Terminology

ARFI elastography is an emerging examination which can quantify tissue elasticity. ARFI elastography has the distinct advantage of being integrated into a conventional US system and can be checked simultaneously during a conventional US study. Furthermore, because ARFI elastography uses elastography with a flexible metering box of the region of the interest, it is suitable for quantifying focal liver mass stiffness.

Peer review

Although this study had limitations and should be interpreted cautiously, their results show the clinical applicability of ARFI elastography as a complementary diagnostic tool for the differential diagnosis of liver masses.

REFERENCES

1 **Assy N**, Nasser G, Djibre A, Beniashvili Z, Elias S, Zidan J. Characteristics of common solid liver lesions and recommendations for diagnostic workup. *World J Gastroenterol* 2009; **15**: 3217-3227 [PMID: 19598296]

2 **Trillaud H**, Bruel JM, Valette PJ, Vilgrain V, Schmutz G, Oyen R, Jakubowski W, Danes J, Valek V, Greis C. Characterization of focal liver lesions with SonoVue-enhanced sonography: international multicenter-study in comparison to CT and MRI. *World J Gastroenterol* 2009; **15**: 3748-3756 [PMID: 19673015]

3 **Vilgrain V**. Advancement in HCC imaging: diagnosis, staging and treatment efficacy assessments: hepatocellular carcinoma: imaging in assessing treatment efficacy. *J Hepatobiliary Pancreat Sci* 2010; **17**: 374-379 [PMID: 19924373 DOI: 10.1007/s00534-009-0230-3]

4 **Hohmann J**, Albrecht T, Hoffmann CW, Wolf KJ. Ultrasonographic detection of focal liver lesions: increased sensitivity and specificity with microbubble contrast agents. *Eur J Radiol* 2003; **46**: 147-159 [PMID: 12714231]

5 **Numminen K**, Isoniemi H, Halavaara J, Tervahartiala P, Makisalo H, Laasonen L, Hockerstedt K. Preoperative assessment of focal liver lesions: multidetector computed tomography challenges magnetic resonance imaging. *Acta Radiol* 2005; **46**: 9-15 [PMID: 15841734]

6 **Gallotti A**, D'Onofrio M, Romanini L, Cantisani V, Pozzi Mucelli R. Acoustic Radiation Force Impulse (ARFI) ultrasound imaging of solid focal liver lesions. *Eur J Radiol* 2012; **81**: 451-455 [PMID: 21330078 DOI: 10.1016/j.ejrad.2010.12.071]

7 **Fahey BJ**, Nightingale KR, Nelson RC, Palmeri ML, Trahey GE. Acoustic radiation force impulse imaging of the abdomen: demonstration of feasibility and utility. *Ultrasound Med Biol* 2005; **31**: 1185-1198 [PMID: 16176786 DOI: 10.1016/j.ultrasmedbio.2005.05.004]

8 **Nightingale K**, Palmeri M, Trahey G. Analysis of contrast in images generated with transient acoustic radiation force. *Ultrasound Med Biol* 2006; **32**: 61-72 [PMID: 16364798 DOI: 10.1016/j.ultrasmedbio.2005.08.008]

9 **Piscaglia F**, Salvatore V, Di Donato R, D'Onofrio M, Gualandi S, Gallotti A, Peri E, Borghi A, Conti F, Fattovich G, Sagrini E, Cucchetti A, Andreone P, Bolondi L. Accuracy of VirtualTouch Acoustic Radiation Force Impulse (ARFI) imaging for the diagnosis of cirrhosis during liver ultrasonography. *Ultraschall Med* 2011; **32**: 167-175 [PMID: 21321842 DOI: 10.1055/s-0029-1245948]

10 **Palmeri ML**, Wang MH, Rouze NC, Abdelmalek MF, Guy CD, Moser B, Diehl AM, Nightingale KR. Noninvasive evaluation of hepatic fibrosis using acoustic radiation force-based shear stiffness in patients with nonalcoholic fatty liver disease. *J Hepatol* 2011; **55**: 666-672 [PMID: 21256907 DOI: 10.1016/j.jhep.2010.12.019]

11 **Kim SU**, Jang HW, Cheong JY, Kim JK, Lee MH, Kim DJ, Yang JM, Cho SW, Lee KS, Choi EH, Park YN, Han KH. The usefulness of liver stiffness measurement using FibroScan in chronic hepatitis C in South Korea: a multicenter, prospective study. *J Gastroenterol Hepatol* 2011; **26**: 171-178 [PMID: 21175811 DOI: 10.1111/j.1440-1746.2010.06385.x]

12 **Sporea I**, Sirli R, Popescu A, Danilă M. Acoustic Radiation Force Impulse (ARFI)--a new modality for the evaluation of liver fibrosis. *Med Ultrason* 2010; **12**: 26-31 [PMID: 21165451]

13 **Horster S**, Mandel P, Zachoval R, Clevert DA. Comparing acoustic radiation force impulse imaging to transient elastography to assess liver stiffness in healthy volunteers with and without valsalva manoeuvre. *Clin Hemorheol Microcirc* 2010; **46**: 159-168 [PMID: 21135491 DOI: 10.3233/ch-2010-1342]

14 **Friedrich-Rust M**, Wunder K, Kriener S, Sotoudeh F, Richter S, Bojunga J, Herrmann E, Poynard T, Dietrich CF, Vermehren J, Zeuzem S, Sarrazin C. Liver fibrosis in viral hepatitis: noninvasive assessment with acoustic radiation force impulse imaging versus transient elastography. *Radiology* 2009; **252**: 595-604 [PMID: 19703889 DOI: 10.1148/radiol.2523081928]

15 **Lupsor M**, Badea R, Stefanescu H, Sparchez Z, Branda H, Serban A, Maniu A. Performance of a new elastographic method (ARFI technology) compared to unidimensional transient elastography in the noninvasive assessment of chronic hepatitis C. Preliminary results. *J Gastrointestin Liver Dis* 2009; **18**: 303-310 [PMID: 19795024]

16 **Boursier J**, Isselin G, Fouchard-Hubert I, Oberti F, Dib N, Lebigot J, Bertrais S, Gallois Y, Calès P, Aubé C. Acoustic radiation force impulse: a new ultrasonographic technology for the widespread noninvasive diagnosis of liver fibrosis. *Eur J Gastroenterol Hepatol* 2010; **22**: 1074-1084 [PMID: 20440210 DOI: 10.1097/MEG.0b013e328339e0a1]

17 **Goertz RS**, Zopf Y, Jugl V, Heide R, Janson C, Strobel D, Bernatik T, Haendl T. Measurement of liver elasticity with acoustic radiation force impulse (ARFI) technology: an alternative noninvasive method for staging liver fibrosis in viral hepatitis. *Ultraschall Med* 2010; **31**: 151-155 [PMID: 20306380 DOI: 10.1055/s-0029-1245244]

18 **Gallotti A**, D'Onofrio M, Pozzi Mucelli R. Acoustic Radiation Force Impulse (ARFI) technique in ultrasound with Virtual Touch tissue quantification of the upper abdomen. *Radiol Med* 2010; **115**: 889-897 [PMID: 20082227 DOI: 10.1007/s11547-010-0504-5]

19 **Takahashi H**, Ono N, Eguchi Y, Eguchi T, Kitajima Y, Kawaguchi Y, Nakashita S, Ozaki I, Mizuta T, Toda S, Kudo S, Miyoshi A, Miyazaki K, Fujimoto K. Evaluation of acoustic radiation force impulse elastography for fibrosis staging of chronic liver disease: a pilot study. *Liver Int* 2010; **30**: 538-545 [PMID: 19874490 DOI: 10.1111/j.1478-3231.2009.02130.x]

20 **Yu H**, Wilson SR. Differentiation of benign from malignant liver masses with Acoustic Radiation Force Impulse technique. *Ultrasound Q* 2011; **27**: 217-223 [PMID: 22124386 DOI: 10.1097/RUQ.0b013e318239422e]

21 **Fahey BJ**, Nelson RC, Bradway DP, Hsu SJ, Dumont DM, Trahey GE. In vivo visualization of abdominal malignancies with acoustic radiation force elastography. *Phys Med Biol* 2008; **53**: 279-293 [PMID: 18182703 DOI: 10.1088/0031-9155/53/1/020]

22 **Cho SH**, Lee JY, Han JK, Choi BI. Acoustic radiation force impulse elastography for the evaluation of focal solid hepatic lesions: preliminary findings. *Ultrasound Med Biol* 2010; **36**: 202-208 [PMID: 20018432 DOI: 10.1016/j.ultrasmedbio.2009.10.009]

23 **Heide R**, Strobel D, Bernatik T, Goertz RS. Characterization of focal liver lesions (FLL) with acoustic radiation force impulse (ARFI) elastometry. *Ultraschall Med* 2010; **31**: 405-409 [PMID: 20652853 DOI: 10.1055/s-0029-1245565]

24 **Davies G**, Koenen M. Acoustic radiation force impulse elastography in distinguishing hepatic haemangiomata from metastases: preliminary observations. *Br J Radiol* 2011; **84**: 939-943 [PMID: 21385910 DOI: 10.1259/bjr/97637841]

25 **Shuang-Ming T**, Ping Z, Ying Q, Li-Rong C, Ping Z, Rui-

Zhen L. Usefulness of acoustic radiation force impulse imaging in the differential diagnosis of benign and malignant liver lesions. *Acad Radiol* 2011; **18**: 810-815 [PMID: 21419668 DOI: 10.1016/j.acra.2011.01.026]

26 **Bruix J**, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051 DOI: 10.1002/hep.20933]

27 **Son CY**, Kim SU, Han WK, Choi GH, Park H, Yang SC, Choi JS, Park JY, Kim do Y, Ahn SH, Chon CY, Han KH. Normal liver elasticity values using acoustic radiation force impulse imaging: a prospective study in healthy living liver and kidney donors. *J Gastroenterol Hepatol* 2012; **27**: 130-136 [PMID: 21679249 DOI: 10.1111/j.1440-1746.2011.06814.x]

28 **Quaia E**, Calliada F, Bertolotto M, Rossi S, Garioni L, Rosa L, Pozzi-Mucelli R. Characterization of focal liver lesions with contrast-specific US modes and a sulfur hexafluoride-filled microbubble contrast agent: diagnostic performance and confidence. *Radiology* 2004; **232**: 420-430 [PMID: 15286314 DOI: 10.1148/radiol.2322031401]

29 **Lim SM**, Kim SU. Acoustic radiation force impulse elastography is useful to exclude nonliver-related ascites. *Eur J Gastroenterol Hepatol* 2011; **23**: 1080-1081 [PMID: 21971343 DOI: 10.1097/MEG.0b013e32834be9e9]

30 **Yoon KT**, Lim SM, Park JY, Kim do Y, Ahn SH, Han KH, Chon CY, Cho M, Lee JW, Kim SU. Liver stiffness measurement using acoustic radiation force impulse (ARFI) elastography and effect of necroinflammation. *Dig Dis Sci* 2012; **57**: 1682-1691 [PMID: 22302243 DOI: 10.1007/s10620-012-2044-4]

31 **Lim SM**, Chung MJ, Han KH, Kim SU. Acoustic radiation force impulse elastography: a better option for patients with extrahepatic cholestasis. *Eur J Gastroenterol Hepatol* 2012; **24**: 215-216 [PMID: 22081004 DOI: 10.1097/MEG.0b013e32834e0789]

32 **Kwon HJ**, Kang MJ, Cho JH, Oh JY, Nam KJ, Han SY, Lee SW. Acoustic radiation force impulse elastography for hepatocellular carcinoma-associated radiofrequency ablation. *World J Gastroenterol* 2011; **17**: 1874-1878 [PMID: 21528062 DOI: 10.3748/wjg.v17.i14.1874]

P- Reviewers Di Carlo I, Montet X **S- Editor** Gou SX
L- Editor A **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i2.227

World J Gastroenterol 2013 January 14; 19(2): 227-234
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

ORIGINAL ARTICLE

Inositol-requiring enzyme 1α is required for gut development in *Xenopus lavies* embryos

Jing Guo, Xin-Xin Li, Jiao-Jiao Feng, Chen-Yang Yin, Xue-Jun Wang, Ning Wang, Li Yuan

Jing Guo, Xin-Xin Li, Jiao-Jiao Feng, Chen-Yang Yin, Xue-Jun Wang, Ning Wang, Li Yuan, Department of Biochemistry and Molecular Biology, Nanjing Medical University, Nanjing 210029, Jiangsu Province, China

Author contributions: Guo J, Li XX, Feng JJ and Yin CY performed the majority of the experiments; Wang XJ and Wang N provided vital reagents and analytical tools and revised the manuscript; Yuan L designed the study and wrote the manuscript; all authors read and approved the final manuscript.

Supported by The National Natural Science Foundation of China, No. 30971680; A Project Funded by the Priority Academic Program Development of Jiangsu Higher Education Institutions, No. JX10131801101

Correspondence to: Li Yuan, PhD, Associate Professor of Biochemistry and Molecular Biology, Department of Biochemistry and Molecular Biology, Nanjing Medical University, Nanjing 210029, Jiangsu Province, China. yuanli@njmu.edu.cn
Telephone: +86-25-86862895 Fax: +86-25-86862728

Received: August 3, 2012 Revised: November 7, 2012
Accepted: December 15, 2012
Published online: January 14, 2013

Abstract

AIM: To investigate the role of inositol-requiring enzyme 1α (IRE1α) in gut development of *Xenopus lavies* embryos.

METHODS: *Xenopus* embryos were obtained with *in vitro* fertilization and cultured in 0.1 × MBSH. One and half nanogram of IRE1α, 1 ng of IRE1α-GR mRNA, 1 ng of IRE1αΔC-GR mRNA, and 50 ng of IRE1α morpholino oligonucleotide (MO) or XBP1(C)MO were injected into four blastomeres at 4-cell stage for scoring the phenotype and marker gene analysis. To rescue the effect of IRE1α MO, 1 ng of IRE1α-GR mRNA was co-injected with 50 ng of MO. For the activation of the GR-fusion proteins, dexamethasone was prepared as 5 mmol/L stock solutions in 100% ethanol and applied to the mRNA injected embryos at desired stages in a concentration of 10 μmol/L in 0.1 × MBSH. Embryos were kept in dexamethasone up to stage 41. Whole-mount *in situ* hybridization was used to determine specific gene expression, such as IRE1α, IRE1β, Xbra and Xsox17α. IRE1α protein expression during *Xenopus* embryogenesis was detected by Western blotting.

RESULTS: In the whole-mount *in situ* hybridization analysis, xenopus IRE1α and IRE1β showed quite different expression pattern during tadpole stage. The relatively higher expression of IRE1α was observed in the pancreas, and significant transcription of IRE1β was found in the liver. IRE1α protein could be detected at all developmental stages analyzed, from stage 1 to stage 42. Gain-of-function assay showed that IRE1α mRNA injected embryos at tailbud stage were nearly normal and the expression of the pan-mesodermal marker gene Xbra and the endodermal gene Xsox17α at stage 10.5 was not significantly changed in embryos injected with IRE1α mRNA as compared to uninjected control embryos. And at tadpole stage, the embryos injected with IRE1α-GR mRNA did not display overt phenotype, such as gut-coiling defect. Loss-of-function assay demonstrated that the IRE1α MO injected embryos were morphologically normal before the tailbud stages. We did not observe a significant change of mesodermal and endodermal marker gene expression, while after stage 40, about 80% of the MO injected embryos exhibited dramatic gut defects in which the guts did not coil, but other structures outside the gastrointestinal tract were relatively normal. To test if the phenotypes were specifically caused by the knockdown of IRE1α, a rescue experiment was performed by co-injection of IRE1α-GR mRMA with IRE1α MO. The data obtained demonstrated that the gut coiling defect was rescued. The deletion mutant of IRE1α was constructed, consisting of the N-terminal part without the C-terminal kinase and RNase domains named IRE1αΔC, to investigate the functional domain of IRE1α. Injection of IRE1αΔC-GR mRNA caused similar morphological alterations with gut malformation by interfering with the function of endogenous xIRE1α. In order to investigate if IRE1α/

XBP1 pathway was involved in gut development, 50 ng of XBP1 MO was injected and the results showed that knockdown of XBP1 resulted in similar morphological alterations with gut-coiling defect at tadpole stage.

CONCLUSION: IRE1α is not required for germ layer formation but for gut development in *Xenopus lavies* and it may function *via* XBP1-dependent pathway.

© 2013 Baishideng. All rights reserved.

Key words: Inositol-requiring enzyme 1α; XBP1; *Xenopus lavies*; Gut; Development

Guo J, Li XX, Feng JJ, Yin CY, Wang XJ, Wang N, Yuan L. Inositol-requiring enzyme 1α is required for gut development in *Xenopus lavies* embryos. *World J Gastroenterol* 2013; 19(2): 227-234 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i2/227.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i2.227

INTRODUCTION

The endoplasmic reticulum (ER) plays a central role in the synthesis and modification of secretary and membrane proteins in all eukaryotic cells[1-3]. Under normal conditions, these proteins are correctly folded and assembled in the ER. However, when cells are exposed to disturbed environment, such as overproduction of ER proteins, viral infection, and glucose deprivation, these proteins accumulate as unfolded or misfolded forms in the ER lumen and, consequently, cause ER stress. To maintain cellular homeostasis, cells induce some adaptive responses to ER stress. One of them is the unfolded protein response (UPR), which up-regulates the transcription of various genes to increase the protein-folding and protein-degradation activity in the ER[4-7]. Inositol requiring enzyme-1 (IRE1) is an ER-located type I transmembrane protein with a kinase domain and RNase domain in the cytosolic region and has a unique function of relieving ER stress in cells. When the amino-terminal luminal region senses perturbations in the ER environment, *via* trans-autophosphorylation and activation of its RNase domain, IRE1 induces unconventional splicing of mRNA coding, a specific transcription factor for activating the UPR[8-10]. IRE1 is highly conserved from yeast to humans, and two IRE1 paralogues have been reported in mammals: IRE1α and IRE1β[11-13]. Upon activation by ER stress, IRE1 performs an unconventional cytoplasmic splicing of XBP1 pre-mRNA, thus allowing the synthesis of active XBP1, which activates UPR target genes to restore the homeostasis of the ER[10,14,15]. The spliced XBP1 mRNA is translated into a functional transcription factor to up-regulate gene expression for ER quality control. IRE1 is also reported to activate proapoptotic JNK signaling under ER stress conditions[16].

IRE1α is also known to be expressed ubiquitously in fetal and adult mice and to be essential for mammalian developmental processes[17]. Therefore, IRE1α inactivation results in widespread developmental defects, leading to embryonic death after 12.5 d of gestation in mice[18]. However, the cause of this embryonic lethality is not fully understood. These lines of evidence suggest that IRE1α has a unique function in mammalian developmental processes, but it has been hitherto unclear in which tissues and how IRE1α functions during embryogenesis.

Xenopus lavies is an excellent model system for studying organ development[19]. However, little information is available about the function of the IRE1/XBP1 pathway during embryogenesis, although loss of function studies revealed that IRE1 or XBP1 is absolutely required for embryonic development of C. elegans[20], Drosophila[21] or mouse[22]. In *Xenopus*, although the transcripts of two isoforms of IRE1, IRE1α and IRE1β show similar spatial expression in pre-neurula embryos, they are also differentially expressed following the onset of neurulation[23]. IRE1α is localized to the nervous system and mesoderm or endoderm-derived organs, such as pronephros and pancreas. However, the role of IRE1α in organogenesis is still unclear. In the present study, both gain and loss of function analyses revealed that IRE1α is required for gut development in *Xenopus*.

MATERIALS AND METHODS

Embryos

Wild type *Xenopus lavies* eggs were obtained by injecting 1000 IU of human chorionic gonadotrophin into the dorsal lymph sacs of adult females 6-8 h before egg collection. Eggs were fertilized *in vitro* with minced testes, dejellied with 2% cysteine hydrochloride (pH 7.8-8.0) 30 min after fertilization, and cultured in 0.1 × MBSH (8.8 mmol/L NaCl, 0.24 mmol/L $NaHCO_3$, 0.1 mmol/LKCl, 0.082 mmol/L $MgSO_4$, 0.041 mmol/L $CaCl_2$, 0.033 mmol/L $Ca(NO_3)_2$, 1 mmol/L HEPES, pH 7.4). Staging of *Xenopus lavies* embryos was according to Nieuwkoop and Faber (1967).

Plasmids and constructs

IRE1α ORF was amplified from a cDNA pool consisting of st.1, st.8, st.10, st.15, st.20 and st.28 cDNAs and subcloned to $pCS2^+$ vector. The construct was named $pCS2^+$-IRE1α. To make expression constructs, complete coding region and the N-terminal region (aa 1-479) were amplified from the $pCS2^+$-IRE1α using PCR and subcloned to the BamHI-EcoRV sites on $pCS2^+$-GR. The resulting constructs were designated as $pCS2^+$IRE1α-GR and $pCS2^+$IRE1αΔC-GR.

In vitro RNA synthesis, antisense morpholino oligonucleotides and microinjection

Plasmids $pCS2^+$IRE1α, $pCS2^+$IRE1α-GR and $pCS2^+$IRE1αΔC-GR were linearized with *Not*I. Capped mRNA for microinjection was synthesized with SP6 mMessage mMachine™ kit (Ambion) and cleaned up with RNeasy kit (Qiagen). The antisense morpholino

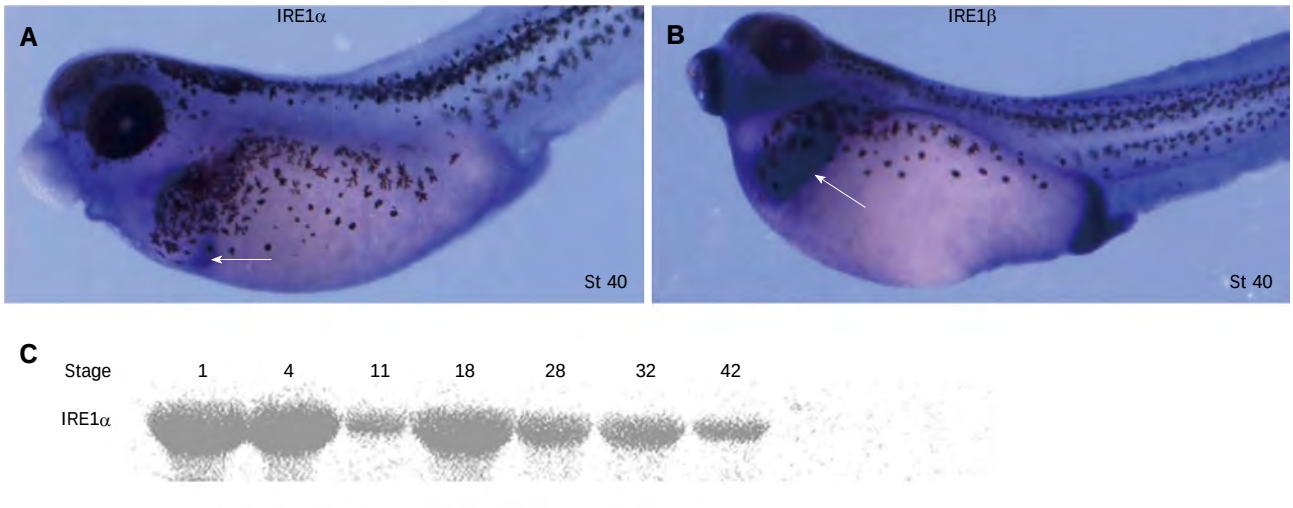


Figure 1 Expression pattern of xIRE1 in *Xenopus lavies* during development. A, B: Whole-mount *in situ* hybridizations revealed relatively high expression of inositol-requiring enzyme (IRE) 1α in pancreas (white arrow in A) and relatively high expression of IRE1β in liver (white arrow in B); C: Western blotting revealed temporal expression of IRE1α during *Xenopus* embryogenesis.

oligonucleotide (Gene Tools) used for IRE1α functional knockdown (IRE1α MO) was: 5'-AAGAGAACCGC-CAGAGGC GCCATG T-3'; and an antisense morpholino oligonucleotide XBP1(C) MO designed to inhibit the cytoplasmic splicing of xXBP1 was: 5'-GACATCT-GGGCCTGCTC CTGC TGCA-3'. One and half nanogram of IRE1α, 1 ng of IRE1α-GR mRNA, 1 ng of IRE1αΔC-GR mRNA, and 50 ng of IRE1α MO or XBP1(C)MO were injected into four blastomeres at 4-cell stage for scoring the phenotype, whole-mount *in situ* hybridization and marker gene analysis. For the activation of the GR-fusion proteins, dexamethasone (Sigma) was prepared as 5 mmol/L stock solutions in 100% ethanol and applied to the control and mRNA injected embryos at desired stages in a concentration of 10 μmol/L in 0.1 × MBS. Embryos were kept in dexamethasone up to stage 41. To rescue the effect of blocking xXBP1 splicing by IRE1α MO, 1 ng of IRE1α-GR mRNA was co-injected with 50 ng of MO.

In vitro translation

In vitro protein translation was performed with TNT coupled Reticulocyte Lysate Systems (Promega) to test the efficiency of IRE1α MO for blocking protein translation. One μg of pCS2$^+$IRE1α plasmid was used either alone or together with 20 μg IRE1α MO for *in vitro* translation. Translation products were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and chemiluminescent detection.

Whole-mount in situ hybridization

Whole-mount *in situ* hybridization was performed according to the protocol described elsewhere[24]. The digoxigenin-labeled antisense probes were prepared as follows: pDrive-IRE1α cut with *Hin*dⅢ, transcribed with T7 RNA polymerase; pCS2$^+$XIRE1β cut with *Xba*I, transcribed with T3 RNA polymerase; pBS-Xbra cut with *Sal*I, transcribed with T7 RNA polymerase; and pCS2$^+$XSox17α cut with *Cla*I, transcribed with T7 RNA polymerase.

Protein extraction and Western blotting

Embryos were homogenized in RIPA lysis buffer (20 mmol/L Tris, pH 8; 2 mmol/L EDTA, pH 8; 0.5% NP-40; 25 mmol/L β glycerophosphate; 100 mmol/L NaF; 100 mmol/L PMSF and phosphatase inhibitor cocktail). Lysates were centrifuged at 4 ℃, 15 000 × *g*, for 20 min, and the supernatants were added to 5 × SDS loading buffer. Proteins were separated by 10% SDS-PAGE and transferred to nitrocellulose membrane (Millipore, Billerica, MA). Immunoblotting membranes were blocked with 5% milk in TBST. After several washes in TBST, membranes were incubated overnight with the first antibody [anti-IRE1α antibody (Santa Cruz Biotechnology, Santa Cruz, CA), 1:500]. Loading controls of presumably constantly expressed proteins such as β-actin were used; however, their variability and increase in development precluded their use[25]. Detection was then done with HRP-labeled secondary antibodies and enhanced chemiluminescence (ECL).

RESULTS

Expression of xIRE1α in Xenopus lavies during development

Two forms of IRE1 genes, IRE1α and IRE1β, exist in *Xenopus lavies*. Whole-mount *in situ* hybridization revealed that IRE1α and IRE1β were expressed in a similar pattern from egg to gastrulation. In tailbud embryos, IRE1α was detected in a domain that probably represented the dorsal pancreas anlage. IRE1β was only observed in the hatching gland and cement gland until the hatched tadpole stage[23]. To further explore the spatial expression patterns of IRE1α and IRE1β in *Xenopus* embryos at later stages, whole-mount *in situ* hybridizations were carried out. During tadpole stages, relatively high expression of IRE1α was observed in the pancreas (Figure 1A), and significant transcription of IRE1β was observed in the

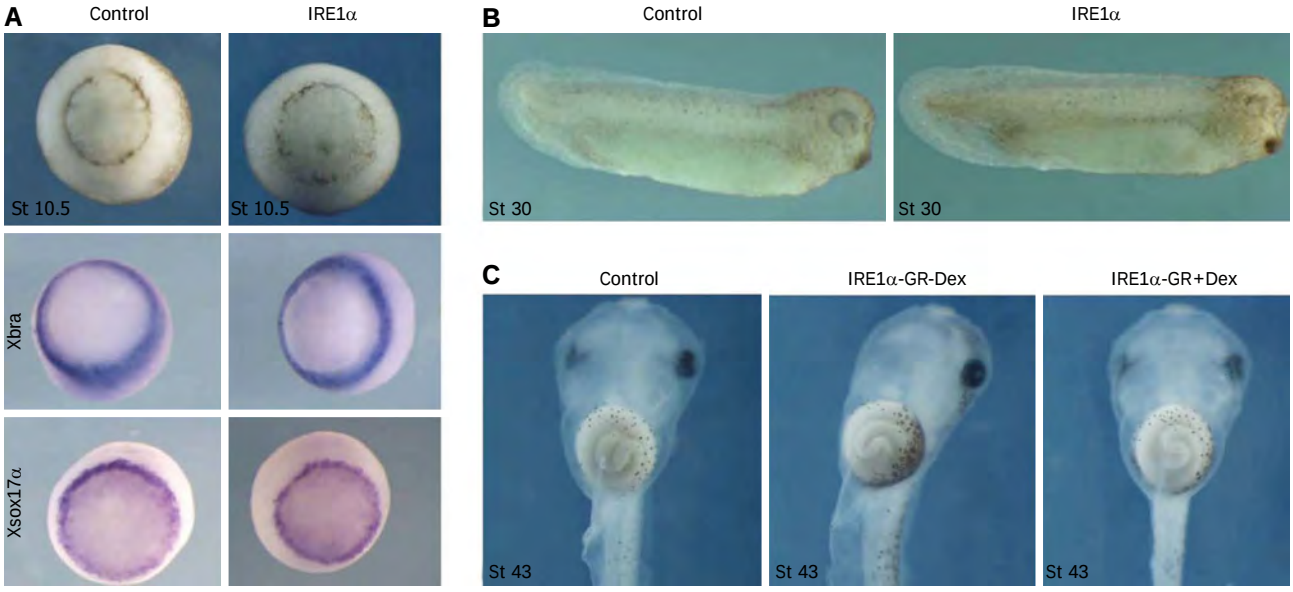


Figure 2 Overexpression of inositol-requiring enzyme 1α affected neither mesoderm and endoderm formation nor gut development. A: Overexpression of inositol-requiring enzyme (IRE) 1α did not change the phenotype and the expression of Xbra and Xsox17α at stage 10.5; B: IRE1α mRNA injected embryos at tailbud stage were nearly normal; C: Embryos injected with IRE1α-GR mRNA did not display any defect until tadpole stage.



Figure 3 Inositol-requiring enzyme 1α morpholino oligonucleotide blocks inositol-requiring enzyme 1α translation in an *in vitro* transcription/translation assay. In this assay, xIRE1α was effectively transcribed/translated from a pCS2+xIRE1α construct. However, translation was dramatically decreased by addition of IRE1α morpholino oligonucleotide (MO). IRE: Inositol-requiring enzyme.

liver (Figure 1B). IRE1α protein could be detected at all developmental stages analyzed (Figure 1C).

IRE1α gain of function

Different doses of IRE1α mRNA were injected into all blastomeres at 4-cell stage. Even at a dose of 1.5 ng, injected embryos at tailbud stage were nearly normal (Figure 2B). Whole-mount *in situ* hybridization of embryos injected with IRE1α mRNA revealed that the expression of the pan-mesodermal marker gene Xbra and the endodermal gene Xsox17α at stage 10.5 was not significantly changed as compared to uninjected control embryos (Figure 2A). To test the function of IRE1α in the later stage of development, the mRNA encoding a dexamethasone inducible variant of IRE1α, referred to as IRE1α-GR, was injected into all four blastomeres at 4-cell stage *Xenopus* embryos. For the activation of the GR-fusion proteins, dexamethasone treatment was administered at embryonic stage 27. No apparent change of phenotype was observed even at tadpole stage (Figure 2C). Therefore, gain of function of IRE1α did not lead to an apparent change of phenotype and we did not observe a significant change of mesodermal and endodermal marker gene expression.

IRE1α MO blocking IRE1α translation

To further explore the function of xIRE1α during embryonic development, we performed a loss of function (LOF) analysis using an antisense morpholino oligonucleotide (IRE1α MO) directed against xIRE1α. *In vitro* protein translation was performed with TNT coupled Reticulocyte Lysate Systems to test the efficiency of IRE1α MO for blocking protein translation. As shown in Figure 3, IRE1α MO totally blocked IRE1α translation.

IRE1α loss of function

The IRE1α MO-injected embryos were morphologically normal before the tailbud stages (Figure 4B) and we did not observe a significant change of mesodermal and endodermal marker gene expression (Figure 4A), but after stage 40, about 80% of the MO (50 ng)-injected embryos exhibited dramatic gut defects in which the guts did not coil, while other structures outside the gastrointestinal tract developed normally (Figure 4). To test if the phenotypes were specifically caused by the knockdown of IRE1α, a rescue experiment was performed by co-injection of IRE1α-GR mRMA with MO. The results indicated that the phenotype could be rescued (Figure 5). Taken together, these data indicate that IRE1α is re-

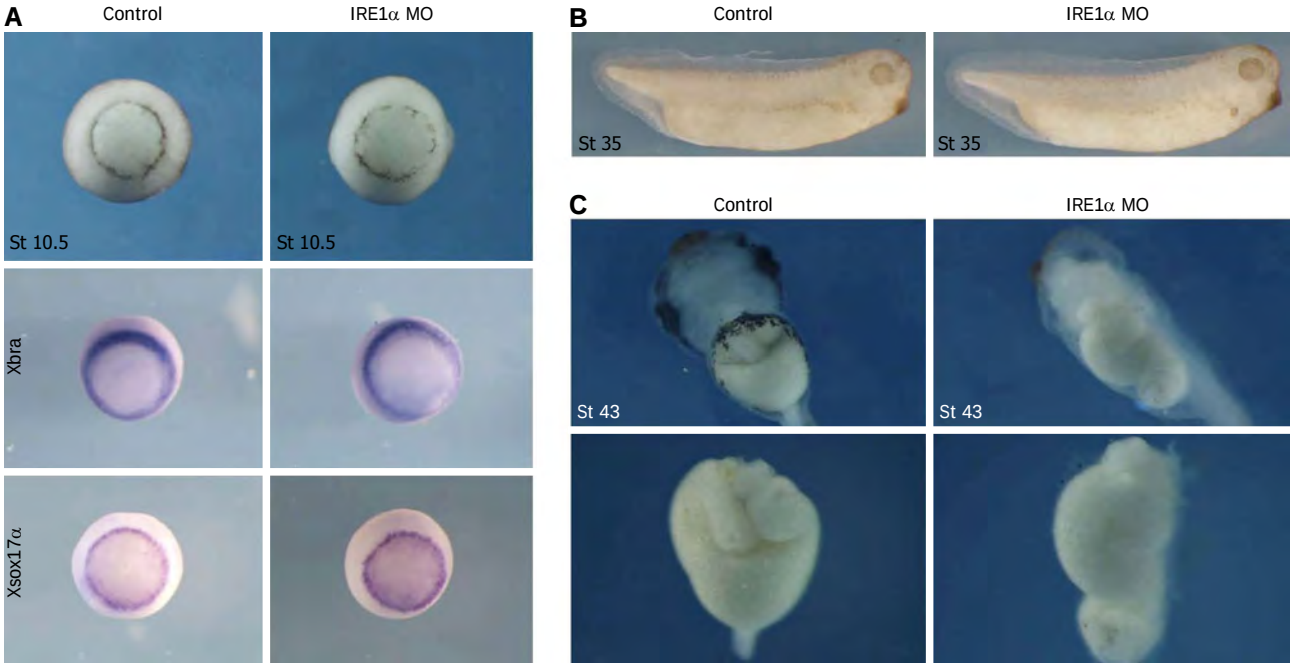


Figure 4 ***Xenopus*** **inositol-requiring enzyme 1α is required for gut development.** A: Knockdown of x IRE1α did not change the phenotype and the expression of Xbra and Xsox17α at stage 10.5; B: The inositol-requiring enzyme (IRE) 1α morpholino oligonucleotide (MO)-injected embryos were morphologically normal before the tailbud stages; C: IRE1α knockdown upon injection of 50 ng of MO resulted in a gut defective phenotype. Surgically-resected guts from embryos were shown in C under panel. Coiled structure of gut was not detected in IRE1α MO-injected embryos at stage 43. MO injection was repeated 5 times in a total of 278 embryos.



Figure 5 **Rescue of xIRE1α knockdown with IRE1α-GR mRNA.** Gut defective phenotype caused by 50 ng of morpholino oligonucleotide (MO) could be rescued by co-injection of 1 ng of inositol-requiring enzyme (IRE) 1α-GR mRNA. Co-injection was done 3 times in a total of 258 embryos.

quired for proper gut development in *Xenopus*.

Deletion mutant of IRE1α

IRE1α is a Ser/Thr protein kinase and endoribonuclease that, upon activation, initiates the unconventional splicing of the mRNA of XBP1[12]. The deletion mutant was constructed consisting of the N-terminal part without the C-terminal kinase and RNase domains (Figure 6A). Injection of IRE1αΔC-GR mRNA caused similar morphological alterations with gut malformation by interfering with the function of endogenous xIRE1α (Figure 6B-D). These data further confirmed the notion that IRE1αΔC represents a dominant-negative form of IRE1α.

XBP1 loss of function

IRE1α is the most evolutionarily conserved branch of the UPR, and upon activation, initiates the unconventional splicing of the mRNA encoding the transcriptional factor XBP1 to attenuate ER stress by mediating UPR. In other words, IRE1α mediates XBP1 splicing. IRE1α MO could knockdown IRE1α expression, and then XBP1 splicing would be repressed. To test if knockdown of XBP1 might cause similar phenotype with IRE1α knockdown, 50 ng of XBP1(C)MO was injected into four blastomeres at 4-cell stage for scoring the phenotype. As shown in Figure 7, injection of XBP1(C)MO caused similar phenotype with gut-coiling defect.

DISCUSSION

In eukaryotic cells, ER is responsible for the early steps in the maturation of most proteins in the secretory pathway, such as folding of the newly synthesized polypeptide chains and post-translational modifications that are essential for protein function[26,27]. Nascent polypeptides are translocated to the ER lumen in an unfolded state, where

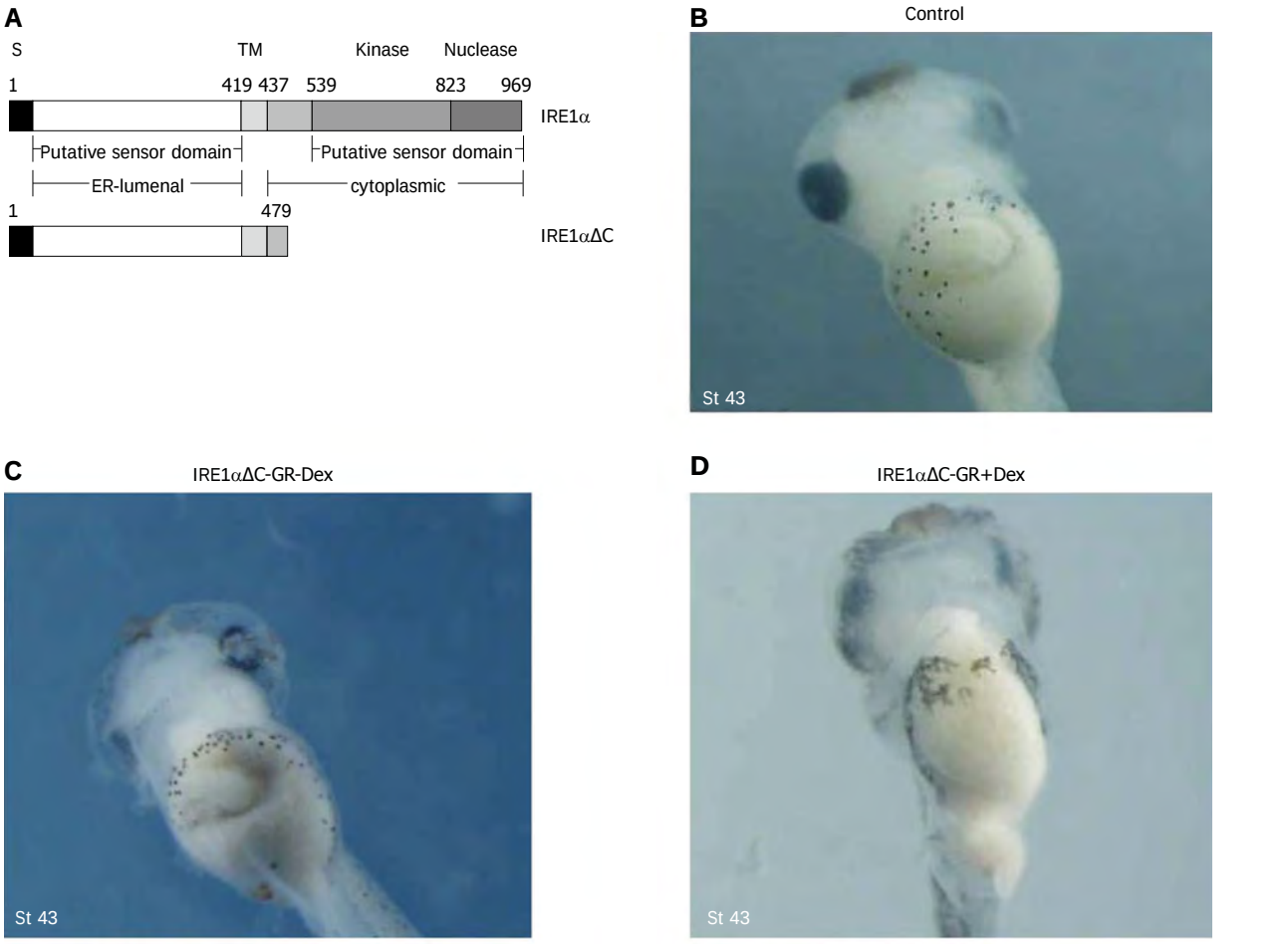


Figure 6 Injection of IRE1α**ΔC-GR mRNA caused morphological alterations with gut malformation.** A: Diagram depicting the construction of deletion mutants. S and TM denote the signal peptide and the transmembrane domain; B-D: Gut defects were also observed with a dominant-negative mutant, IRE1αΔC, lacking the cytoplasmic kinase and RNase domains.



Figure 7 XBP1 knockdown affected gut development. Injection of XBP1(C) morpholino oligonucleotide (MO) caused similar phenotype with gut-coiling defect at stage 43.

they are processed for folding. However, the function of ER will be disrupted when the inflow of unfolded polypeptide chains exceeds the folding or processing capacity of the ER[28,29]. This ER stress in turn leads to the activation of a series of adaptive pathways known as UPR to maintain ER homeostasis[30]. The adaptive process, UPR, has at least two distinct components[4]. The first consists of the rapid and transient attenuation of new protein synthesis and can be considered to be an attempt on the part of the cell to limit the load on the folding apparatus in the ER. The second component consists of the upregulation of expression of genes whose products promote protein folding in the ER and degradation of malfolded proteins. The latter UPR signaling pathways are transduced by three ER resident transmembrane proteins IRE1, PERK and ATF6 upon activation[14], among which IRE1 functions as an endoribonuclease (RNase) to process XBP1 pre-mRNA to a mature form. Spliced XBP1 is a transcriptional activator that plays a fundamental role in the activation of a wide variety of UPR target genes[31,32]. Two mammalian homologues of yeast IRE1 have been identified: IRE1α and IRE1β. IRE1α is expressed in most cells and tissues, with highest levels of expression in the pancreas and placenta[17]. IRE1β expression is prominent only in intestinal epithelial cells[13].

Accumulation of malfolded proteins in the ER occurs under many pathophysiological conditions, in addition, it also takes place during embryonic development. During embryogenesis of vertebrates, such as *Xenopus*, germ layer induction, pattern formation, and morphoge-

netic movement are known to be mediated by secreted proteins, including fibroblast growth factors (FGFs)[33], TGF-β/nodal/BMPs, and Wnts[34,35]. Therefore, dysfunction of the ER should also interfere with the secretion of these proteins and consequently disrupt early embryonic development[36].

Although the effect of ER stress on cellular physiology has been extensively investigated, little is known so far about how it affects early embryonic development. The previous study has verified the conservation of IRE1/XBP1 pathway in *Xenopus* embryos and the importance of IRE1β for mesoderm formation in *Xenopus* embryos[23]. In this study, we demonstrated that (1) IRE1α mainly expressed in pancreas at tadpole stages and IRE1α protein could be detected at all developmental stages analyzed (Figure 1); (2) although it did not cause overt phenotypes upon overexpression (Figure 2), specific knockdown of IRE1α (Figure 4) or XBP1 (Figure 7) led to a gut-coiling defect, and injection of xIRE1αΔC-GR mRNA caused similar morphological alterations with gut malformation (Figure 6); and (3) IRE1α-GR mRNA can rescue IRE1 knockdown phenotypes (Figure 5).

In conclusion, our loss and gain of function data support the notion that IRE1α is required for gut development in *Xenopus* embryos. Our results demonstrate that homeostasis of ER and xIRE1α functions are required for gut development in *Xenopus* embryos. We infer from our results that IRE1 regulates gut development through IRE1α-XBP1 pathway.

COMMENTS

Background

Inositol-requiring enzyme 1α (IRE1α) is an endoplasmic reticulum (ER)-located type I transmembrane protein with a kinase domain and RNase domain in the cytosolic region. IRE1α induces the unconventional splicing of XBP1 mRNA under ER stress condition. However, a XBP1-independent IRE1α function also exists. IRE1α is expressed ubiquitously in fetal and adult mice and is essential for mammalian developmental processes. However, the function of IRE1α in specific organs and tissues remains incompletely understood.

Research frontiers

IRE1α is confirmed to be essential during mammalian development. However, the IRE1α conventional knockout mice showed embryonic lethality, and it has been reported that during development IRE1α is required for B-cell differentiation, placental development and embryonic viability. And the function of IRE1α in specific organs and tissues deserves to be illustrated.

Innovations and breakthroughs

Compared with previous studies, this study used the *Xenopus lavies* as animal model to study the function of IRE1α during their development. After overexpressing and knockdown of the IRE1α, the phenotype could be analyzed. And the results showed that at early stage, IRE1α did not play a significant role in germ layer formation, however, at the stage of organogenesis, knockdown of IRE1α or XBP1 caused gut-coiling defect. The results suggested that IRE1α is not required for germ layer formation, but for gut development in *Xenopus lavies* and it may function *via* XBP1-dependent pathway.

Applications

The results in this study showed that IRE1α does play a role in organogenesis, however, two questions remain to be answered: which organs or tissues in gut are affected and what is the underlying mechanism.

Peer review

This is an interesting article. The authors show in a systematic manner that the role of IRE1a in the gut development of *Xenopus*. They also show that it acts through the XBP1 processing.

REFERENCES

1 **Malhotra JD**, Kaufman RJ. The endoplasmic reticulum and the unfolded protein response. *Semin Cell Dev Biol* 2007; **18**: 716-731 [PMID: 18023214 DOI: 10.1016/j.semcdb.2007.09.003]

2 **Anelli T**, Sitia R. Protein quality control in the early secretory pathway. *EMBO J* 2008; **27**: 315-327 [PMID: 18216874 DOI: 10.1038/sj.emboj.7601974]

3 **Schröder M**, Kaufman RJ. The mammalian unfolded protein response. *Annu Rev Biochem* 2005; **74**: 739-789 [PMID: 15952902 DOI: 10.1146/annurev.biochem.73.011303.074134]

4 **Credle JJ**, Finer-Moore JS, Papa FR, Stroud RM, Walter P. On the mechanism of sensing unfolded protein in the endoplasmic reticulum. *Proc Natl Acad Sci USA* 2005; **102**: 18773-18784 [PMID: 16365312 DOI: 10.1073/pnas.0509487102]

5 **Xu C**, Bailly-Maitre B, Reed JC. Endoplasmic reticulum stress: cell life and death decisions. *J Clin Invest* 2005; **115**: 2656-2664 [PMID: 16200199 DOI: 10.1172/JCI26373]

6 **Wu J**, Kaufman RJ. From acute ER stress to physiological roles of the Unfolded Protein Response. *Cell Death Differ* 2006; **13**: 374-384 [PMID: 16397578 DOI: 10.1038/sj.cdd.4401840]

7 **Boyce M**, Yuan J. Cellular response to endoplasmic reticulum stress: a matter of life or death. *Cell Death Differ* 2006; **13**: 363-373 [PMID: 16397583 DOI: 10.1038/sj.cdd.4401817]

8 **Kimata Y**, Ishiwata-Kimata Y, Ito T, Hirata A, Suzuki T, Oikawa D, Takeuchi M, Kohno K. Two regulatory steps of ER-stress sensor Ire1 involving its cluster formation and interaction with unfolded proteins. *J Cell Biol* 2007; **179**: 75-86 [PMID: 17923530 DOI: 10.1083/jcb.200704166]

9 **Oikawa D**, Kimata Y, Kohno K, Iwawaki T. Activation of mammalian IRE1alpha upon ER stress depends on dissociation of BiP rather than on direct interaction with unfolded proteins. *Exp Cell Res* 2009; **315**: 2496-2504 [PMID: 19538957 DOI: 10.1016/j.yexcr.2009.06.009]

10 **Calfon M**, Zeng H, Urano F, Till JH, Hubbard SR, Harding HP, Clark SG, Ron D. IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature* 2002; **415**: 92-96 [PMID: 11780124 DOI: 10.1038/415092a]

11 **Shen X**, Zhang K, Kaufman RJ. The unfolded protein response--a stress signaling pathway of the endoplasmic reticulum. *J Chem Neuroanat* 2004; **28**: 79-92 [PMID: 15363493 DOI: 10.1016/j.jchemneu.2004.02.006]

12 **Liu CY**, Kaufman RJ. The unfolded protein response. *J Cell Sci* 2003; **116**: 1861-1862 [PMID: 12692187 DOI: 10.1242/jcs.00408]

13 **Wang XZ**, Harding HP, Zhang Y, Jolicoeur EM, Kuroda M, Ron D. Cloning of mammalian Ire1 reveals diversity in the ER stress responses. *EMBO J* 1998; **17**: 5708-5717 [PMID: 9755171 DOI: 10.1093/emboj/17.19.5708]

14 **Yoshida H**, Matsui T, Yamamoto A, Okada T, Mori K. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* 2001; **107**: 881-891 [PMID: 11779464 DOI: 10.1016/S0092-8674(01)00611-0]

15 **Oikawa D**, Tokuda M, Hosoda A, Iwawaki T. Identification of a consensus element recognized and cleaved by IRE1 alpha. *Nucleic Acids Res* 2010; **38**: 6265-6273 [PMID: 20507909 DOI: 10.1093/nar/gkq452]

16 **Urano F**, Wang X, Bertolotti A, Zhang Y, Chung P, Harding HP, Ron D. Coupling of stress in the ER to activation of JNK protein kinases by transmembrane protein kinase IRE1. *Science* 2000; **287**: 664-666 [PMID: 10650002 DOI: 10.1126/science.287.5453.664]

17 **Tirasophon W**, Welihinda AA, Kaufman RJ. A stress response pathway from the endoplasmic reticulum to the nucleus requires a novel bifunctional protein kinase/endoribonuclease (Ire1p) in mammalian cells. *Genes Dev* 1998; **12**: 1812-1824 [PMID: 9637683 DOI: 10.1101/gad.12.12.1812]

18 **Zhang K**, Wong HN, Song B, Miller CN, Scheuner D, Kaufman RJ. The unfolded protein response sensor IRE-1alpha is required at 2 distinct steps in B cell lymphopoiesis. *J Clin Invest* 2005; **115**: 268-281 [PMID: 15690081 DOI: 10.1172/JCI200521848]

19 **Kashiwagi K**, Kashiwagi A, Kurabayashi A, Hanada H, Nakajima K, Okada M, Takase M, Yaoita Y. Xenopus tropicalis: an ideal experimental animal in amphibia. *Exp Anim* 2010; **59**: 395-405 [PMID: 20660986 DOI: 10.1538/expanim.59.395]

20 **Shen X**, Ellis RE, Lee K, Liu CY, Yang K, Solomon A, Yoshida H, Morimoto R, Kurnit DM, Mori K, Kaufman RJ. Complementary signaling pathways regulate the unfolded protein response and are required for C. elegans development. *Cell* 2001; **107**: 893-903 [PMID: 11779465 DOI: 10.1016/S0092-8674(01)00612-2]

21 **Souid S**, Lepesant JA, Yanicostas C. The xbp-1 gene is essential for development in Drosophila. *Dev Genes Evol* 2007; **217**: 159-167 [PMID: 17206451 DOI: 10.1007/s00427-006-0124-1]

22 **Reimold AM**, Etkin A, Clauss I, Perkins A, Friend DS, Zhang J, Horton HF, Scott A, Orkin SH, Byrne MC, Grusby MJ, Glimcher LH. An essential role in liver development for transcription factor XBP-1. *Genes Dev* 2000; **14**: 152-157 [PMID: 10652269 DOI: 10.1101/gad.14.2.152]

23 **Yuan L**, Cao Y, Oswald F, Knöchel W. IRE1beta is required for mesoderm formation in Xenopus embryos. *Mech Dev* 2008; **125**: 207-222 [PMID: 18191552 DOI: 10.1016/j.mod.2007.11.010]

24 **Gawantka V**, Pollet N, Delius H, Vingron M, Pfister R, Nitsch R, Blumenstock C, Niehrs C. Gene expression screening in Xenopus identifies molecular pathways, predicts gene function and provides a global view of embryonic patterning. *Mech Dev* 1998; **77**: 95-141 [PMID: 9831640 DOI: 10.1016/S0925-4773(98)00115-4]

25 **Yashpal NK**, Li J, Wheeler MB, Wang R. Expression of {beta}1 integrin receptors during rat pancreas development--sites and dynamics. *Endocrinology* 2005; **146**: 1798-1807 [PMID: 15618357 DOI: 10.1210/en.2004-1292]

26 **Novosyadlyy R**, Kurshan N, Lann D, Vijayakumar A, Yakar S, LeRoith D. Insulin-like growth factor-I protects cells from ER stress-induced apoptosis via enhancement of the adaptive capacity of endoplasmic reticulum. *Cell Death Differ* 2008; **15**: 1304-1317 [PMID: 18437163 DOI: 10.1038/cdd.2008.52]

27 **Merquiol E**, Uzi D, Mueller T, Goldenberg D, Nahmias Y, Xavier RJ, Tirosh B, Shibolet O. HCV causes chronic endoplasmic reticulum stress leading to adaptation and interference with the unfolded protein response. *PLoS One* 2011; **6**: e24660 [PMID: 21949742 DOI: 10.1371/journal.pone.0024660]

28 **Back SH**, Lee K, Vink E, Kaufman RJ. Cytoplasmic IRE1alpha-mediated XBP1 mRNA splicing in the absence of nuclear processing and endoplasmic reticulum stress. *J Biol Chem* 2006; **281**: 18691-18706 [PMID: 16644724 DOI: 10.1074/jbc.M602030200]

29 **Lin JH**, Li H, Yasumura D, Cohen HR, Zhang C, Panning B, Shokat KM, Lavail MM, Walter P. IRE1 signaling affects cell fate during the unfolded protein response. *Science* 2007; **318**: 944-949 [PMID: 17991856 DOI: 10.1126/science.1146361]

30 **Yuan L**, Cao Y, Knöchel W. Endoplasmic reticulum stress induced by tunicamycin disables germ layer formation in Xenopus laevis embryos. *Dev Dyn* 2007; **236**: 2844-2851 [PMID: 17849439 DOI: 10.1002/dvdy.21299]

31 **Iwawaki T**, Akai R, Yamanaka S, Kohno K. Function of IRE1 alpha in the placenta is essential for placental development and embryonic viability. *Proc Natl Acad Sci USA* 2009; **106**: 16657-16662 [PMID: 19805353 DOI: 10.1073/pnas.0903775106]

32 **Back SH**, Schröder M, Lee K, Zhang K, Kaufman RJ. ER stress signaling by regulated splicing: IRE1/HAC1/XBP1. *Methods* 2005; **35**: 395-416 [PMID: 15804613 DOI: 10.1016/j.ymeth.2005.03.001]

33 **Böttcher RT**, Niehrs C. Fibroblast growth factor signaling during early vertebrate development. *Endocr Rev* 2005; **26**: 63-77 [PMID: 15689573 DOI: 10.1210/er.2003-0040]

34 **Clevers H**. Wnt/beta-catenin signaling in development and disease. *Cell* 2006; **127**: 469-480 [PMID: 17081971 DOI: 10.1016/j.cell.2006.10.018]

35 **Kimelman D**. Mesoderm induction: from caps to chips. *Nat Rev Genet* 2006; **7**: 360-372 [PMID: 16619051 DOI: 10.1038/nrg1837]

36 **Kaufman RJ**. Orchestrating the unfolded protein response in health and disease. *J Clin Invest* 2002; **110**: 1389-1398 [PMID: 12438434 DOI: 10.1172/JCI0216886]

P- Reviewer Venugopal SK **S- Editor** Song XX
L- Editor A **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i2.235

World J Gastroenterol 2013 January 14; 19(2): 235-240
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Partially hydrolyzed guar gum in pediatric functional abdominal pain

Claudio Romano, Donatella Comito, Annalisa Famiani, Sabrina Calamarà, Italia Loddo

Claudio Romano, Donatella Comito, Pediatric Department, University of Messina, Messina 98100, Italy
Annalisa Famiani, Sabrina Calamarà, Italia Loddo, Pediatric Department, University of Messina, Messina 98100, Italy
Author contributions: Romano C and Comito D conducted the trial and recruited the cases; Famiani A, Calamarà S and Loddo I were involved in the elaboration of the data.
Correspondence to: Claudio Romano, Medical Doctor, PhD, Chief of Endosopy and Gastroenterology Unit, Pediatric Department, University of Messina, Messina 98100, Italy. romanoc@unime.it
Telephone: +39-90-2212918 Fax: +39-90-2217005
Received: August 13, 2012 Revised: November 2, 2012
Accepted: November 11, 2012
Published online: January 14, 2013

Abstract

AIM: To assess the effects of partially hydrolyzed guar gum (PHGG) diet supplement in pediatric chronic abdominal pain (CAP) and irritable bowel syndrome (IBS).

METHODS: A randomized, double-blind pilot study was performed in sixty children (8-16 years) with functional bowel disorders, such as CAP or IBS, diagnosed according to Rome Ⅲ criteria. All patients underwent ultrasound, blood and stool examinations to rule out any organic disease. Patients were allocated to receive PHGG at dosage of 5 g/d (n = 30) or placebo (fruit-juice n = 30) for 4 wk. The evaluation of the efficacy of fiber supplement included IBS symptom severity score (Birmingham IBS Questionnaire), severity of abdominal pain (Wong-Baker Face Pain Rating Score) and bowel habit (Bristol Stool Scale). Symptom scores were completed at 2, 4, and 8 wk. The change from baseline in the symptom severity scale at the end of treatment and at 4 wk follow-up after treatment was the primary endpoint. The secondary endpoint was to evaluate compliance to supplementation with the PHGG in the pediatric population. Differences within groups during the treatment period and follow-up were evaluated by the Wilcoxon signed-rank test.

RESULTS: The results of the study were assessed considering some variables, such as frequency and intensity of symptoms with modifications of the bowel habit**.** Both groups were balanced for baseline characteristics and all patients completed the study. Group A (PHGG group) presented a higher level of efficacy compared to group B (control group), (43% *vs* 5%, P = 0.025) in reducing clinical symptoms with modification of Birmingham IBS score (median 0 ± 1 *vs* 4 ± 1, P = 0.025), in intensity of CAP assessed with the Wong-Baker Face Pain Rating Score and in normalization of bowel habit evaluated with the Bristol Stool Scale (40% *vs* 13.3%, P = 0.025). In IBS subgroups, statistical analysis shown a tendency toward normalization of bowel movements, but there was no difference in the prevalence of improvement in two bowel habit subsets. PHGG was therefore better tolerated without any adverse effects.

CONCLUSION: Although the cause of pediatric functional gastrointestinal disorders is not known, the results show that complementary therapy with PHGG may have beneficial effects on symptom control.

© 2013 Baishideng. All rights reserved.

Key words: Functional bowel disorders; Partially hydrolyzed guar gum; Pediatric chronic abdominal pain; Fiber diet

Romano C, Comito D, Famiani A, Calamarà S, Loddo I. Partially hydrolyzed guar gum in pediatric functional abdominal pain. *World J Gastroenterol* 2013; 19(2): 235-240 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i2/235.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i2.235

INTRODUCTION

Functional gastrointestinal disorders (FGIDs) are defined as a variable combination of chronic or recurrent gastrointestinal (GI) symptoms; they are age dependent and not explained by structural or biochemical abnormalities[1]. Chronic abdominal pain (CAP) is the most common condition in FGIDs. It is usually functional without objective evidence of an underlying organic disorder. Apley *et al*[2] introduced the term recurrent abdominal pain (RAP) for the first time in pediatric literature, using it to describe a condition whereby children have experienced at least 3 bouts of pain, severe enough to affect activities, over a period of at least 3 mo. In 1999, the Pediatric Rome Working Group introduced standardized symptom-based criteria for pediatric FGIDs with the publication of the Rome Ⅱ criteria[3]. In 2006, the "Rome Committee"[4] defined new diagnostic criteria (Roma Ⅲ) for pediatric FGIDs, differentiating functional abdominal pain (FAP) from dyspepsia and irritable bowel syndrome (IBS) in that the pain is at a different site with normal bowel habits. The exact prevalence of CAP in children is not known. It seems to account for 2%-4% of all pediatric office visits[5]. Several studies suggested that 13% of middle-school students and 17% of high school students have weekly experience of abdominal pain[6,7]. According to these criteria, IBS is an FGID characterized by abdominal pain or discomfort, accompanied by altered bowel habits (constipation, IBS-c or diarrhea, IBS-d or alternating)[8]. It has been shown that some factors, especially psychological factors, dietary habits, and frequency of exercise, are associated with onset and course of IBS[9-11]. The term "functional" is not real but conceptual and the pathogenesis can be correlated with alterations of visceral sensitivity, increased intestinal permeability, chronic inflammation and presence of a genetic predisposition[12]. Pharmacotherapy generally cannot be recommended for children with FAP, except in the context of clinical trials. Drugs should only be given in exceptional cases[13,14]. Up to 40% of children undergo alternative or complementary therapy such as reassurance, phytotherapy, dietary restrictions or homeopathy[15]. The putative benefit of such methods has not been documented by controlled clinical trials.

The beneficial effects of water-soluble dietary fibers have received attention as complementary therapy in FGIDs, especially in FAP and IBS, for their ability to modify bowel pattern, accelerate oral-to-anal transit and decrease intracolonic pressure and alleviate pain[16,17]. Partially hydrolyzed guar gum (PHGG) is a vegetal, water-soluble, non-viscous, non-gelling dietary fiber that is derived from guar gum, a water-soluble, viscous, gelling polysaccharide found in the seeds of the guar plant. The saccharide component of guar gum is galactomannan[18]. Parisi *et al*[19] showed, in an adult open trial, that PHGG supplementation is followed by a decrease of IBS symptoms, such as abdominal pain and bowel habit. Feldman *et al*[20], in a small, prospective, randomized, double-blind, controlled trial, have revealed that fiber supplementation can improve symptoms in children with FAP. Despite this, there have been no recently available published randomized controlled trials (RCTs) to support the use of fiber in the treatment of CAP in a pediatric population.

The aim of this study was to assess the effect of PHGG diet supplement on CAP and IBS symptoms in paediatric population.

MATERIALS AND METHODS

Sixty patients were prospectively enrolled in the study and randomly assigned to one of 2 study arms (PHGG group or group A: 30 patients; placebo group or group B: 30 patients). Median age was 12.8 years (range 8-16 years) with a greater predominance of females (62% girls and 38% boys). CAP and IBS patients were defined according to the Rome Ⅲ criteria.

All patients were identified into two subgroups: 21/30 (70%) and 19/30 (63%) with constipation-predominant IBS in group A and B respectively; 9/30 (30%) and 11/30 (37%) with diarrhoea-predominant IBS in group A and B respectively. At baseline, the two groups were not statistically different, with respect to age, sex, alterations in bowel movements, incidence and intensity of self-reported symptoms. Subjects' overall baseline demographic and clinical characteristics are summarized in Table 1. All patients underwent ultrasound, blood and stool examinations to exclude organic disease. Seven days before joining the study, patients were asked to not use any medication. The study was performed in accordance with the Declaration of Helsinki and was approved by the local ethics committee. Informed consent was obtained for each patient. All patients completed the trial without any dropouts. Figure 1 is a flow diagram showing the subjects' progression through the study. Patients were consecutively recruited from November 2010 to May 2011, in the Pediatric Gastroenterology Unit of the University of Messina, and randomly assigned to two groups (1:1, PHGG group or group A: 30 patients; placebo group or group B: 30 patients) to receive either a beverage of PHGG (Benefibra, Novartis Consumer Health) at a dosage of 5 g/d in 50 mL of fruit-juice (n = 30) or matching placebo (fruit-juice, n = 30) for 4 wk. For technical reasons of non-laboratory reproducibility of an inert and odorless powder, the placebo consisted of a fruit juice. As in other studies, PHGG was mixed with fruit juice during meals or between meals.

The manufacturer had no role in the conception, design or conduct of the study or in the analysis or interpretation of the data. Randomization was based on a computer-generated list. Supplementation was stopped after 4 wk and patients were followed up for a further 4 wk. GI symptoms were assessed with the "Birmingham IBS Symptom Questionnaire", "Wong Baker Faces Pain Rating Score" and "Bristol Stool Scale". The Birmingham IBS symptoms score consists of 11 questions based

Table 1 Baseline demographic and clinical data

	PHGG group A	Placebo group B	*P* value
n	30	30	
Age (yr)	12.3 ± 2.0	13.1 ± 1.5	0.16
Sex (male/female)	12/18	11/19	0.88
Self-reported pain	3.7 ± 1.2	3.5 ± 1.5	0.15
c-IBS	21/30 (70%)	19/30 (63%)	0.75
d-IBS	9/30 (30%)	11/30 (37%)	0.64

Values are mean ± SD or *n* (%). PHGG: Partially hydrolyzed guar gum; IBS: Irritable bowel syndrome.

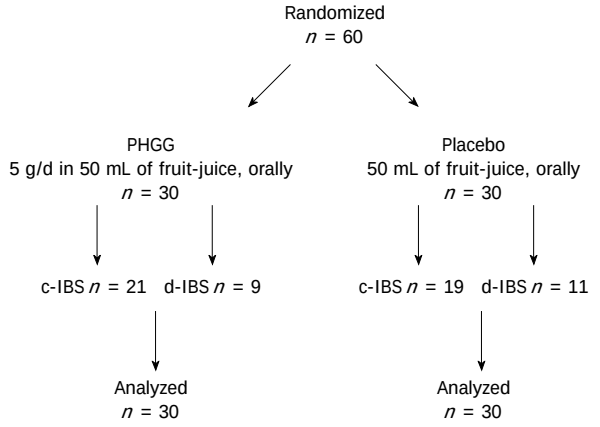


Figure 1 Flow diagram of the progress through the study. PHGG: Partially hydrolyzed guar gum; IBS: Irritable bowel syndrome.

on the frequency of IBS related symptoms. Each question had a standard response scale with symptoms all being measured on a 6-point Likert scale ranging from 0 = none of the time, to 5 = all of the time (Table 2). Wong-Baker Faces Pain Rating Score was used to evaluate CAP severity with a variable score from 0 = no hurt, to 5 = hurts worst. The Bristol Stool Scale classifies the form and consistency of stools into 7 categories (from separate hard lump to entirely liquid stools).

All patients were assessed clinically at 2, 4, and 8 wk (T1, T2, T3) by means of a physical examination and the scoring systems from baseline (Table 3). At T2, compliance with treatment (worse, unchanged, better) was also assessed. Adverse events or any use of other drugs were recorded. Primary outcome was reduction in the frequency and intensity of clinical symptoms and correlation with the improvement of character of stool. Secondary outcome was the evaluation of compliance and safety of PHGG in children.

Statistical analysis

Data are given as mean ± SD. Differences between groups were evaluated by the Kruskall-Wallis one-way analysis of variance (ANOVA). Nominal variables were analyzed with Pearson's chi-square test and Fisher's exact test when, in a 2 × 2 table, one cell had an expected frequency ⩽ 5. Differences within groups during the treatment period and follow-up were evaluated by the Wilcoxon signed-rank test. The statistical level of significance was set at the 5% level ($P < 0.05$). The system utilized was IBM SPSS Statistics Processor.

Table 2 Modified by Birmingham Score Questionnaire[36]

Constipation	Diarrhoea	Pain
Hard bowel motions	Loose, mushy or watery bowel motions	Discomfort or pain
Straining	Diarrhoea	Discomfort or pain after eating
Constipation	Leaked or soiled	Sleep problem
	Urgency	
	Mucus or slime	

Each question had a standard response scale with symptoms all being measured on a 6-point Likert Scale ranging from 0 = none of the time to 5 = all of the time.

RESULTS

The results of the study were assessed considering some main variables, such as improving frequency and intensity of the symptoms and modifications of the bowel habit.

Overall rating of frequency symptoms

At the enrolment visit (T0), mean score evaluation of principal IBS related symptoms (Birmingham Score) confirmed that symptoms were present almost every day, together with a strong functional disability in both groups. Overall, 15 of the 60 (24%) participants with IBS reported treatment success. Those in the PHGG group were more likely to have treatment success than those in the control group (43% *vs* 5%, $P = 0.025$). Responders with significant reduction of Birmingham IBS score (median 0 ± 1 *vs* 4 ± 1, $P = 0.025$) was shown in the PHGG group *vs* placebo at both 4 wk and 8 wk (Table 3). The total score and the three subscale scores for constipation, diarrhoea and pain symptoms of the Birmingham score were significantly improved at the 4 and 8 wk evaluations compared to the baseline in the PHGG group. The supplementation response was comparable both in IBS-d and IBS-c subgroups. In group B, no significant difference was found in comparisons at any evaluation time point for any subscale score.

Bowel habits

At baseline all patients were shown a wide range of alterations in bowel movements, evaluated with Bristol Stool Scale, without any difference in the two treatment groups. Effects of PHGG supplementation (5 g/d) for 4 wk on fecal output in IBS-d and IBS-c subsets *vs* placebo were also evaluated. In group A, there was a tendency toward normalization of bowel movements, which is highlighted by the progressive normalization of Bristol Stool Scale at type 3 or 4 (Table 3). In particular, 16 (26.6%) of 60 patients had normalized bowel habits: in the PHGG group the prevalence of improvement was 40% (12 patients), while it was 13.3% (4 patients) in the placebo group ($P = 0.025$). This result remained constant during the follow-up 4 wk. There was no difference in the prevalence of

Table 3 Outcome measures at baseline (T0), at 4 wk of supplementation (T2) and after 8 wk (mean ± SD)

	Group A PHGG 5g/d (n = 30)			Group B Placebo (n = 30)		
	Baseline	4 wk (T2)	8 wk (T3)	Baseline	4 wk (T2)	8 wk (T3)
Birmingham Score[a]	28.5 ± 7.16	24.3 ± 6.02	23.0 ± 6.15	29.5 ± 6.94	28.4 ± 8.39	28.7 ± 7.54
Bristol Stool Score[a]						
IBS-c	1.00 ± 1.02	2.02 ± 1.50	2.32 ± 1.50	1.16 ± 0.89	1.76 ± 1.04	1.65 ± 1.08
IBS-d	5.02 ± 0.63	4.01 ± 0.16	4.07 ± 0.12	5.54 ± 0.32	4.86 ± 0.96	4.89 ± 0.73
Wong-Baker Score[a]	2.15 ± 0.14	1.86 ± 0.14	1.63 ± 0.16	2.16 ± 0.17	2.04 ± 0.17	2.05 ± 0.19

[a]$P < 0.05$ *vs* placebo (Wilcoxon 2-sample test). PHGG: Partially hydrolyzed guar gum; IBS: Irritable bowel syndrome.

improvement in two bowel habit subsets ($P > 0.05$).

Intensity of the abdominal pain

There was no difference in pain intensity reported at baseline between the groups as Wong-Baker Face Pain Rating Score. During the course of study, there was a decrease in the intensity of pain in the group of children given PHGG, which was not seen in the placebo-supplemented group (Table 3). However, this result was not statistically significant ($P > 0.05$), compared with baseline at wk 4 and 8. Improvement of clinical symptoms in group A was correlated with a change of bowel habit and persisted 4 wk (T8) after cessation of PHGG supplementation. The clinical response was comparable both in IBS-d and IBS-c subgroups (Table 3). Analysis of the data confirmed optimal compliance and safety of PHGG dietary supplementation.

DISCUSSION

CAP is common in children and adolescents. In most children, CAP is functional without objective evidence of an underlying organic disorder. Children with CAP are more likely than children without CAP to have headache, joint pain, anorexia, vomiting, nausea and altered bowel habit assignable to IBS[21]. Physicians must decide whether to order diagnostic tests or use conservative management. The presence of alarm symptoms or signs suggests higher pretest probability and prevalence of organic disease and may justify the performance of diagnostic tests. CAP can cause long absences from school and markedly worsens quality of life of the children and parents[22,23].

In a recent American study, the diagnostic evaluation of CAP in a tertiary center in United States was found to cost approximately $6000 per patient[24]. The first treatment step is an age-appropriate assessment through the reassurance of the child and family on the absence of organic causes, but this does not mean that abdominal pain is not a real problem. Cognitive behavioral therapy, however, is an effective form of alternative treatment[25,26].

A thorough review of literature, with a focus on RCTs, revealed a paucity of studies examining effectiveness of pharmacologic and dietary interventions. Definitive statements concerning therapeutic efficacy are quite limited. Huertas-Ceballos *et al*[15], in a meta-analysis, failed to reveal any therapeutic benefit from a low-lactose or high-fiber diet for children with CAP. Therapeutic trials in adults with CAP associated with IBS symptoms have revealed a high rate of the placebo-response, confirming that non-pharmacological therapies alone are often adequate for many patients. PHGG is a soluble fiber with important properties, such as non-viscous texture, normal fermentation, non gelling, high hydrophilic potential and no interference with micronutrient absorption[27]. There is clear evidence that fiber decreases whole gut transit time, accelerates oral-to-anal transit, and decreases intracolonic pressure reducing abdominal pain. Fiber may represent a mainstay in the FAP and IBS therapeutic algorithm. Results of fiber supplementation in the adult population in patients with FAP and IBS has produced contrasting results, and the main reason for the variation is correlated with different types of fiber used[28,29]. The main distinction between soluble and insoluble fiber is essential as only soluble fiber such as PHGG dissolves in water and is widely metabolized in the large bowel, thus producing short-chain fatty acids, leading to selective stimulation of microbial growth[30,31]. PHGG may also act as prebiotic, thus modulating intestinal microbiota. Weaver *et al*[32], in experimental studies in rats, demonstrated that PHGG administration was accompanied by a rise in butyrate concentrations of colonocytes. Tuohy *et al*[33] showed that PHGG supplementation in healthy volunteers caused selective increase in the percentage of *Bifidobacteria* and *Lactobacilli* with beneficial modulation of microbiota that has been reported to ameliorate IBS symptoms, with a decrease in pain and flatulence[34].

Bijkerk *et al*[35] observed in an adult population that, although general fiber supplementation globally alleviates IBS symptoms, the beneficial effect is mainly associated with the use of soluble fiber rather than insoluble fiber. This study demonstrated that soluble fiber is effective in decreasing global IBS symptoms[36] but was no better than a placebo. Some of these above mentioned studies on the use of fiber in adult populations were biased as they confirmed that the placebo response in IBS patients ranged from 20%-50%. In our study, the placebo response was much lower than expected.

In 2012, a systematic review identified 3 RCTs evalu-

ating fiber supplementation in children with FGIDs. Patients were supplemented with different dietary fiber types for 4-6 wk[57]. Among these, the Feldman[20] study, a randomized, double-blind, placebo-controlled trial, is the only study in children with CAP (26 for group) recruited from primary care practices and supplemented with soluble fibers. Improvement of symptoms in treated patients with fiber not was significant *vs* the placebo-group. In patients with IBS symptoms with modification of the bowel habit, water-soluble fibers, such as PHGG, decrease symptoms also with a prebiotic effect, beneficial modification of the intestinal microflora and selective increase of *Lactobacilli* and *Bifidobacteria*[38]. PHGG was therefore tolerated and preferred by patients, indicating higher success of soluble fiber than bran or insoluble fiber. The present findings confirm the beneficial effects of PHGG at 5 g/d and in the short term (4 wk). Our study can be considered the first prospective, randomized, controlled, single-blind, clinical trial conducted with this particular fiber supplementation (PHGG) in pediatric CAP and IBS. Some limitations should caution against generalizing from the results of this study, such as the classification at baseline of CAP according to severity of symptoms (mild, moderate and severe) and lack of knowledge of dietary habits in patients enrolled. Given the good results obtained for the first time, it is important to confirm these preliminary data on a greater number of patients and also to consider the active role of liquid fiber in improvement of symptoms. The efficacy of this approach has proven how dietary management is more effective than pharmacological therapy in children with CAP and IBS.

In summary, fiber supplementation can be considered an important option in pediatric CAP and IBS. Water-soluble fiber, such as PHGG, is preferable to insoluble fiber. Moreover, initial studies have shown that fiber may act as a prebiotic, thus increasing the therapeutic benefits. Further placebo-controlled studies are needed to evaluate whether PHGG can also be seen as a maintenance therapy of CAP.

COMMENTS

Background

Functional bowel disorders, such as chronic abdominal pain (CAP), are frequent in children and similar to adult irritable bowel syndrome (IBS). Some children with CAP develop substantial disability and limitations in physical and psychosocial functions. There is little evidence of the efficacy of conventional medical treatment while there is a moderate evidence for the efficacy of complementary therapy (diet, fibers, low-lactose intake) in the adult population. Water-soluble fibers, such as oats, barley and gums in psyllium, can be safe in IBS symptoms.

Research frontiers

Water-soluble fibers are known for their ability to modify bowel patterns, accelerate oral-to-anal transit, decrease intracolonic pressure and alleviate pain. Functional abdominal disorders, such as abdominal pain and IBS, are frequent also in pediatric populations and should stimulate the trend to conservative therapy.

Innovations and breakthroughs

To date, there has been a limited number of studies regarding specific optional treatment in CAP and IBS. This is the first study in a pediatric population that showed a clinically significant improvement of the symptoms in pediatric functional gastrointestinal disease with dietary manipulation. The small sample size and a low placebo effect may indicate a requirement for request powered and well designed randomized controlled trials on the clinical effectiveness and safety of dietary treatment.

Applications

The findings in this study indicate that fiber supplementation can be considered an important therapeutic option in pediatric IBS.

Peer review

It is very well written and the topic is very interesting for the readers. This is an important topic for gastroenterologists, clinicians, surgeons, Critical Care doctors and nutritionists.

REFERENCES

1 **Clouse RE**, Mayer EA, Aziz Q, Drossman DA, Dumitrascu DL, Mönnikes H, Naliboff BD. Functional abdominal pain syndrome. *Gastroenterology* 2006; **130**: 1492-1497 [PMID: 16678562 DOI: 10.1053/j.gastro.2005.11.062.]

2 **Apley J**, Naish N. Recurrent abdominal pains: a field survey of 1,000 school children. *Arch Dis Child* 1958; **33**: 165-170 [PMID: 13534750 DOI: 10.1136/adc.33.168.165]

3 **Rasquin-Weber A**, Hyman PE, Cucchiara S, Fleisher DR, Hyams JS, Milla PJ, Staiano A. Childhood functional gastrointestinal disorders. *Gut* 1999; **45** Suppl 2: II60-II68 [PMID: 10457047 DOI: 10.1136/gut.45.2008.ii60]

4 **Rasquin A**, Di Lorenzo C, Forbes D, Guiraldes E, Hyams JS, Staiano A, Walker LS. Childhood functional gastrointestinal disorders: child/adolescent. *Gastroenterology* 2006; **130**: 1527-1537 [PMID: 16678566]

5 **Starfield B**, Hoekelman RA, McCormick M, Benson P, Mendenhall RC, Moynihan C, Radecki S. Who provides health care to children and adolescents in the United States? *Pediatrics* 1984; **74**: 991-997 [PMID: 6504643]

6 **Walker LS**, Lipani TA, Greene JW, Caines K, Stutts J, Polk DB, Caplan A, Rasquin-Weber A. Recurrent abdominal pain: symptom subtypes based on the Rome II Criteria for pediatric functional gastrointestinal disorders. *J Pediatr Gastroenterol Nutr* 2004; **38**: 187-191 [PMID: 14734882 DOI: 10.1097/00005176-200402000-00016]

7 **Son YJ**, Jun EY, Park JH. Prevalence and risk factors of irritable bowel syndrome in Korean adolescent girls: a school-based study. *Int J Nurs Stud* 2009; **46**: 76-84 [PMID: 18722617 DOI: 10.1016/j.ijnurstu.2008.07.006]

8 **Drossman DA**, Corazziari E, Delvaux M, Spiller R, Talley NJ, Thompson WG. [Appendix B: Rome III diagnostic criteria for functional gastrointestinal disorders.] *Rev Gastroenterol Mex* 2010; **75**: 511-516 [PMID: 21169122]

9 **Faresjö A**, Grodzinsky E, Johansson S, Wallander MA, Timpka T, Akerlind I. Psychosocial factors at work and in every day life are associated with irritable bowel syndrome. *Eur J Epidemiol* 2007; **22**: 473-480 [PMID: 17484023 DOI: 10.1007/s10654-007-9133-2]

10 **Saito YA**, Locke GR, Weaver AL, Zinsmeister AR, Talley NJ. Diet and functional gastrointestinal disorders: a population-based case-control study. *Am J Gastroenterol* 2005; **100**: 2743-2748 [PMID: 16393229 DOI: 10.1111/j.1572-0241.2005.00288.x]

11 **Kim YJ**, Ban DJ. Prevalence of irritable bowel syndrome, influence of lifestyle factors and bowel habits in Korean college students. *Int J Nurs Stud* 2005; **42**: 247-254 [PMID: 15708012 DOI: 10.1016/j.ijnurstu.2004.06.015]

12 **Faure C**, Wieckowska A. Somatic referral of visceral sensations and rectal sensory threshold for pain in children with functional gastrointestinal disorders. *J Pediatr* 2007; **150**: 66-71 [PMID: 17188617 DOI: 10.1016/j.jpeds.2006.08.072]

13 **Youssef NN**, Di Lorenzo C. The role of motility in functional abdominal disorders in children. *Pediatr Ann* 2001; **30**: 24-30 [PMID: 11195731]

14 **Ford AC**, Talley NJ, Spiegel BM, Foxx-Orenstein AE, Schiller L, Quigley EM, Moayyedi P. Effect of fibre, antispasmodics, and peppermint oil in the treatment of irritable bowel syn-

drome: systematic review and meta-analysis. *BMJ* 2008; **337**: a2313 [PMID: 19008265 DOI: 10.1136/bmj.a2313]
15 **Huertas-Ceballos A**, Logan S, Bennett C, Macarthur C. Pharmacological interventions for recurrent abdominal pain (RAP) and irritable bowel syndrome (IBS) in childhood. *Cochrane Database Syst Rev* 2008; (**1**): CD003017 [PMID: 18254013]
16 **Connell AM**. The effects of dietary fiber on gastrointestinal motor function. *Am J Clin Nutr* 1978; **31**: S152-S156 [PMID: 101074]
17 **Heizer WD**, Southern S, McGovern S. The role of diet in symptoms of irritable bowel syndrome in adults: a narrative review. *J Am Diet Assoc* 2009; **109**: 1204-1214 [PMID: 19559137 DOI: 10.1016/j.jada.2009.04.012]
18 **Zuckerman MJ**. The role of fiber in the treatment of irritable bowel syndrome: therapeutic recommendations. *J Clin Gastroenterol* 2006; **40**: 104-108 [PMID: 16394869 DOI: 10.1097/01.mcg.0000196405.15110.bb]
19 **Parisi G**, Bottona E, Carrara M, Cardin F, Faedo A, Goldin D, Marino M, Pantalena M, Tafner G, Verdianelli G, Zilli M, Leandro G. Treatment effects of partially hydrolyzed guar gum on symptoms and quality of life of patients with irritable bowel syndrome. A multicenter randomized open trial. *Dig Dis Sci* 2005; **50**: 1107-1112 [PMID: 15986863 DOI: 10.1007/s10620-005-2713-7]
20 **Feldman W**, McGrath P, Hodgson C, Ritter H, Shipman RT. The use of dietary fiber in the management of simple, childhood, idiopathic, recurrent, abdominal pain. Results in a prospective, double-blind, randomized, controlled trial. *Am J Dis Child* 1985; **139**: 1216-1218 [PMID: 2998181]
21 **Berger MY**, Gieteling MJ, Benninga MA. Chronic abdominal pain in children. *BMJ* 2007; **334**: 997-1002 [PMID: 17494020 DOI: 10.1136/bmj.39189.465718.BE]
22 **Whitehead WE**, Burnett CK, Cook EW, Taub E. Impact of irritable bowel syndrome on quality of life. *Dig Dis Sci* 1996; **41**: 2248-2253 [PMID: 8943980 DOI: 10.1007/BF02071408]
23 **Chassany O**, Geneve J, Abitbol JL. Specific quality of life questionnaire in irritable bowel syndrome. *Gastroenterology* 1995; **108**: A581 [DOI: 10.1016/0016-5085(95)26636-4]
24 **Di Lorenzo C**, Colletti RB, Lehmann HP, Boyle JT, Gerson WT, Hyams JS, Squires RH, Walker LS, Kanda PT. Chronic Abdominal Pain In Children: a Technical Report of the American Academy of Pediatrics and the North American Society for Pediatric Gastroenterology, Hepatology and Nutrition. *J Pediatr Gastroenterol Nutr* 2005; **40**: 249-261 [PMID: 15735476 DOI: 10.1097/01.MPG.0000154661.39488.AC]
25 **Chiou E**, Nurko S. Management of functional abdominal pain and irritable bowel syndrome in children and adolescents. *Expert Rev Gastroenterol Hepatol* 2010; **4**: 293-304 [PMID: 20528117 DOI: 10.1586/egh.10.28]
26 **Lackner JM**, Mesmer C, Morley S, Dowzer C, Hamilton S. Psychological treatments for irritable bowel syndrome: a systematic review and meta-analysis. *J Consult Clin Psychol* 2004; **72**: 1100-1113 [PMID: 15612856 DOI: 10.1037/0022-006X.72.6.1100]
27 **Bijkerk CJ**, Muris JW, Knottnerus JA, Hoes AW, de Wit NJ. Systematic review: the role of different types of fibre in the treatment of irritable bowel syndrome. *Aliment Pharmacol Ther* 2004; **19**: 245-251 [PMID: 14984370 DOI: 10.1111/j.0269-2813.2004.01862.x]
28 **Giannini EG**, Mansi C, Dulbecco P, Savarino V. Role of partially hydrolyzed guar gum in the treatment of irritable bowel syndrome. *Nutrition* 2006; **22**: 334-342 [PMID: 16413751 DOI: 10.1016/j.nut.2005.10.003]
29 **Giaccari S**, Grasso G, Tronci S, Allegretta L, Sponziello G, Montefusco A, Siciliano IG, Guarisco R, Candiani C, Chiri S. [Partially hydrolyzed guar gum: a fiber as coadjuvant in the irritable colon syndrome]. *Clin Ter* 2001; **152**: 21-25 [PMID: 11382164]
30 **Marteau P**, Flourié B, Cherbut C, Corrèze JL, Pellier P, Seylaz J, Rambaud JC. Digestibility and bulking effect of ispaghula husks in healthy humans. *Gut* 1994; **35**: 1747-1752 [PMID: 7829013]
31 **Parisi GC**, Zilli M, Miani MP, Carrara M, Bottona E, Verdianelli G, Battaglia G, Desideri S, Faedo A, Marzolino C, Tonon A, Ermani M, Leandro G. High-fiber diet supplementation in patients with irritable bowel syndrome (IBS): a multicenter, randomized, open trial comparison between wheat bran diet and partially hydrolyzed guar gum (PHGG). *Dig Dis Sci* 2002; **47**: 1697-1704 [PMID: 12184518]
32 **Weaver GA**, Tangel C, Krause JA, Alpern HD, Jenkins PL, Parfitt MM, Stragand JJ. Dietary guar gum alters colonic microbial fermentation in azoxymethane-treated rats. *J Nutr* 1996; **126**: 1979-1991 [PMID: 8759370]
33 **Tuohy KM**, Kolida S, Lustenberger AM, Gibson GR. The prebiotic effects of biscuits containing partially hydrolysed guar gum and fructo-oligosaccharides--a human volunteer study. *Br J Nutr* 2001; **86**: 341-348 [PMID: 11570986]
34 **Slavin JL**, Greenberg NA. Partially hydrolyzed guar gum: clinical nutrition uses. *Nutrition* 2003; **19**: 549-552 [PMID: 12781858]
35 **Bijkerk CJ**, de Wit NJ, Muris JW, Whorwell PJ, Knottnerus JA, Hoes AW. Soluble or insoluble fibre in irritable bowel syndrome in primary care? Randomised placebo controlled trial. *BMJ* 2009; **339**: b3154 [PMID: 19713235 DOI: 10.1136/bmj.b3154]
36 **Roalfe AK**, Roberts LM, Wilson S. Evaluation of the Birmingham IBS symptom questionnaire. *BMC Gastroenterol* 2008; **8**: 30 [PMID: 18651941 DOI: 10.1186/1471-230X-8-30]
37 **Horvath A**, Dziechciarz P, Szajewska H. Systematic Review of Randomized Controlled Trials: Fiber Supplements for Abdominal Pain-Related Functional Gastrointestinal Disorders in Childhood. *Ann Nutr Metab* 2012; **61**: 95-101 [PMID: 22889919]
38 **Gibson GR**, Roberfroid MB. Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr* 1995; **125**: 1401-1412 [PMID: 7782892]

P- Reviewers Khan I, Nielsen OH, Bian ZX, Rodriguez DC
S- Editor Wen LL **L- Editor** O'Neill M **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i2.241

World J Gastroenterol 2013 January 14; 19(2): 241-248
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Atorvastatin and rosuvastatin do not prevent thioacetamide induced liver cirrhosis in rats

Haim Shirin, Efrat Sharvit, Hussein Aeed, Dov Gavish, Rafael Bruck

Haim Shirin, Gastroenterology Institute, Assaf Harofeh Medical Center, Zerifin 70300, Israel
Hussein Aeed, Department of Gastroenterology, The E. Wolfson Medical Center, Holon 58100, Israel
Dov Gavish, Department of Internal Medicine "A", The E. Wolfson Medical Center, Holon 58100, Israel
Rafael Bruck, Efrat Sharvit, Department of Gastroenterology, Tel Aviv Sourasky Medical Center, Tel Aviv 64239, Israel
Haim Shirin, Dov Gavish, Rafael Bruck, Sackler School of Medicine Tel-Aviv University, Tel-Aviv 69975, Israel
Author contributions: Aeed H performed the majority of the in vivo experiments; Sharvit E performed the majority of the in vitro experiments; Gavish D was involved in designing and editing the manuscript; Shirin H and Bruck R designed the study and wrote the manuscript.
Correspondence to: Haim Shirin, MD, Gastroenterology Institute, Assaf Harofeh Medical Center, Zerifin 70300, Israel. haimsh@asaf.health.gov.il
Telephone: +972-8-9779722 Fax: +972-8-9542047
Received: March 25, 2012 Revised: August 31, 2012
Accepted: September 19, 2012
Published online: January 14, 2013

Abstract

AIM: To examine whether the administration of atorvastatin and rosuvastatin would prevent experimentally-induced hepatic cirrhosis in rats.

METHODS: Liver cirrhosis was induced by injections of thioacetamide (TAA). Rats were treated concurrently with TAA alone or TAA and either atorvastatin (1,10 and 20 mg/kg) or rosuvastatin (1, 2.5, 5, 10 and 20 mg/kg) given daily by nasogastric gavage.

RESULTS: Liver fibrosis and hepatic hydroxyproline content, in the TAA-treated group was significantly higher than those of the controls [11.5 ± 3.2 *vs* 2.6 ± 0.6 mg/g protein (P = 0.02)]. There were no differences in serum aminotransferase levels in the TAA controls compared to all the groups treated concomitantly by statins. Both statins used in our study did not prevent liver fibrosis or reduce portal hypertension, and had no effect on hepatic oxidative stress. Accordingly, the hepatic level of malondialdehyde was not lower in those groups treated by TAA + statins compared to TAA only. *In vitro* studies, using the BrdU method have shown that atorvastatin had no effect of hepatic stellate cells proliferation. Nevertheless, statin treatment was not associated with worsening of liver damage, portal hypertension or survival rate.

CONCLUSION: Atorvastatin or rosuvastatin did not inhibit TAA-induced liver cirrhosis or oxidative stress in rats. Whether statins may have therapeutic applications in hepatic fibrosis due to other etiologies deserve further investigation.

© 2013 Baishideng. All rights reserved.

Key words: Liver cirrhosis; Statins; Thioacetamide

Shirin H, Sharvit E, Aeed H, Gavish D, Bruck R. Atorvastatin and rosuvastatin do not prevent thioacetamide induced liver cirrhosis in rats. *World J Gastroenterol* 2013; 19(2): 241-248 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i2/241.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i2.241

INTRODUCTION

Liver cirrhosis is one of the leading causes of morbidity and mortality worldwide. Hepatic stellate cells (HSC) play a major role in the pathogenesis of hepatic fibrosis[1]. Injury to hepatocytes results in generation of lipid peroxides, which may have a direct stimulatory effect on matrix production by activated HSC[2]. It has been suggested that aldehyde-protein adducts, including products of lipid peroxidation, modulate collagen gene expression in human fibroblasts[3,4] and may be a link between tissue injury and hepatic fibrosis[2,5]. Several

studies demonstrated that activation of HSC in culture is provoked by generation of free radicals and is blocked by anti-oxidants. This activation may involve the transcription factor c-myb and nuclear factor kappa B (NF-κB)[6,7]. Accordingly, antioxidants have been suggested as therapeutic modalities in experimental models[8-10], and in patients with chronic liver injury[11].

3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) are used extensively to reduce serum cholesterol in an effort to reduce atherosclerotic cardiovascular morbidity and mortality. In addition to their cholesterol-lowering effect, statins demonstrate other biological effects (pleiotropic effects) that some of them may lead to clinical benefits. Those include anti-inflammatory[12] and antioxidant effects[13], inhibition of PDGF-stimulated proliferation and upregulation of tumor growth factor (TGF)-β signaling in murine mesangial cells and cultured heart cells[14-16].

Over the past decade the potential effect of statins as anti-fibrotic agents has received increasing attention. The rationale for the anti fibrotic efficacy is based on the ability of statin compounds to: (1) decrease the growth of human Ito cells in vitro, independently of their effect on cholesterol synthesis[17]; (2) inhibit the proliferation rate of HSC and reduction of the collagen protein steady state levels by both simvastatin and lovastatin[18]; (3) inhibit steatosis, hepatic fibrosis and carcinogenesis in a rat model of non-alcoholic steatohepatitis (NASH)[19]; and (4) enhance hepatic nitric oxide production and decrease hepatic vascular resistance in patients with cirrhosis and portal hypertension[20]. In addition to inhibiting stellate cell activation, the anti-oxidative activity and the attenuation of inflammation by specific statin derivatives may also contribute to the inhibition of fibrosis. Despite the existing data suggesting that statin derivatives can inhibit fibrosis by various mechanisms, treatment with simvastatin or pravastatin did not decrease fibrosis-induced by bile duct ligation (BDL) or carbon tetrachloride (CCl_4) in rats[21,22]. However, one recent study showed that very early atorvastatin treatment inhibits HSC activation and fibrosis in the BDL model *in vivo*[23].

To further elucidate the anti-fibrotic activity of statins in the liver, we examined the effects of both atorvastatin and rosuvastatin in a well characterized model of chronic thioacetamide (TAA)-induced administration in which cirrhosis is mainly produced *via* the formation of reactive oxygen species. TAA undergoes an extensive metabolism to acetamide shortly after administration, and to the hepatotoxic reactive metabolite thioacetamide-S-oxide by the mixed function oxidase system[24-26]. We hypothesized that inhibition of HSC activity in addition to the anti inflammatory and anti oxidative effects induced by statins may prevent the hepatic damage induced by TAA in rats.

Our results indicate that both atorvastatin and rosuvastatin did not diminish neither oxidative stress nor the development of TAA-induced cirrhosis in rats, and also had no effect on the proliferation of cultured HSC.

MATERIALS AND METHODS

Materials and animals

TAA, atorvastatin and rosuvastatin was purchased from Sigma (Sigma Chemical Co., St. Louis, MO). Male Wistar rats (250-300 g), obtained from Tel-Aviv University Animal Breeding Center, were kept in the animal breeding house of the E. Wolfson Medical Center and fed a Purina chow *ad libitum*. Animals were kept with a 12-h light-dark cycle at constant temperature and humidity and the rats had free access to tap water during the study period. Use of animals was in accordance with the National Institutes of Health Policy on the care and use of laboratory animals and was approved by the Animal Use and Care Committee of the E. Wolfson Medical Center.

Induction of liver cirrhosis

For induction of liver cirrhosis, rats were given intraperitoneal injections of thioacetamide, 200 mg/kg, twice a week for 12 wk, as previously described[27]. Control rats were treated with intraperitoneal injections of NaCl 0.9%.

Analysis of liver histopathology

The rats were sacrificed at the completion of the treatment protocols, their livers were removed, and midsections of the left lobes of the livers were processed for light microscopy. This processing consisted of fixing the specimens in a 5% neutral formol solution, embedding the specimens in paraffin, making sections of 5 μm thickness, and staining the sections with hematoxylin and eosin and Masson Trichrome. The tissue slices were scanned and scored blindly by two expert pathologists. The degree of fibrosis was expressed as the mean of 10 different fields in each slide, which had been classified on a scale of 0-4 according to Batts and Ludwig[28].

Measurement of hepatic hydroxyproline levels

Quantitative determination of hepatic hydroxyproline content was performed as previously described[29].

Measurement of hepatic malondialdehyde

For the determination of the hepatic content of malondialdehyde, liver tissue (5 g) was cut into small pieces using a razor blade, and homogenized after dilution in H_2O 1:10 w/v. Liver homogenate was centrifuged in 900 g for 5 min, and then the supernatant was collected and centrifuged in 20 000 rpm in Sorvall for 30 min using plastic tubes. The clear supernatant was obtained and malondialdehyde was measured and expressed as nmole/g wet tissue using the thiobarbituric acid method[30]. Briefly, to 1 mL of 10% liver homogenate with 1.15% KCl were added 2 mL of fresh solution 15% w/v TCA, 0.375% w/v thiobarbituric acid, 0.25 mL/L HCl. The mixture was heated at 95 ℃ for 15 min. The solution was cooled to room temperature using tap water and centrifuged at 300 rpm for 10 min. Absorption of the pink supernatant was determined spectrophoto-

metrically at 532 nm.

Effect of atorvastatin on proliferation of primary hepatic stellate cells

Hepatic stellate cells were isolated from male rat using sequential pronase-collagenase digestion followed by Nycodenz (Sigma-Aldrich, Inc., St. Louis, MO, United States) density gradient centrifugation essentially as described previously with minor modifications[1]. Briefly, the liver of male rat Wistar (300-400 g) was minced with scalpels and incubated with 100 mL of freshly prepared and filtered-GBSS with 65 mg pronase (Roche Molecular Biochemicals, Indianapolis, IN, United States), 50 mg collagenase (Worthington Biochemical Corporationv, Lakewood, NJ, United States), and 0.5mL 2.7%$CaCl_2$ for 15 min at 37 ℃ with 200 rpm shaking. Then 50 mL of freshly prepared and filtered-GBSS was added containing 12.5 mg pronase, 12.5 mg collagenase, 20 μg/mL DNAse I (Sigma-Aldrich, Inc., St. Louis, MO, United States) and 0.25 mL 2.7% $CaCl_2$ for 30 min at 37 ℃ with 200 rpm shaking. The digested tissue was filtered through a sterile 150-μm metal mesh, and the cells were centrifuged at for 2000 rpm for 7 min. The digested liver hepatic stellate cells were isolated on a 17.5% Nycodenz gradient centrifuged at 2700 rpm for 20 min. A stellate cell-enriched fraction was present in the upper layer. Cells were washed twice by centrifugation (1200 rpm, 4 ℃, 5 min) in DMEM with 10% fetal calf serum (FCS), 100 μg/mL penicillin and 100 μg/mL of streptomycin.

Reagents

Atorvastatin (Sigma-Aldrich, Inc., St. Louis, MO, United States) stock solution was dissolved in DDW to a concentration of 5×10^{-4} mol/L. PDGF-BB (Peprotech Inc. NJ, United States) was dissolved in DDW to a concentration of 1μg/mL. All the reagents were aliquot and stored in -20 ℃ until use.

Proliferation assays

The proliferation of HSC was examined by BrdU method (Exalpha biological, Inc. Watertown, MA, United States). Primary HSC were cultured for 14 d, after which they were trypsinized and plated at a density of 20 000 cell/well in 96 well plates in DMEM containing 10% FCS. The cells were incubated for 24 h, and then they were serum starved in DMEM + 0.5% FCS overnight. At the following day, the various stimuli were added in medium containing 0.5% FCS. HSC were exposed to either 30 ng/mL of PDGF (Peprotech, Inc. NJ, United States), and different concentration of Atorvastatin (5×10^{-8} mol/L, 10^{-7} mol/L, 5×10^{-7} mol/L) alone, or combination of the two. After 24 h the cells were tested for proliferation according to the manufacture instruction.

Western blotting

HSCs were plated on 100 mm plates at a density of 2×10^6 cells/plate. After 24 h, the medium was changed to starvation medium (DMEM + 0.5% FCS) overnight. The following day, cells were incubated for 24 h with the different treatments according to which experiments were performed. Total proteins were extracted by incubating the cells for 30 min on ice in RIPA buffer containing a 1:100 dilution of a protease inhibitor cocktail (Sigma-Aldrich, Inc., St. Louis, MO, United States). After 20 min centrifugation at 14 000 rpm at 4 ℃, extracts were normalized to total protein content, determined using the BCA Reagent (Sigma-Aldrich, Inc., St. Louis, MO, United States). Equal amounts of total protein-were separated in 4%-12% bis-tris (BT) gels (NuPAGE, Gibco-BRL Life Technologies, Grand Island, NY), blotted onto Hybond C extra membranes, blocked overnight in 5% milk, and incubated with antibodies against α smooth muscle actin (αSMA), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (Santa Cruz Biotechnology, Santa Cruz, CA, United States) and then incubated with horseradish peroxidase-conjugated secondary antibody. Afterwards, signals were detected by chemiluminescent. Expression of proteins was normalized to the expression of GAPDH.

Experimental design (in vivo experiments)

Control groups: (1) normal controls: injections of NaCl 0.9%; and (2) cirrhotic controls: TAA (200 mg/kg *ip*, twice weekly) injections for 12 wk.

Atorvastatin groups: (1) TAA + Atorvastatin 1 mg/kg daily; (2) TAA + Atorvastatin 10 mg/kg daily; and (3) TAA+ Atorvastatin 20 mg/kg daily.

Rosuvastatin groups: (1) TAA + Rosuvastatin 2.5 mg/kg daily; (2) TAA + Rosuvastatin 5 mg/kg daily; (3) TAA + Rosuvastatin 10 mg/kg daily; and (4) TAA + Rosuvastatin 20 mg/kg daily.

Each subgroup included 6-7 rats. The statins were given *po*, started concurrently with TAA and continued during the study. When the treatments were completed after 12 wk the rats were sacrificed, their livers were removed and the weights of their spleens measured.

Statistical analysis

The data are presented as median (range). The significance of differences among different groups was determined by a student's *t*-test.

RESULTS

Induction of liver cirrhosis by thioacetamide

Intraperitoneal administration of TAA for 12 wk, resulted in a uniform coarse granulation of the surface of the rats' liver. Microscopic analysis revealed liver cirrhosis, characterized by mixed-sized fibrotic nodules in these TAA-treated rats. Neither atorvastatin nor rosuvastatin had any effect on liver enzymes when given alone or in addition to TAA.

Table 1 Effects of rosuvastatin and atorvastatin treatment on oxidative stress and liver fibrosis in thioacethamide-treated rats

	ALT	MDA nmole/g liver	Hydroxyproline (mg/g protein)	Fibrosis score (0-4)	Spleen weight (mg)
Rosuvastatin dose (mg/kg)					
Control	59 (44, 69)	30.6 (20.5, 31.1)	2.67 (1.9, 3.3)	0	1200 (1100, 1400)
Ros 2.5	52 (47, 58)	24.1 (19.8, 33.9)	2.7 (2.2, 3.5)	0	1020 (950, 1150)
Ros 5	50 (41, 57)	31.3 (26.9, 36.7)	2.8 (2.3, 3.1)	0	1100 (950, 1300)
R 10	56 (46, 64)	33.7 (27.7, 37.8)	2.3 (1.8, 3.7)	0	1000 (800, 1200)
R 20	44 (41, 47)	29.4 (23.9, 39.6)	3.9 (2.4, 4.2)	0	1200 (900, 1200)
TAA	63 (37, 79)	48.7 (38.4, 50.2)	11.2 (4.5, 15.4)	4 (1.5, 4)	1900 (1600, 2200)
TAA + R 2.5	53 (47, 63)	22 (19, 31.2)	7.5 (3.7, 13.1)	2.5 (1, 4)	1450 (1050, 1950)
TAA + R 5	54 (48, 67)	36.5 (24.8, 52.1)	11.0 (4.7, 16.2)	3.25 (1, 4)	1230 (1000, 1350)
TAA + R 10	51 (43, 60)	37.8 (27.5, 44.9)	11.8 (8.3, 16.2)	3 (2, 4)	1200 (1100, 1500)
TAA + R 20	51.5 (48, 55)	39.2 (29.6, 46.8)	12.3 (3.1, 14.3)	3.5 (1, 4)	1050 (900, 1300)
Atorvastatin dose (mg/kg)					
Control	59 (44, 69)	30.6 (20.5, 31.1)	2.67 (1.9, 3.3)	0	1200 (1100, 1400)
Ato 1	61 (55, 81)	32.8 (27.4, 33.6)	2.5 (2.1, 3.1)	0	1300 (1100, 1400)
Ato 10	56 (53, 59)	32.9 (29.1, 35.2)	3.4 (1.6, 3.7)	0	1200 (1100, 1300)
Ato 20	58 (48, 73)	30.1 (29.8, 35.3)	2.3 (1.95, 3)	0	1100 (1100, 1200)
TAA	63 (37, 79)	48.7 (38.4, 50.2)	11.2 (4.5, 15.4)	4 (1.5, 4)	1900 (1600, 2200)
TAA + Ato 1	49 (22, 70)	46.6 (35.8, 63.1)	9.1 (6.7, 16.1)	3 (2, 4)	1600 (900, 3000)
TAA + Ato 10	53 (47, 62)	43.4 (36.2, 53.1)	12.5 (6.9, 15.4)	4 (2, 4)	1500 (1400, 1700)
TAA + Ato 20	57 (43, 67)	48.2 (34.2, 58.5)	10.9 (5.3, 16.8)	4 (3, 4)	1500 (1300, 1700)

n = 7, in the groups that received thioacethamide (TAA), n = 4 in the control, rosuvastatin only treated groups and atorvastatin only treated groups. TAA, 200 mg/kg *ip* twice weekly for 12 wk. Rosuvastatin and atorvastatin had given daily by nasogastric gavage. Ros: Rosuvastatin; ALT:Alanine transaminase; MDA: Malondialdehyde.

Inhibition of thioacetamide-induced liver cirrhosis by statins

Compared to the rats which received TAA only, neither atorvastatin nor rosuvastatin had protective effect on the histopathologic score after 12 wk and TAA-induced liver cirrhosis was not inhibited by all the doses that were examined. Hepatic fibrosis was also quantitated by the measurement of hepatic hydroxyproline levels. The mean hydroxyproline levels of the TAA-treated group were similar to those of the TAA plus atorvastatin or rosuvastatin at all doses used.

Spleen weights

An indirect measure of portal hypertension was obtained by measuring the weights of the rats' spleens at the end of the experiment. Characteristic hemodynamic changes have previously been shown after 3 mo of TAA administration, i.e., upon TAA-induced liver cirrhosis[27]. These changes include portal hypertension and hyperdynamic circulation which are accompanied with a significant increase in spleen weight. After 12 wk, the mean spleen weight of rats receiving TAA was about 30% higher than those receiving injections of 0.9% NaCl or statins only. The mean spleen weight of rats that received statins in addition to TAA was not lower than that of TAA alone (Table 1).

Hepatic levels of malondialdehyde and lipid peroxides

The hepatic levels of malondialdehyde measured after 12 wk, were not significantly different in the rats treated with TAA and statins compared to TAA only. Table 1 summarize the rosuvastatin and atorvastatin treatment effects on oxidative stress and liver fibrosis in TAA-treated rats [median (range)], n = 6-7.

Effect of Atorvastatin on hepatic stellate cells proliferation and smooth muscle actin expression

Atorvastatin in different concentrations had no effect on HSC proliferation as examined by the BrdU method (Figure 1) and no effect on the expression of smooth muscle actin determined by western blot analysis (Figure 2).

DISCUSSION

Our major finding in the present study is that atorvastatin and rosuvastatin do not have a therapeutic value as a potential anti-oxidant or anti-fibrotic agents targeting increased oxidative stress or liver fibrosis induced by TAA in rats.

There are various experimental observations regarding the direct anti-fibrotic activity of the statins by the inhibition of stellate cell proliferation; lovastatin inhibits pancreatic stellate cell activation and alpha-smooth muscle actin expression[31] and both simvastatin and lovastatin interferes with HSC activation in vitro[20,21]. Fluvastatin reduces renal fibroblast proliferation and collagen type Ⅲ production[32] and suppresses oxidative stress and kidney fibrosis after ureteral obstruction[33]. It is also possible that the anti-fibrotic effects of statins are mediated through mechanisms that stimulate fibroblast apoptosis *in vitro* and *in vivo* as it was shown in lung and renal fibroblasts[32,34]. Alternatively, the anti-fibrotic effects of statins may be mediated through their newly recognized anti inflammatory[12] and antioxidant mechanisms[35-37]. These include the inhibition of myeloperoxidase derived and nitric oxide derived oxidants[36],

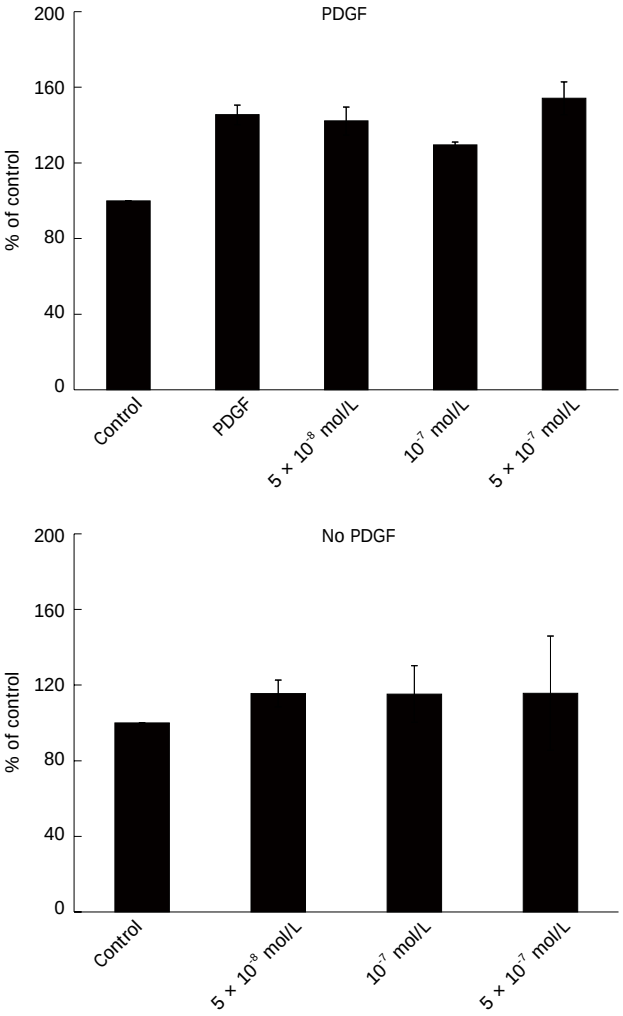


Figure 1 Effect of atorvastatin on hepatic stellate cells proliferation. PDGF: Platelet derived growth factor.

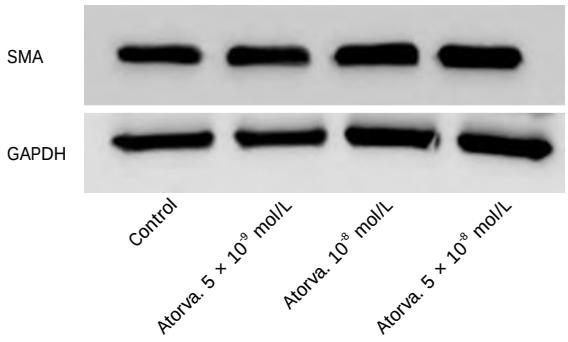


Figure 2 Atorvastatin effect on the expression of smooth muscle actin. GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; SMA: Smooth muscle actin.

S-nitrosylation and activation of thioredoxin in endothelial cells[37], decreased expression of essential NAD(P)H oxidase subunits and upregulation of catalase expression in vascular smooth muscle cells[35]. Statins also induce the expression of a protein with antioxidant and anti-inflammatory functions, heme oxygenase-1 (HO-1), *in vitro* and *in vivo*[33,38]. However, this effect is tissue specific demonstrating significant increase in liver HO-1 with simvastatin and lovastatin but not atorvastatin and rosuvastatin. The involvement of hydroxyl radicals and oxidative stress in TAA induced cirrhosis[39], the anti-oxidative effects of different statins, including atorvastatin, and the antifibrotic effect of atorvastatin in a rat model if BDL as was reported recently by Trebicka *et al*[23], provided a good rationale for the assumption that statins may reduce or prevent fibrogenesis in this specific animal model.

The explanation for the unexpected failure of atorvastatin and rosuvastatin to inhibit fibrosis in this model of cirrhosis are not clear. Two studies demonstrated anti fibrogenic effects of atorvastatin in a CCl_4 induced fibrosis[40,41]. Gardner *et al*[41] have shown that atorvastatin exhibited statistically significant, although modest, suppression of CCl_4-induced fibrogenesis after 3 wk of treatment. This was shown only using a novel technique for measuring hepatic collagen synthesis *in vivo* through metabolic labeling with heavy water (^2H_2O). Histopathology of the same tissues revealed no significant differences in fibrosis scores among groups that received co-treatment with atorvastatin, emphasizing the importance of the fibrosis measuring method.

Nevertheless the evidence that fibrosis was not inhibited by atorvastatin and with a more potent cholesterol metabolism pathway inhibitor, rosuvastatin, suggests that this effect is not selective and occurs independently of their HMG-CoA inhibition. Since we used much higher doses than those used clinically (up to 20 mg/kg) it does not seem that we are dealing with under dose treatment. Appropriate timing for the administration of the statins may also be critical. Later therapy in the BDL model, for example, lacked significant effects on fibrosis with no change in hepatic inflammation[23]. The statin treatment in our study began in parallel to the administration of TAA. Although, it is possible that pretreatment by statins would be more effective, the fact that there was no improvement, neither in MDA levels nor in fibrosis after three months, do not support this hypothesis. Another possibility to explain these negative results might be the selection of the wrong statins. Indeed, the two statins that inhibited HSC proliferation in vitro were lovastatin and simvastatin[18] and atorvastatin *in vivo*. In addition pitavastatin was the one that inhibited hepatic fibrosis in a choline-deficient L-amino acid defined diet liver fibrosis[19]. However, despite these effects, even the latter mechanisms were not efficient to prevent or inhibit liver fibrosis, as it was demonstrated with simvastatin or pravastatin in two different animal models of cirrhosis (bile duct ligation and CCl_4), in rats[21,22]. Moreover, antioxidant therapy in human clinical trials also lack or have minimal effect in chronic liver disease[42,43]. Finally, statin-induced protein kinase C (PKC) activation in activated HSCs may interrupt statin-induced HSC apoptosis, thereby reducing its antifibrotic efficacy. Indeed Yang *et al*[44] recently demonstrated that simultaneous treatment with pravastatin and PKC inhibitor may

synergistically enhance antifibrotic efficacy in hepatic fibrosis induced by intraperitoneal injection of carbon tetrachloride or thioacetamide in mice. Finally, we also have to consider the possibility that the negative results may be due to the small numbers (type 2 error).

To further explore the effect of statins on liver fibrosis we examined the effect of several atorvastain concentrations on the proliferation of primary HSC. We observed that atorvastatin had no inhibitory effect on HSC proliferation either in the presence or in the absence of PDGF (Figure 1). This finding is in discordance with several previous studies that demonstrated decreased HSC activation by statins[17,18]. Moreover, using western blot analysis we found that atorvastatin (5×10^{-8}-10^{-9} mol/L) had no effect on the expression of α smooth muscle actin further supporting the lack of effect shown in the proliferation assay.

It is of interest that treatment with atorvastatin or rosuvastatin in all doses did not reduce spleen weights, a parameter of portal hypertension. This is in contrast to the recently described therapeutic effects of simvastatin in patients with cirrhosis and portal hypertension. In patients with cirrhosis and portal hypertension simvastatin enhanced hepatic nitric oxide production and decreased hepatic resistance[20]. Similarly, in cirrhotic rats, induced by BDL, atorvastatin has been shown to lower intrahepatic resistance and to decrease portal hypertension[45].

Finally, oral atorvastatin and rosuvastatin were both well tolerated with no side effects, toxicity or mortality. Furthermore, transaminase levels, hepatic MDA and hydroxyproline and liver histopathology score did not aggravate. These data suggest that the use of atorvastatin and rosuvastatin is not associated with an increased risk of hepatoxicity in damaged liver.

In summary, our results show that atorvastatin and rosuvastatin have no effect on TAA-induced liver cirrhosis. Obviously further studies are required to evaluate whether statins may have therapeutic applications, in the development of hepatic fibrosis induced by other etiologies.

COMMENTS

Background

Liver cirrhosis is one of the leading causes of morbidity and mortality worldwide. Hepatic stellate cells (HSC) play a major role in the pathogenesis of hepatic fibrosis. Several studies demonstrated that activation of HSC in culture is provoked by generation of free radicals. Accordingly, antioxidants have been suggested as therapeutic modalities in experimental models, and in patients with chronic liver injury.

Research frontiers

Previous studies mainly focused on the potential effect of statins as anti-fibrotic agents. However, whether the anti-oxidative activity and the attenuation of inflammation by specific statin derivatives may also contribute to the inhibition of stellate cell activation and fibrosis remain unclear. The authors hypothesized that inhibition of HSC activity in addition to the anti inflammatory and anti oxidative effects induced by statins may prevent the hepatic damage induced by TAA in rats.

Innovations and breakthroughs

To further elucidate the anti-fibrotic activity of statins in the liver, the authors examined the effects of both atorvastatin and rosuvastatin in thioacetamide (TAA)-induced liver fibrosis in which cirrhosis is mainly produced *via* the formation of reactive oxygen species. The major finding was that both statins do not have a therapeutic value as a potential anti-oxidant or anti-fibrotic agents targeting increased oxidative stress or liver fibrosis induced by TAA.

Applications

The results show that atorvastatin and rosuvastatin have no effect on cirrhosis induced mainly by oxidative stress. Further studies are required to evaluate clarify the mechanism by which statins may have therapeutic applications, in hepatic fibrosis induced by other etiologies.

Terminology

3-Hydroxy-3-methylglutaryl-coenzyme A (HMG-CoA) reductase inhibitors (statins) are used extensively to reduce serum cholesterol in an effort to reduce atherosclerotic cardiovascular morbidity and mortality. In addition to their cholesterol-lowering effect, statins demonstrate also anti-inflammatory and antioxidant effects. TAA-induced cirrhosis is mainly produced *via* the formation of reactive oxygen species. TAA undergoes an extensive metabolism to acetamide shortly after administration, and to the hepatotoxic reactive metabolite thioacetamide-S-oxide by the mixed function oxidase system.

Peer review

Well written manuscript which presented that atorvastatin or rosuvastatin did not inhibit the formation of TAA-induced liver cirrhosis in rats. Further, no induction of oxidative stress or effect on HSC proliferation have been noted. The model is well presented, the experiments seems to be correct. Even if results are negative, and in partial or total contrast with previous studies, the study appears well conducted and the results discussed with honesty, and caution.

REFERENCES

1 **Tsukada S**, Parsons CJ, Rippe RA. Mechanisms of liver fibrosis. *Clin Chim Acta* 2006; **364**: 33-60 [PMID: 16139830 DOI: 10.1016/j.cca.2005.06.014]

2 **Bedossa P**, Houglum K, Trautwein C, Holstege A, Chojkier M. Stimulation of collagen alpha 1(I) gene expression is associated with lipid peroxidation in hepatocellular injury: a link to tissue fibrosis? *Hepatology* 1994; **19**: 1262-1271 [PMID: 8175151 DOI: 10.1016/0270-9139(94)90876-1]

3 **Brenner DA**, Chojkier M. Acetaldehyde increases collagen gene transcription in cultured human fibroblasts. *J Biol Chem* 1987; **262**: 17690-17695 [PMID: 3693368]

4 **Houglum K**, Brenner DA, Chojkier M. d-alpha-tocopherol inhibits collagen alpha 1(I) gene expression in cultured human fibroblasts. Modulation of constitutive collagen gene expression by lipid peroxidation. *J Clin Invest* 1991; **87**: 2230-2235 [PMID: 2040703 DOI: 10.1172/JCI115258]

5 **Houglum K**, Bedossa P, Chojkier M. TGF-beta and collagen-alpha 1 (I) gene expression are increased in hepatic acinar zone 1 of rats with iron overload. *Am J Physiol* 1994; **267**: G908-G913 [PMID: 7977754]

6 **Meyer M**, Caselmann WH, Schlüter V, Schreck R, Hofschneider PH, Baeuerle PA. Hepatitis B virus transactivator MHBst: activation of NF-kappa B, selective inhibition by antioxidants and integral membrane localization. *EMBO J* 1992; **11**: 2991-3001 [PMID: 1639069]

7 **Lee KS**, Buck M, Houglum K, Chojkier M. Activation of hepatic stellate cells by TGF alpha and collagen type I is mediated by oxidative stress through c-myb expression. *J Clin Invest* 1995; **96**: 2461-2468 [PMID: 7593635 DOI: http:]

8 **Liu SL**, Degli Esposti S, Yao T, Diehl AM, Zern MA. Vitamin E therapy of acute CCl4-induced hepatic injury in mice is associated with inhibition of nuclear factor kappa B binding. *Hepatology* 1995; **22**: 1474-1481 [PMID: 7590666 DOI: 10.1002/hep.1840220522]

9 **Brown KE**, Poulos JE, Li L, Soweid AM, Ramm GA, O'Neill R, Britton RS, Bacon BR. Effect of vitamin E supplementation on hepatic fibrogenesis in chronic dietary iron overload. *Am J Physiol* 1997; **272**: G116-G123 [PMID: 9038884]

10 **Boigk G**, Stroedter L, Herbst H, Waldschmidt J, Riecken EO, Schuppan D. Silymarin retards collagen accumulation

in early and advanced biliary fibrosis secondary to complete bile duct obliteration in rats. *Hepatology* 1997; **26**: 643-649 [PMID: 9303494 DOI: 10.1002/hep.510260316]
11 **Houglum K**, Venkataramani A, Lyche K, Chojkier M. A pilot study of the effects of d-alpha-tocopherol on hepatic stellate cell activation in chronic hepatitis C. *Gastroenterology* 1997; **113**: 1069-1073 [PMID: 9322499 DOI: 10.1053/gast.1997.v113.pm9322499]
12 **Crisby M**. Modulation of the inflammatory process by statins. *Timely Top Med Cardiovasc Dis* 2005; **9**: E3 [PMID: 15824762]
13 **Takemoto M**, Liao JK. Pleiotropic effects of 3-hydroxy-3-methylglutaryl coenzyme a reductase inhibitors. *Arterioscler Thromb Vasc Biol* 2001; **21**: 1712-1719 [PMID: 11701455 DOI: 10.1161/hq1101.098486]
14 **Yoshimura A**, Nemoto T, Sugenoya Y, Inui K, Watanabe S, Inoue Y, Sharif S, Yokota N, Uda S, Morita H, Ideura T. Effect of simvastatin on proliferative nephritis and cell-cycle protein expression. *Kidney Int Suppl* 1999; **71**: S84-S87 [PMID: 10412745 DOI: 10.1046/j.1523-1755.1999.07121.x]
15 **Park HJ**, Galper JB. 3-Hydroxy-3-methylglutaryl CoA reductase inhibitors up-regulate transforming growth factor-beta signaling in cultured heart cells via inhibition of geranylgeranylation of RhoA GTPase. *Proc Natl Acad Sci U S A* 1999; **96**: 11525-11530 [PMID: 10500210 DOI: 10.1073/pnas.96.20.11525]
16 **Nogaki F**, Muso E, Yashiro M, Kasuno K, Kamata T, Ono T, Sasayama S. Direct inhibitory effects of simvastatin on matrix accumulation in cultured murine mesangial cells. *Kidney Int Suppl* 1999; **71**: S198-S201 [PMID: 10412775 DOI: 10.1046/j.1523-1755.1999.07151.x]
17 **Mallat A**, Preaux AM, Blazejewski S, Dhumeaux D, Rosenbaum J, Mavier P. Effect of simvastatin, an inhibitor of hydroxy-methylglutaryl coenzyme A reductase, on the growth of human Ito cells. *Hepatology* 1994; **20**: 1589-1594 [PMID: 7982659 DOI: 10.1002/hep.1840200631]
18 **Rombouts K**, Kisanga E, Hellemans K, Wielant A, Schuppan D, Geerts A. Effect of HMG-CoA reductase inhibitors on proliferation and protein synthesis by rat hepatic stellate cells. *J Hepatol* 2003; **38**: 564-572 [PMID: 12713866 DOI: 10.1016/S0168-8278(03)00051-5]
19 **Miyaki T**, Nojiri S, Shinkai N, Kusakabe A, Matsuura K, Iio E, Takahashi S, Yan G, Ikeda K, Joh T. Pitavastatin inhibits hepatic steatosis and fibrosis in non-alcoholic steatohepatitis model rats. *Hepatol Res* 2011; **41**: 375-385 [PMID: 21276150 DOI: 10.1111/j.1872-034X.2010.00769.x]
20 **Zafra C**, Abraldes JG, Turnes J, Berzigotti A, Fernández M, Garca-Pagán JC, Rodés J, Bosch J. Simvastatin enhances hepatic nitric oxide production and decreases the hepatic vascular tone in patients with cirrhosis. *Gastroenterology* 2004; **126**: 749-755 [PMID: 14988829 DOI: 10.1053/j.gastro.2003.12.007]
21 **Oberti F**, Pilette C, Rifflet H, Maïga MY, Moreau A, Gallois Y, Girault A, le Bouil A, Le Jeune JJ, Saumet JL, Feldmann G, Calès P. Effects of simvastatin, pentoxifylline and spironolactone on hepatic fibrosis and portal hypertension in rats with bile duct ligation. *J Hepatol* 1997; **26**: 1363-1371 [PMID: 9210625 DOI: 10.1016/S0168-8278(97)80473-4]
22 **Huang HC**, Chang CC, Wang SS, Chan CY, Lee FY, Chuang CL, Hsin IF, Teng TH, Lin HC, Lee SD. Pravastatin for thioacetamide-induced hepatic failure and encephalopathy. *Eur J Clin Invest* 2012; **42**: 139-145 [PMID: 21749370 DOI: 10.1111/j.1365-2362.2011.02566.x]
23 **Trebicka J**, Hennenberg M, Odenthal M, Shir K, Klein S, Granzow M, Vogt A, Dienes HP, Lammert F, Reichen J, Heller J, Sauerbruch T. Atorvastatin attenuates hepatic fibrosis in rats after bile duct ligation via decreased turnover of hepatic stellate cells. *J Hepatol* 2010; **53**: 702-712 [PMID: 20633948 DOI: 10.1016/j.jhep.2010.04.025]
24 **Natarajan SK**, Thomas S, Ramamoorthy P, Basivireddy J, Pulimood AB, Ramachandran A, Balasubramanian KA. Oxidative stress in the development of liver cirrhosis: a comparison of two different experimental models. *J Gastroenterol Hepatol* 2006; **21**: 947-957 [PMID: 16724977 DOI: 10.1111/j.1440-1746.2006.04231.x]
25 **Müller D**, Sommer M, Kretzschmar M, Zimmermann T, Buko VU, Lukivskaya O, Dargel R. Lipid peroxidation in thioacetamide-induced macronodular rat liver cirrhosis. *Arch Toxicol* 1991; **65**: 199-203 [PMID: 2053847 DOI: 10.1007/BF02307309]
26 **Porter WR**, Neal RA. Metabolism of thioacetamide and thioacetamide S-oxide by rat liver microsomes. *Drug Metab Dispos* 1978; **6**: 379-388 [PMID: 28917]
27 **Hori N**, Okanoue T, Sawa Y, Mori T, Kashima K. Hemodynamic characterization in experimental liver cirrhosis induced by thioacetamide administration. *Dig Dis Sci* 1993; **38**: 2195-2202 [PMID: 8261820 DOI: 10.1007/BF01299895]
28 **Batts KP**, Ludwig J. Chronic hepatitis. An update on terminology and reporting. *Am J Surg Pathol* 1995; **19**: 1409-1417 [PMID: 7503362 DOI: 10.1097/00000478-199512000-00007]
29 **Woessner JF**. The determination of hydroxyproline in tissue and protein samples containing small proportions of this imino acid. *Arch Biochem Biophys* 1961; **93**: 440-447 [PMID: 13786180 DOI: 10.1016/0003-9861(61)90291-0]
30 **Wills ED**. Lipid peroxide formation in microsomes. General considerations. *Biochem J* 1969; **113**: 315-324 [PMID: 4390101]
31 **Jaster R**, Brock P, Sparmann G, Emmrich J, Liebe S. Inhibition of pancreatic stellate cell activation by the hydroxymethylglutaryl coenzyme A reductase inhibitor lovastatin. *Biochem Pharmacol* 2003; **65**: 1295-1303 [PMID: 12694870 DOI: 10.1016/S0006-2952(03)00075-3]
32 **Ikeuchi H**, Kuroiwa T, Yamashita S, Hiramatsu N, Maeshima A, Kaneko Y, Hiromura K, Ueki K, Nojima Y. Fluvastatin reduces renal fibroblast proliferation and production of type III collagen: therapeutic implications for tubulointerstitial fibrosis. *Nephron Exp Nephrol* 2004; **97**: e115-e122 [PMID: 15331935 DOI: 10.1159/000079176]
33 **Moriyama T**, Kawada N, Nagatoya K, Takeji M, Horio M, Ando A, Imai E, Hori M. Fluvastatin suppresses oxidative stress and fibrosis in the interstitium of mouse kidneys with unilateral ureteral obstruction. *Kidney Int* 2001; **59**: 2095-2103 [PMID: 11380811 DOI: 10.1046/j.1523-1755.2001.0590062095.x]
34 **Tan A**, Levrey H, Dahm C, Polunovsky VA, Rubins J, Bitterman PB. Lovastatin induces fibroblast apoptosis in vitro and in vivo. A possible therapy for fibroproliferative disorders. *Am J Respir Crit Care Med* 1999; **159**: 220-227 [PMID: 9872842]
35 **Wassmann S**, Laufs U, Müller K, Konkol C, Ahlbory K, Bäumer AT, Linz W, Böhm M, Nickenig G. Cellular antioxidant effects of atorvastatin in vitro and in vivo. *Arterioscler Thromb Vasc Biol* 2002; **22**: 300-305 [PMID: 11834532 DOI: 10.1161/hq0202.104081]
36 **Shishehbor MH**, Brennan ML, Aviles RJ, Fu X, Penn MS, Sprecher DL, Hazen SL. Statins promote potent systemic antioxidant effects through specific inflammatory pathways. *Circulation* 2003; **108**: 426-431 [PMID: 12860913 DOI: 10.1161/01.CIR.0000080895.05158.8B]
37 **Haendeler J**, Hoffmann J, Zeiher AM, Dimmeler S. Antioxidant effects of statins via S-nitrosylation and activation of thioredoxin in endothelial cells: a novel vasculoprotective function of statins. *Circulation* 2004; **110**: 856-861 [PMID: 15289372 DOI: 10.1161/01.CIR.0000138743.09012.93]
38 **Hsu M**, Muchova L, Morioka I, Wong RJ, Schröder H, Stevenson DK. Tissue-specific effects of statins on the expression of heme oxygenase-1 in vivo. *Biochem Biophys Res Commun* 2006; **343**: 738-744 [PMID: 16563347 DOI: 10.1016/j.bbrc.2006.03.036]
39 **Bruck R**, Shirin H, Aeed H, Matas Z, Hochman A, Pines M, Avni Y. Prevention of hepatic cirrhosis in rats by hydroxyl radical scavengers. *J Hepatol* 2001; **35**: 457-464 [PMID: 11682029 DOI: 10.1016/S0168-8278(01)00163-5]

40 **Schwartz YSh**, Dushkin MI, Komarova NI, Vorontsova EV, Kuznetsova IS. Cholesterol-induced stimulation of postinflammatory liver fibrosis. *Bull Exp Biol Med* 2008; **145**: 692-695 [PMID: 19110552 DOI: 10.1007/s10517-008-0175-6]

41 **Gardner JL**, Turner SM, Bautista A, Lindwall G, Awada M, Hellerstein MK. Measurement of liver collagen synthesis by heavy water labeling: effects of profibrotic toxicants and antifibrotic interventions. *Am J Physiol Gastrointest Liver Physiol* 2007; **292**: G1695-G1705 [PMID: 17347453 DOI: 10.1152/ajpgi.00209.2006]

42 **Medina J**, Moreno-Otero R. Pathophysiological basis for antioxidant therapy in chronic liver disease. *Drugs* 2005; **65**: 2445-2461 [PMID: 16296871 DOI: 10.2165/00003495-200565170-00003]

43 **Dey A**, Cederbaum AI. Alcohol and oxidative liver injury. *Hepatology* 2006; **43**: S63-S74 [PMID: 16447273 DOI: 10.1002/hep.20957]

44 **Yang JI**, Yoon JH, Bang YJ, Lee SH, Lee SM, Byun HJ, Myung SJ, Kim W, Lee HS. Synergistic antifibrotic efficacy of statin and protein kinase C inhibitor in hepatic fibrosis. *Am J Physiol Gastrointest Liver Physiol* 2010; **298**: G126-G132 [PMID: 19910526 DOI: 10.1152/ajpgi.00299.2009]

45 **Trebicka J**, Hennenberg M, Laleman W, Shelest N, Biecker E, Schepke M, Nevens F, Sauerbruch T, Heller J. Atorvastatin lowers portal pressure in cirrhotic rats by inhibition of RhoA/Rho-kinase and activation of endothelial nitric oxide synthase. *Hepatology* 2007; **46**: 242-253 [PMID: 17596891 DOI: 10.1002/hep.21673]

P- Reviewers Mueller S, Angeli P, Abraham P, Schaff Z
S- Editor Shi ZF **L- Editor** A **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i2.249

World J Gastroenterol 2013 January 14; 19(2): 249-257
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Hyperglycemia is a significant prognostic factor of hepatocellular carcinoma after curative therapy

Takanori Hosokawa, Masayuki Kurosaki, Kaoru Tsuchiya, Shuya Matsuda, Masaru Muraoka, Yuichiro Suzuki, Nobuharu Tamaki, Yutaka Yasui, Toru Nakata, Takashi Nishimura, Shoko Suzuki, Ken Ueda, Hiroyuki Nakanishi, Jun Itakura, Yuka Takahashi, Namiki Izumi

Takanori Hosokawa, Masayuki Kurosaki, Kaoru Tsuchiya, Shuya Matsuda, Masaru Muraoka, Yuichiro Suzuki, Nobuharu Tamaki, Yutaka Yasui, Toru Nakata, Takashi Nishimura, Shoko Suzuki, Ken Ueda, Hiroyuki Nakanishi, Jun Itakura, Yuka Takahashi, Namiki Izumi, Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital, Tokyo 180-8610, Japan

Author contributions: Hosokawa T and Kurosaki M contributed equally to this work; Kurosaki M and Izumi N made substantial contributions to the conception and design of the study; Tsuchiya K, Matsuda S, Muraoka M, Suzuki Y, Tamaki N, Yasui Y, Nakata T, Nishimura T, Suzuki S, Ueda K, Nakanishi H, Itakura J, Takahashi Y and Izumi N collected the clinical data; Hosokawa T and Kurosaki M contributed to the analysis and interpretation of the data; Hosokawa T wrote the draft of the manuscript; Kurosaki M and Izumi N made critical revisions of the manuscript; and Izumi N obtained a research fund.

Supported by A Grant-in-Aid from the Ministry of Health, Labor and Welfare, Japan

Correspondence to: Namiki Izumi, MD, Division of Gastroenterology and Hepatology, Musashino Red Cross Hospital, 1-26-1 Kyonan-cho, Musashino-shi, Tokyo 180-8610, Japan. nizumi@musashino.jrc.or.jp
Telephone: +81-422-323111 Fax: +81-422-329551

Received: June 17, 2012 Revised: September 6, 2012
Accepted: October 16, 2012
Published online: January 14, 2013

Abstract

AIM: To evaluate whether metabolic factors are related to distant recurrence of hepatocellular carcinoma (HCC) and survival after curative treatment.

METHODS: This retrospective study included 344 patients whose HCC was treated curatively by radiofrequency ablation (RFA) therapy. The mean age was 67.6 years and the mean observation period was 4.04 years. The etiological background of liver disease was hepatitis B virus infection in 30, hepatitis C virus infection in 278, excessive alcohol drinking in 9, and other in 27 patients. The Child-Pugh classification grade was A (n = 307) or B (n = 37). The number of HCC nodules was one in 260, two in 61, and three in 23 patients. For surveillance of HCC recurrence after curative therapy with RFA, patients were radiologically evaluated every 3 mo. Factors associated with distant recurrence of HCC or survival were studied.

RESULTS: Inadequate maintenance of blood glucose in diabetic patients was associated with higher incidence of distant recurrence. The 1-, 2-, and 3-year recurrence rates were significantly higher in diabetic patients with inadequate maintenance of blood glucose compared with the others: 50.6% *vs* 26.8%, 83.5% *vs* 54.4%, and 93.8% *vs* 73.0%, respectively (P = 0.0001). Inadequate maintenance of blood glucose was an independent predictor of distant recurrence [adjusted relative risk 1.97 (95%CI, 1.33-2.91), (P = 0.0007)] after adjustment for other risk factors, such as number of HCC nodules [2.03 (95%CI, 1.51-2.73), P < 0.0001] and initial level of serum alpha fetoprotein (AFP) [1.43 (95%CI, 1.04-1.97), P = 0.028]. Obesity was not an independent predictor of recurrence. The incidence of distant recurrence did not differ between diabetic patients with adequate maintenance of blood glucose and non-diabetic patients. Among 232 patients who had HCC recurrence, 138 had a second recurrence. The 1-, 2-, and 3-year rates of second recurrence were significantly higher in diabetic patients with inadequate maintenance of blood glucose than in the others: 9.0% *vs* 5.9%, 53.1% *vs* 24.3%, and 69.6% *vs* 42.3%, respectively (P = 0.0021). Inadequate maintenance of blood glucose in diabetic patients [1.99 (95%CI, 1.23-3.22), P = 0.0049] and presence of multiple HCC nodules [1.53 (95%CI, 1.06-2.22), P = 0.024] were again significantly associated with second HCC recurrence. Inadequate maintenance of blood glucose in diabetic

patients was also a significant predictor of poor survival [2.77 (95%CI, 1.38-5.57), P = 0.0046] independent of excessive alcohol drinking [6.34 (95%CI, 1.35-29.7), P = 0.019], initial level of serum AFP [3.40 (95%CI, 1.88-6.18), P < 0.0001] and Child-Pugh classification grade B [2.24 (95%CI, 1.12-4.46), P = 0.022]. Comparing diabetic patients with inadequate maintenance of blood glucose *vs* the others, the 1-, 2-, and 3-year survival rates were significantly lower in diabetic patients with inadequate maintenance of blood glucose: 92% *vs* 99%, 85% *vs* 96%, and 70% *vs* 92%, respectively (P = 0.0003).

CONCLUSION: Inadequate maintenance of blood glucose in diabetic patients is a significant risk factor for recurrence of HCC and for poor survival after curative RFA therapy.

© 2013 Baishideng. All rights reserved.

Key words: Hyperglycemia; Hepatocellular carcinoma; Recurrence; Radio frequency ablation; Survival

Hosokawa T, Kurosaki M, Tsuchiya K, Matsuda S, Muraoka M, Suzuki Y, Tamaki N, Yasui Y, Nakata T, Nishimura T, Suzuki S, Ueda K, Nakanishi H, Itakura J, Takahashi Y, Izumi N. Hyperglycemia is a significant prognostic factor of hepatocellular carcinoma after curative therapy. *World J Gastroenterol* 2013; 19(2): 249-257 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i2/249.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i2.249

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide[1] and its incidence has been increasing in many countries[2]. Surgical resection, liver transplantation, and local ablation therapy, such as radiofrequency ablation (RFA) therapy, have been considered as efficient curative therapies for HCC. RFA therapy is now widely performed in patients with small HCC[3] and a randomized controlled study demonstrated that the survival rates were similar in patients with small HCC receiving RFA or surgical resection[4]. A characteristic of HCC is its high rate of recurrence after curative resection or local ablation therapy, reaching approximately 80% within 5 years[5-7]. Identification of factors related to recurrence of HCC and therapeutic intervention targeting these factors may lead to prevention of frequent recurrence of HCC and improved survival.

Tumor factors, such as the number of HCC nodules and their size, are associated with the recurrence of HCC and survival prognosis[8-10]. Another factor that is associated with the recurrence of HCC and survival is the hepatic reserve function at the time of HCC therapy[8,10,11]. Hepatitis C virus (HCV) and hepatitis B virus (HBV) infection are the major causes responsible for 80% of HCC cases[2] and antiviral therapy targeting HCV[12,13] or HBV[14] has been shown to decrease HCC recurrence, and improve hepatic reserve function and survival. Non-alcoholic steatohepatitis (NASH) has also received attention as a cause of HCC[15]. Metabolic factors, such as obesity and diabetes, are closely linked to the etiology of NASH. These metabolic factors have also been identified as risk factors for several other types of cancer. Obesity is associated with increased mortality rates of several cancers[16,17] and diabetes is also reported as a risk factor for liver, pancreatic, renal, and colon cancers[18,19]. If these metabolic factors are related to the recurrence of HCC, therapeutic intervention targeting these factors may lead to prevention of frequent recurrence of HCC and improved survival. The impact of diabetes on the recurrence of HCC after treatment has been discussed, but with conflicting results[20-23].

In this study, factors contributing to the recurrence and prognosis of HCC after curative treatment were analyzed. We found that inadequate maintenance of blood glucose was related to the high rate of HCC recurrence and poor survival.

MATERIALS AND METHODS

Patients whose HCC was treated by RFA at the Musashino Red Cross Hospital were studied retrospectively for factors associated with recurrence of HCC and survival. The inclusion criteria were as follows: (1) HCC treated curatively with RFA at the Musashino Red Cross Hospital between 1999 and 2007; (2) maximum diameter of HCC nodule $\leqslant$ 3 cm; (3) number of HCC nodules $\leqslant$ 3; (4) no previous history of treatment for HCC; and (5) follow-up observation for at least 6 mo after RFA therapy. 344 patients met these criteria, including 140 women and 204 men, with a mean age of 67.6 years and mean observation time of 4.04 years. The clinical characteristics of the patients are summarized in Table 1. The etiological background of liver disease was HBV infection in 30, HCV infection in 278, excessive alcohol drinking (intake of ethanol $\geqslant$ 60 g/d for $\geqslant$ 5 years continuously) in 9, and non-B non-C non-alcoholic etiology in 27 patients. The Child-Pugh classification grade was either A (n = 307) or B (n = 37). The number of HCC nodules was one in 260, two in 61, and three in 23 patients. Thus, 260 patients had a single lesion, and 84 had multiple lesions. The maximum diameter of HCC nodules was 19.9 $\pm$ 0.3 mm.

Obesity was defined as a body mass index > 25 kg/m^2 according to the definition of the Japan Society for the Study of Obesity[24]. Blood glucose was measured monthly for 6 mo after HCC treatment and the average value was determined. Inadequate maintenance of blood glucose was defined as an average value of blood glucose $\geqslant$ 200 mg/dL. The level of hemoglobin A1c (HbA1c) was not used in the present study because the lifespan of erythrocytes is shortened due to hypersplenism in patients with chronic hepatitis or cirrhosis, leading to lower HbA1c levels relative to the blood glucose level[25]. Diagnosis of type 2 diabetes was made according to the

Table 1 Characteristics of patients undergoing curative radiofrequency ablation for hepatocellular carcinoma *n* (%)

Variable	Value
Sex (male/female)	204/140
Age(yr)	67.6 ± 8.4
Etiology of liver disease: HBV/HCV/NBNC	30/278/36
AST (IU/L)	84.0 ± 34.5
ALT (IU/L)	73.2 ± 36.5
GGT (IU/L)	82.9 ± 96.8
T-Chol (mg/dL)	157.8 ± 32.0
TG (mg/dL)	112.3 ± 55.7
Mean blood sugar (mg/dL)	139.3 ± 44.0
Diabetes mellitus	159 (48)
BMI > 25 kg/m^2	86 (25)
Maximum diameter of HCC nodule (mm)	19.9 ± 0.3
Number of HCC nodules: single/2 or 3	260/84
AFP (ng/mL)	214 ± 1025
Alcohol drinking > 60 g/d	9 (2.6)
Child-Pugh grade: A/B	307/37

HBV: Hepatitis B virus; HCV: Hepatitis C virus; NBNV: Neither HBV nor HCV; AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; GGT: γ-glutamyltransferase; T-Chol: Total cholesterol; TG: Triglyceride; BMI: Body mass index; AFP: α-fetoprotein; HCC: Hepatocellular carcinoma.

American Diabetes Association criteria of a fasting blood glucose level ≥ 126 mg/dL (≥ 7.0 mmol/L) and/or HbA1c level ≥ 6.5[26]. After initial treatment of HCC by RFA, the ablated area was confirmed by contrast-enhanced computed tomography (CT) within one week. If the ablated area was not sufficient, then RFA therapy was repeated until the HCC nodule was completely ablated.

HCC surveillance and diagnosis of recurrence

Diagnosis of HCC was based on abdominal ultrasonography, contrast-enhanced CT, magnetic resonance imaging (MRI), or angiography. Classical HCC was diagnosed for tumors showing vascular enhancement with washout on at least two types of diagnostic imaging. Tumor biopsy was used to diagnose tumors with non-classical imaging findings.

For surveillance of HCC recurrence after curative therapy with RFA, patients were evaluated by abdominal ultrasonography, contrast-enhanced CT, or contrast-enhanced MRI every three months. Recurrence of HCC was diagnosed based on a new lesion detected by ultrasonography showing vascular enhancement with washout on CT or MRI. If the tumor was not hypervascular, a tumor biopsy was performed to confirm the diagnosis.

Statistical analysis

For analysis of survival and recurrence, the time of initial RFA treatment was defined as day zero. Survival rate and recurrence rate were analyzed by the Kaplan-Meier method and log rank test. Multivariate analysis was performed using a Cox proportional hazard model. Data were analyzed using StatView Version 5.0 (SAS Institute Inc, Cary, North Carolina, United States) and IBM-SPSS statistics version 18 (IBM SPSS Inc, Chicago, IL, United States). Statistical significance was set at $P < 0.05$.

RESULTS

Factors associated with HCC recurrence

Of the 344 patients whose HCC was curatively treated by RFA, 232 had HCC recurrence. The 1-, 2-, and 3-year recurrence rates were 29.3%, 57.5%, and 75.2%, respectively. On univariate analysis, inadequate maintenance of blood glucose, higher initial level of serum AFP and multiple HCC nodules were significantly associated with HCC recurrence. Obesity ($P = 0.06$) and diabetes ($P = 0.65$) were not significantly associated with HCC recurrence.

Thirty-seven patients had diabetes with inadequate maintenance of blood glucose, 122 patients had diabetes with adequate maintenance of blood glucose, and 185 patients did not have diabetes. The HCC recurrence rate was significantly higher in diabetic patients with inadequate maintenance of blood glucose than in the others ($P = 0.0001$) (Figure 1A).

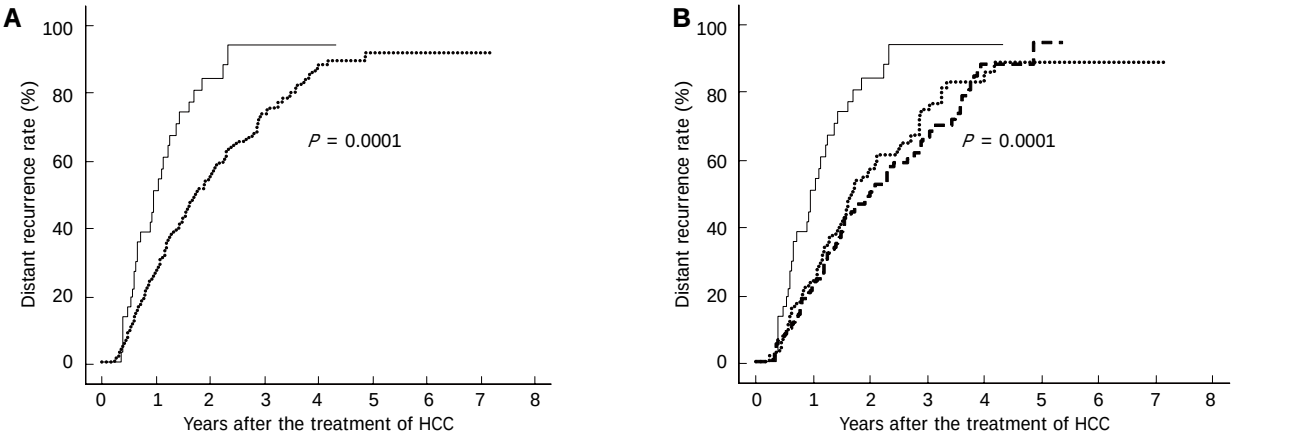
Comparing patients with diabetes ($n = 159$) and patients who did not have diabetes ($n = 185$), there was no significant difference in the recurrence rate ($P = 0.65$). Upon comparison of the three groups, i.e., the diabetes with inadequate maintenance of blood glucose group, the diabetes with adequate maintenance of blood glucose group, and the non-diabetes group, the recurrence rate was significantly higher in the diabetes with inadequate maintenance of blood glucose group than in the other two groups ($P = 0.0001$) (Figure 1B). On the other hand, there was no significant difference in the HCC recurrence rate between the diabetes patients with adequate maintenance of blood glucose group and the non-diabetes group.

In terms of the number of HCC nodules, namely, single ($n = 260$) *vs* multiple ($n = 84$), the recurrence rate was significantly higher in patients with multiple HCC nodules ($P = 0.0001$). Within each subgroup of patients with single and multiple HCC nodules, diabetes with inadequate maintenance of blood glucose was significantly associated with recurrence of HCC (single, $P = 0.006$; multiple, $P = 0.025$) (Figure 2A, B). In terms of the initial level of serum AFP ≥100 ng/mL ($n = 70$) *vs* < 100 ng/mL ($n = 274$), the recurrence rate was significantly higher in patients with AFP ≥ 100 g/mL ($P = 0.018$). Within each subgroup of patients with AFP ≥ 100 ng/mL and < 100 ng/mL, diabetes with inadequate maintenance of blood glucose was associated with a higher rate of recurrence (AFP ≥ 100 ng/mL, $P = 0.005$; AFP < 100 ng/mL, $P = 0.017$) (Figure 2C, D).

Independent risk factors for distant recurrence of HCC on multivariate analysis were inadequate maintenance of blood glucose in diabetic patients [adjusted relative risk, 1.97 (95%CI, 1.33-2.91), $P = 0.0007$], multiple HCC nodules [2.03 (1.51-2.73), $P < 0.0001$], and AFP ≥ 100 ng/mL [1.43 (1.04-1.97), $P = 0.028$] (Table 2).

Factors associated with second recurrence

Among the 232 patients who had HCC recurrence, 138 had a second recurrence. Regarding second recurrence, inadequate maintenance of blood glucose in diabetic pa-



Recurrence rate at	1-year	2-year	3-year
Inadequate maintenance of blood glucose	50.6%	83.5%	93.8%
Others	26.8%	54.4%	73.0%

Recurrence rate at	1-year	2-year	3-year
Diabetes with inadequate maintenance of blood glucose	50.6%	83.5%	93.8%
Diabetes with adequate maintenance of blood glucose	21.9%	48.7%	66.5%
Non-diabetes	23.6%	55.5%	74.4%

Figure 1 Kaplan-Meier curves showing a higher rate of hepatocellular carcinoma recurrence in diabetic patients with hyperglycemia. A: The cumulative incidence of the recurrence of hepatocellular carcinoma (HCC) was significantly higher in diabetic patients with inadequate maintenance of blood glucose (blood glucose ⩾ 200 mg/dL solid line) than in the others (dotted line) (P = 0.0001); B: The HCC recurrence rate was significantly higher in diabetic patients with inadequate maintenance of blood glucose (solid line) than in diabetic patients with adequate maintenance of blood glucose (blood glucose < 200 mg/dL, broken line) or non-diabetic patients (dotted line) (P = 0.0001). There was no significant difference in HCC recurrence rate between diabetic patients with adequate maintenance of blood glucose and non-diabetic patients.

Table 2 Multivariate analysis of factors associated with recurrence of hepatocellular carcinoma

Factors	Odds ratio (95%CI)	*P*-value
First recurrence		
Inadequate maintenance of blood glucose	1.97 (1.33-2.91)	0.0007
Multiple HCC nodules	2.03 (1.51-2.73)	< 0.0001
AFP ⩾ 100 ng/mL	1.43 (1.04-1.97)	0.028
Second recurrence		
Inadequate maintenance of blood glucose (mg/dL)	1.99 (1.23-3.22)	0.0049
Multiple HCC nodules	1.53 (1.06-2.22)	0.024

Inadequate maintenance of blood glucose was defined as an average of casual blood glucose of ⩾ 200 mg/dL. HCC: Hepatocellular carcinoma; AFP: α-fetoprotein.

tients and multiple HCC nodules were again significantly associated with HCC recurrence. Obesity (P = 0.18), diabetes (P = 0.31) and initial level of serum AFP (P = 0.08) were not associated with second recurrence. In terms of the number of HCC nodules, namely, single *vs* multiple, the 1-, 2-, and 3-year recurrence rates were significantly higher in patients with multiple lesions (6.4% *vs* 6.1%, 39.3% *vs* 23.1%, and 52.5% *vs* 42.3%, respectively, P = 0.013). Upon comparing diabetic patients with inadequate maintenance of blood glucose *vs* the others, the rate of second recurrence was significantly higher in diabetic patients with inadequate maintenance of blood glucose (P = 0.0021) (Figure 3A). Upon comparing patients with diabetes *vs* patients who did not have diabetes, the rates of second recurrence were not significantly different (P = 0.31). Upon comparison of the three groups, i.e., the diabetes with inadequate maintenance of blood glucose group, the diabetes with adequate maintenance of blood glucose group, and the non-diabetes group, the second recurrence rate was again significantly higher in the diabetes with inadequate maintenance of blood glucose group than in the other two groups (P = 0.0035) (Figure 3B). On the other hand, there was no significant difference in the second recurrence rate between the diabetes with adequate maintenance of blood glucose group and the non-diabetes group.

Independent risk factors for second recurrence of HCC on multivariate analysis were inadequate maintenance of blood glucose [1.99 (95%CI, 1.23-3.22), P = 0.0049] and multiple HCC nodules [1.53 (95%CI, 1.06-2.22), P = 0.024] (Table 2).

Factors associated with survival

There were 52 HCC-related or hepatic failure deaths. On univariate analysis, inadequate maintenance of blood glucose, excessive alcohol drinking, higher initial level of serum AFP and Child-Pugh classification grade B were significantly associated with survival. Obesity (P = 0.81) and diabetes (P = 0.11) were not significantly associated with survival.

Upon comparing diabetic patients with inadequate maintenance of blood glucose *vs* the others, the survival rate was significantly lower in patients with inadequate maintenance of blood glucose (P = 0.0003) (Figure 4A). Upon comparing diabetic patients *vs* non-diabetic patients, the survival rates were not significantly different

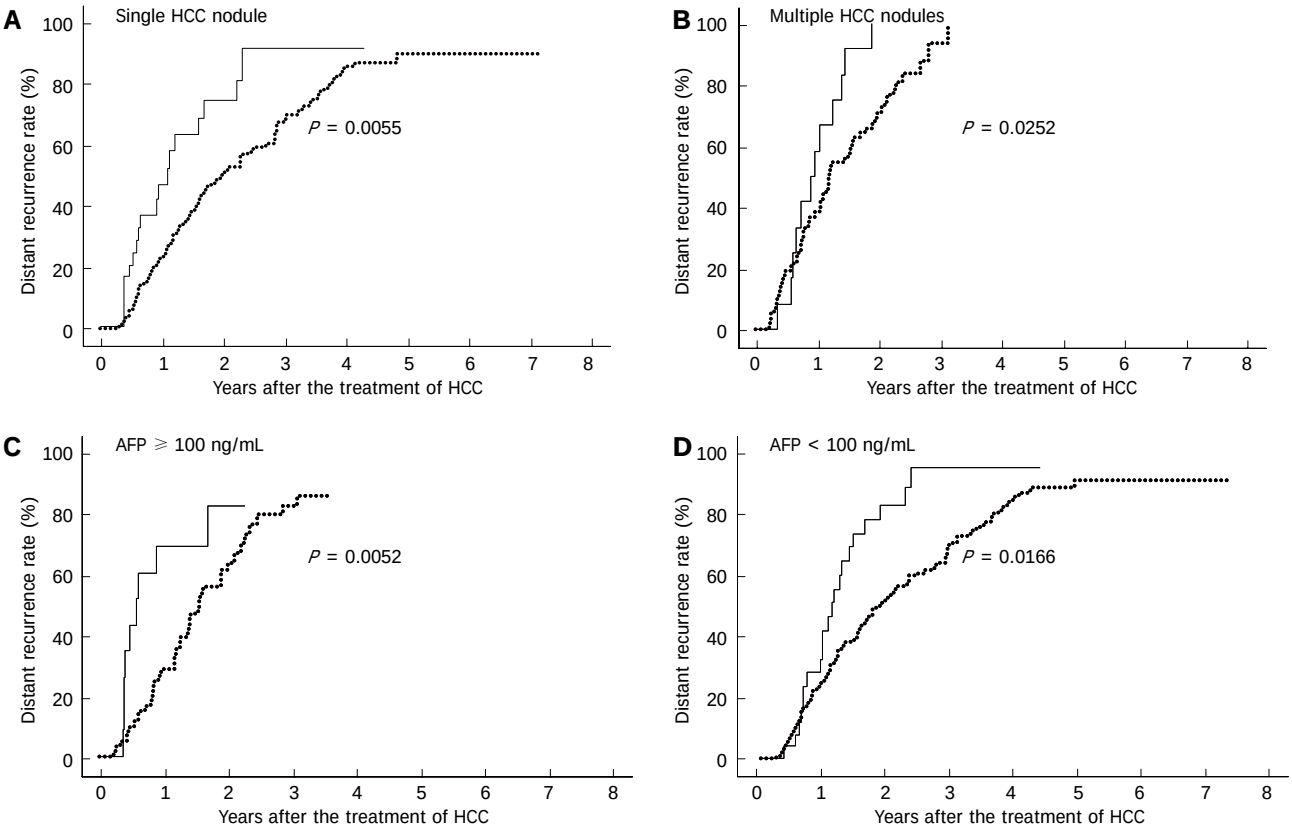


Figure 2 **Diabetic patients with inadequate maintenance of blood glucose have higher rate of hepatocellular carcinoma recurrence after stratification by other risk factors.** A: $P = 0.006$ for single hepatocellular carcinoma (HCC) nodule; B: $P = 0.025$ for multiple HCC nodules; C: $P = 0.005$ for AFP ⩾ 100 ng/mL; D: $P = 0.017$ for α-fetoprotein (AFP) < 100 ng/mL. The cumulative incidence of the recurrence of HCC was significantly higher in diabetic patients with inadequate maintenance of blood glucose (solid line) than in the others (dotted line). after stratification by number of HCC nodules and by initial level of AFP.

Table 3 Multivariable analysis of factors associated with survival

Factors	Odds ratio (95%CI)	*P*-value
Inadequate maintenance of blood glucose	2.77 (1.38-5.57)	0.0046
Alcohol drinking ⩾ 60 g/d	6.34 (1.35-29.7)	0.019
Child Pugh grade B	2.24 (1.12-4.46)	0.022
AFP ⩾ 100 ng/mL	3.40 (1.88-6.18)	< 0.0001

Inadequate maintenance of blood glucose was defined as an average of casual blood glucose of ⩾ 200 mg/dL. AFP: α-fetoprotein.

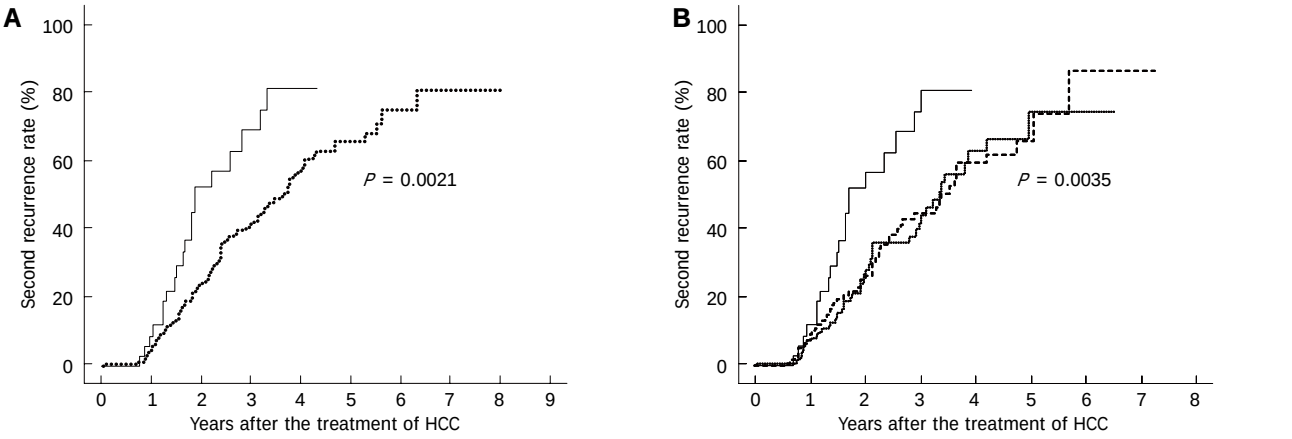
($P = 0.11$). of the survival rate was compared among the three groups, i.e., the diabetes with inadequate maintenance of blood glucose group, the diabetes with adequate maintenance of blood glucose group, and the non-diabetes group. The survival rate was significantly poorer in the diabetes with inadequate maintenance of blood glucose group than in the other two groups ($P = 0.0003$) (Figure 4B), while it did not differ between the diabetes with adequate maintenance of blood glucose group and the non-diabetes group.

The number of HCC nodules, which was a significant factor for HCC recurrence, was not related to survival ($P = 0.34$). Patients with excessive alcohol drinking had poor survival prognosis compared to those with non-excessive or no alcohol drinking ($P = 0.046$). Survival was better in patients in Child-Pugh A class than in patients in Child-Pugh B class ($P = 0.0082$). AFP ⩾ 100 ng/mL was associated with poor survival compared with AFP < 100 ng/mL ($P < 0.0001$).

On multivariate analysis, inadequate maintenance of blood glucose was a significant predictor of poor survival [2.77 (95%CI, 1.38-5.57), $P = 0.0046$] independent of excessive alcohol drinking [6.34 (95%CI, 1.35-29.7), $P = 0.019$], initial level of serum AFP ⩾ 100 ng/mL [3.40 (95%CI, 1.88-6.18), $P < 0.0001$] and Child-Pugh classification grade B [2.24 (95%CI, 1.12-4.46), $P = 0.022$] (Table 3).

DISCUSSION

The impact of metabolic factors, such as hyperglycemia, diabetes and obesity, on distant recurrence and survival after curative RFA therapy for HCC was analyzed retrospectively. We identified that inadequate maintenance of blood glucose in diabetic patients was a significant and independent risk factor for early recurrence of HCC and a risk factor for poor survival, whereas obesity and diabetes were not. Diabetic patients with inadequate maintenance of blood glucose had a higher rate of HCC recurrence and poorer survival compared with diabetic patients with adequate maintenance of blood glucose and non-diabetic patients. In other words, even in patients with diabetes, if



Second recurrence rate at	1-year	2-year	3-year
Inadequate maintenance of blood glucose	9.0%	53.1%	69.6%
Others	5.9%	24.3%	42.3%

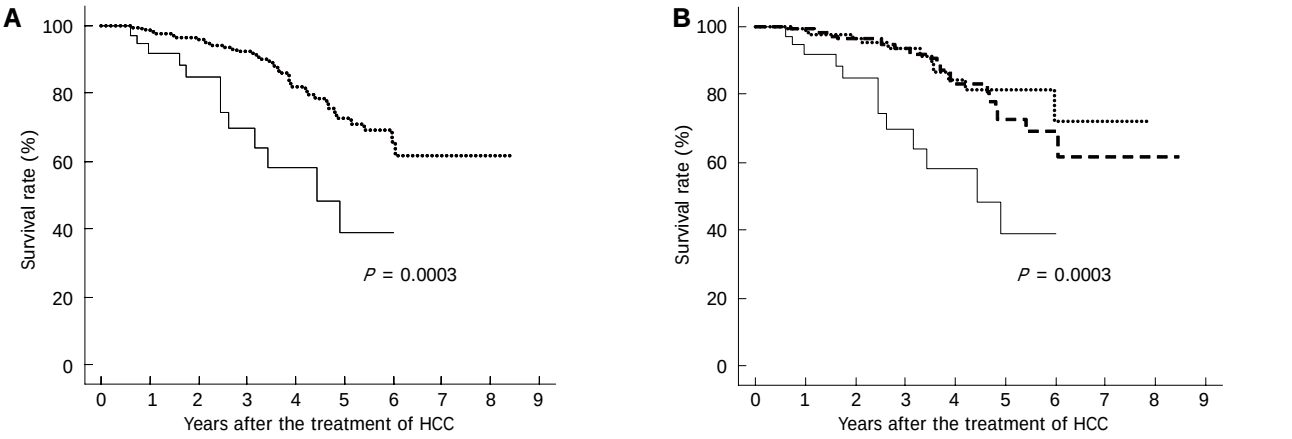
Second recurrence rate at	1-year	2-year	3-year
Diabetes with inadequate maintenance of blood glucose	9.0%	53.1%	69.6%
Diabetes with adequate maintenance of blood glucose	5.9%	22.1%	43.7%
Non-diabetes	5.6%	21.5%	36.5%

Figure 3 Kaplan-Meier curves showing a higher rate of second recurrence of hepatocellular carcinoma in diabetic patients with inadequate maintenance of blood glucose. A: The cumulative incidence of the second recurrence of hepatocellular carcinoma (HCC) was significantly higher in diabetic patients with inadequate maintenance of blood glucose (blood glucose ⩾ 200 mg/dL solid line) than in the others (dotted line) (P = 0.002); B: The rate of second recurrence of HCC was significantly higher in diabetic patients with inadequate maintenance of blood glucose (solid line) than in diabetic patients with adequate maintenance of blood glucose (blood glucose < 200 mg/dL, broken line) or non-diabetic patients (dotted line) (P = 0.004). There was no significant difference in the rate of second recurrence of HCC between diabetic patients with adequate maintenance of blood glucose and non-diabetic patients.

the blood glucose was adequately maintained, the HCC recurrence rate and survival did not differ significantly compared with those in non-diabetic patients. These results indicate the possibility that adequate management of hyperglycemia may lead to reduction in the risk of HCC recurrence and improvement of overall survival.

The contribution of diabetes to the development of HCC has been confirmed in several reports[27-30]. The impact of diabetes on the recurrence of HCC after treatment has also been discussed, but with conflicting results[20-22]. A recent study from Taiwan demonstrated that diabetes may not affect the intra-hepatic HCC recurrence and survival after RFA[23]. The results of the present study also indicated that diabetes itself is not a significant risk factor if the level of blood glucose is adequately managed. Rather, hyperglycemia was a significant risk factor for the recurrence of HCC. There may be several mechanisms involved in the relationship between hyperglycemia and HCC recurrence. Hyperglycemia promotes cancer cell proliferation in pancreatic cancer cells and breast cancer cells[31-33] through accelerated cell cycle progression or through the production of reactive oxygen species, leading to activation of protein kinase C and increased DNA synthesis in cancer cells[34]. A previous study in hepatitis C patients indicated that hyperglycemia after challenge with 75-g oral glucose tolerance test was associated with the risk for HCC while hyperglycemia at fasting was not[35]. A possible reason for this result may be that patients with post-challenge hyperglycemia may have higher fluctuations in daily glucose levels that lead to oxidative stress[35], because it was reported that acute fluctuations in blood glucose levels cause greater oxidative stress than sustained chronic hyperglycemia[36]. Taken together, a possible mechanism for the relationship between higher level of casual blood glucose and development of HCC in the present study may be that daily fluctuations in serum glucose levels caused greater oxidative stress. Alternatively, hyper-insulinemia or increased level of insulin-like growth factor, which are caused by hyperglycemia, may be related to carcinogenesis[37-39]. Insulin levels were not measured in our study; therefore, the effects of insulin could not be identified.

Discussions are now taking place on methods of treating diabetes from the standpoint of cancer prevention. Control of hyperglycemia could reduce cancer incidence, which means that hyperglycemia could directly contribute to the development of cancer[39]. The results of our study also showed that adequate management of hyperglycemia may lead to reduction in the risk of HCC recurrence and improvement of overall survival. Improvement in insulin resistance is undoubtedly the most important factor for the treatment of diabetes, but glycemic control is often difficult to achieve with dietary therapy, exercise, or insulin resistance-improving drugs alone. It was reported that metformin may be associated with a lower risk of cancer[38] and there is a theoretical concern that exogenous insulin may be associated with an increased risk of cancer[40]. In fact, a recent study reported that insulin therapy in patients with HCV infection is linked with the development of HCC[41]. On the other hand, with insulin treatment, concomitant use of metformin has been reported to offset the carcinogenic risk of insulin[42]. Whether glycemic control should be a priority, or whether avoiding hyper-insulinemia because



Second recurrence rate at	1-year	2-year	3-year
Inadequate maintenance of blood glucose	91.7%	84.6%	69.7%
Others	98.7%	95.9%	92.4%

Second recurrence rate at	1-year	2-year	3-year
Diabetes with inadequate maintenance of blood glucose	91.7%	84.6%	69.7%
Diabetes with adequate maintenance of blood glucose	99.2%	96.3%	93.7%
Non-diabetes	99.2%	96.4%	93.3%

Figure 4 Patients with inadequate maintenance of blood glucose have a lower survival rate. A: The survival rate after curative local ablation therapy for hepatocellular carcinoma (HCC) was significantly lower in diabetic patients with inadequate maintenance of blood glucose (blood glucose ≥ 200 mg/dL solid line) than in the others (dotted line) (P = 0.0003); B: The survival rate was significantly lower in diabetic patients with inadequate maintenance of blood glucose (solid line) than in diabetic patients with adequate maintenance of blood glucose (blood glucose < 200 mg/dL, broken line) or non-diabetic patients (dotted line) (P = 0.0003). There was no significant difference in survival rate between diabetic patients with adequate maintenance of blood glucose and non-diabetic patients.

of therapy should be a priority, is an issue for future investigation.

In terms of survival of HCC patients, associations with liver function and tumor factors have been reported[10], but conflicting results have been reported for the relationship with diabetes[20,21]. These two studies involved heterogeneous groups of HCC patients treated with various therapies, including surgery, local ablation therapy and transcatheter arterial embolization. This heterogeneity may have led to the conflicting results, because the survival of HCC patients may be strongly affected by the initial treatment. Our study involved a homogeneous patient population, i.e., all patients were initially treated curatively by RFA. The results of our study suggest that glycemic control in diabetic patients, more so than diabetes itself, plays a role in survival. The mechanism by which glycemic control and survival are related is unknown, but frequent recurrence of HCC in hyperglycemic patients and the accumulation of damage in liver function because of repeated treatment intervention for HCC may lead to worsening survival.

In conclusion, inadequate maintenance of blood glucose in diabetic patients was a significant and independent risk factor for early recurrence of HCC and for poor survival. Adequate management of hyperglycemia in diabetic patients may lead to reduction in the risk of HCC recurrence and improvement in overall survival.

COMMENTS

Background

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide. Radiofrequency ablation (RFA) therapy is an efficient curative therapy for HCC, but long-term survival is limited because of the high rate of distant recurrence of approximately 80% within 5 years. Identification of factors related to recurrence of HCC and therapeutic intervention targeting these factors may lead to prevention of frequent recurrence of HCC and improved survival.

Research frontiers

Metabolic factors, such as obesity and diabetes, have been identified as risk factors for several types of cancer, such as cancer of the liver, pancreas, kidney, and colon. These metabolic factors may be related to recurrence of HCC. The impact of diabetes on the recurrence of HCC after treatment has been discussed, but with conflicting results.

Innovations and breakthroughs

The authors identified that inadequate maintenance of blood glucose in diabetic patients was a significant and independent risk factor for early recurrence of HCC and a risk factor for poor survival, whereas diabetes was not. In other words, even in patients with diabetes, if the blood glucose was adequately maintained, then the HCC recurrence rate and survival did not differ significantly from those in non-diabetic patients.

Applications

The results of the present study indicate the possibility that adequate management of hyperglycemia in diabetic patients may lead to reduction in the risk of HCC recurrence and improvement of overall survival.

Peer review

This is an important study in which the effect of inadequate maintenance of blood glucose in diabetes has been shown as a significant risk factor for distant recurrence of hepatocellular carcinoma and poor survival after curative radiofrequency ablation therapy.

REFERENCES

1 **Parkin DM**, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108 [PMID: 15761078 DOI: 10.3322/canjclin.55.2.74]

2 **Bosch FX**, Ribes J, Díaz M, Cléries R. Primary liver cancer: worldwide incidence and trends. *Gastroenterology* 2004; **127**: S5-S16 [PMID: 15508102 DOI: 10.1053/j.gastro.2004.09.011]

3 **Arii S**, Sata M, Sakamoto M, Shimada M, Kumada T, Shiina S, Yamashita T, Kokudo N, Tanaka M, Takayama T, Kudo M.

Management of hepatocellular carcinoma: Report of Consensus Meeting in the 45th Annual Meeting of the Japan Society of Hepatology (2009). *Hepatol Res* 2010; **40**: 667-685 [PMID: 20633193 DOI: 10.1111/j.1872-034X.2010.00673.x]

4 **Chen MS**, Li JQ, Zheng Y, Guo RP, Liang HH, Zhang YQ, Lin XJ, Lau WY. A prospective randomized trial comparing percutaneous local ablative therapy and partial hepatectomy for small hepatocellular carcinoma. *Ann Surg* 2006; **243**: 321-328 [PMID: 16495695 DOI: 10.1097/01.sla.0000201480.65519.b8]

5 **Tateishi R**, Shiina S, Teratani T, Obi S, Sato S, Koike Y, Fujishima T, Yoshida H, Kawabe T, Omata M. Percutaneous radiofrequency ablation for hepatocellular carcinoma. An analysis of 1000 cases. *Cancer* 2005; **103**: 1201-1209 [PMID: 15690326 DOI: 10.1002/cncr.20892]

6 **Izumi N**, Asahina Y, Noguchi O, Uchihara M, Kanazawa N, Itakura J, Himeno Y, Miyake S, Sakai T, Enomoto N. Risk factors for distant recurrence of hepatocellular carcinoma in the liver after complete coagulation by microwave or radiofrequency ablation. *Cancer* 2001; **91**: 949-956 [PMID: 11251946]

7 **Curley SA**, Izzo F, Ellis LM, Nicolas Vauthey J, Vallone P. Radiofrequency ablation of hepatocellular cancer in 110 patients with cirrhosis. *Ann Surg* 2000; **232**: 381-391 [PMID: 10973388 DOI: 10.1097/00000658-200009000-00010]

8 **Poon RT**, Fan ST, Lo CM, Liu CL, Wong J. Intrahepatic recurrence after curative resection of hepatocellular carcinoma: long-term results of treatment and prognostic factors. *Ann Surg* 1999; **229**: 216-222 [PMID: 10024103 DOI: 10.1097/00000658-199902000-00009]

9 **Tung-Ping Poon R**, Fan ST, Wong J. Risk factors, prevention, and management of postoperative recurrence after resection of hepatocellular carcinoma. *Ann Surg* 2000; **232**: 10-24 [PMID: 10862190 DOI: 10.1097/00000658-200007000-00003]

10 **Kudo M**, Chung H, Haji S, Osaki Y, Oka H, Seki T, Kasugai H, Sasaki Y, Matsunaga T. Validation of a new prognostic staging system for hepatocellular carcinoma: the JIS score compared with the CLIP score. *Hepatology* 2004; **40**: 1396-1405 [PMID: 15565571 DOI: 10.1002/hep.20486]

11 **Fuke H**, Sugimoto K, Shiraki K, Tanaka J, Beppu T, Yoneda K, Yamamoto N, Ito K, Takaki H, Nakatsuka A, Yamakado K, Takeda K, Takei Y. Predictive factors for distant recurrence of HCV-related hepatocellular carcinoma after radiofrequency ablation combined with chemoembolization. *Aliment Pharmacol Ther* 2008; **27**: 1253-1260 [PMID: 18221404 DOI: 10.1111/j.1365-2036.2008.03627.x]

12 **Hagihara H**, Nouso K, Kobayashi Y, Iwasaki Y, Nakamura S, Kuwaki K, Toshimori J, Miyatake H, Ohnishi H, Shiraha H, Yamamoto K. Effect of pegylated interferon therapy on intrahepatic recurrence after curative treatment of hepatitis C virus-related hepatocellular carcinoma. *Int J Clin Oncol* 2011; **16**: 210-220 [PMID: 21152943 DOI: 10.1007/s10147-010-0150-x]

13 **Miyake Y**, Takaki A, Iwasaki Y, Yamamoto K. Meta-analysis: interferon-alpha prevents the recurrence after curative treatment of hepatitis C virus-related hepatocellular carcinoma. *J Viral Hepat* 2010; **17**: 287-292 [PMID: 19732321 DOI: 10.1111/j.1365-2893.2009.01181.x]

14 **Wong JS**, Wong GL, Tsoi KK, Wong VW, Cheung SY, Chong CN, Wong J, Lee KF, Lai PB, Chan HL. Meta-analysis: the efficacy of anti-viral therapy in prevention of recurrence after curative treatment of chronic hepatitis B-related hepatocellular carcinoma. *Aliment Pharmacol Ther* 2011; **33**: 1104-1112 [PMID: 21488914 DOI: 10.1111/j.1365-2036.2011.04634.x]

15 **Bugianesi E**, Leone N, Vanni E, Marchesini G, Brunello F, Carucci P, Musso A, De Paolis P, Capussotti L, Salizzoni M, Rizzetto M. Expanding the natural history of nonalcoholic steatohepatitis: from cryptogenic cirrhosis to hepatocellular carcinoma. *Gastroenterology* 2002; **123**: 134-140 [PMID: 12105842 DOI: 10.1016/S1590-8658(02)90208-2]

16 **Calle EE**, Rodriguez C, Walker-Thurmond K, Thun MJ. Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. *N Engl J Med* 2003; **348**: 1625-1638 [PMID: 12711737 DOI: 10.1056/NEJMoa021423]

17 **Renehan AG**, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet* 2008; **371**: 569-578 [PMID: 18280327 DOI: 10.1016/S0140-6736(08)60269-X]

18 **Inoue M**, Iwasaki M, Otani T, Sasazuki S, Noda M, Tsugane S. Diabetes mellitus and the risk of cancer: results from a large-scale population-based cohort study in Japan. *Arch Intern Med* 2006; **166**: 1871-1877 [PMID: 17000944]

19 **Hu FB**, Manson JE, Liu S, Hunter D, Colditz GA, Michels KB, Speizer FE, Giovannucci E. Prospective study of adult onset diabetes mellitus (type 2) and risk of colorectal cancer in women. *J Natl Cancer Inst* 1999; **91**: 542-547 [PMID: 10088625 DOI: 10.1093/jnci/91.6.542]

20 **Toyoda H**, Kumada T, Nakano S, Takeda I, Sugiyama K, Kiriyama S, Tanikawa M, Sone Y, Hisanaga Y. Impact of diabetes mellitus on the prognosis of patients with hepatocellular carcinoma. *Cancer* 2001; **91**: 957-963 [PMID: 11251947]

21 **Huo TI**, Wu JC, Lui WY, Huang YH, Lee PC, Chiang JH, Chang FY, Lee SD. Differential mechanism and prognostic impact of diabetes mellitus on patients with hepatocellular carcinoma undergoing surgical and nonsurgical treatment. *Am J Gastroenterol* 2004; **99**: 1479-1487 [PMID: 15307864 DOI: 10.1111/j.1572-0241.2004.30024.x]

22 **Komura T**, Mizukoshi E, Kita Y, Sakurai M, Takata Y, Arai K, Yamashita T, Ohta T, Shimizu K, Nakamoto Y, Honda M, Takamura T, Kaneko S. Impact of diabetes on recurrence of hepatocellular carcinoma after surgical treatment in patients with viral hepatitis. *Am J Gastroenterol* 2007; **102**: 1939-1946 [PMID: 17573788 DOI: 10.1111/j.1572-0241.2007.01354.x]

23 **Chen WT**, Macatula TC, Lin CC, Lin CJ, Lin SM. Diabetes may not affect outcomes in hepatocellular carcinoma after radio-frequency ablation. *Hepatogastroenterology* 2011; **58**: 551-557 [PMID: 21661430]

24 **Youkou A**, Hasegawa T, Suzuki K, Koya T, Sakagami T, Toyabe S, Arakawa M, Gejyo F, Narita I, Suzuki E. Influence of obesity on control in asthmatic Japanese patients defined by the Japanese definition of obesity. *Intern Med* 2011; **50**: 1911-1916 [PMID: 21921368 DOI: 10.2169/internalmedicine.50.5474]

25 **Aizawa N**, Enomoto H, Imanishi H, Saito M, Iwata Y, Tanaka H, Ikeda N, Sakai Y, Takashima T, Iwai T, Moriwaki E, Shimomura S, Iijima H, Nakamura H, Nishiguchi S. Elevation of the glycated albumin to glycated hemoglobin ratio during the progression of hepatitis C virus related liver fibrosis. *World J Hepatol* 2012; **4**: 11-17 [PMID: 22312451 DOI: 10.5754/hge12064]

26 **American Diabetes Association**. Diagnosis and classification of diabetes mellitus. *Diabetes Care* 2010; **33** Suppl 1: S62-S69 [PMID: 20042775]

27 **Davila JA**, Morgan RO, Shaib Y, McGlynn KA, El-Serag HB. Diabetes increases the risk of hepatocellular carcinoma in the United States: a population based case control study. *Gut* 2005; **54**: 533-539 [PMID: 15753540 DOI: 10.1136/gut.2004.052167]

28 **Lai MS**, Hsieh MS, Chiu YH, Chen TH. Type 2 diabetes and hepatocellular carcinoma: A cohort study in high prevalence area of hepatitis virus infection. *Hepatology* 2006; **43**: 1295-1302 [PMID: 16729295 DOI: 10.1002/hep.21208]

29 **El-Serag HB**, Hampel H, Javadi F. The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence. *Clin Gastroenterol Hepatol* 2006; **4**: 369-380 [PMID: 16527702 DOI: 10.1016/j.cgh.2005.12.007]

30 **Hassan MM**, Hwang LY, Hatten CJ, Swaim M, Li D, Abbruzzese JL, Beasley P, Patt YZ. Risk factors for hepatocellular carcinoma: synergism of alcohol with viral hepatitis

and diabetes mellitus. *Hepatology* 2002; **36**: 1206-1213 [PMID: 12395331 DOI: 10.1053/jhep.2002.36780]
31 **Liu H**, Ma Q, Li J. High glucose promotes cell proliferation and enhances GDNF and RET expression in pancreatic cancer cells. *Mol Cell Biochem* 2011; **347**: 95-101 [PMID: 20960036 DOI: 10.1007/s11010-010-0617-0]
32 **Okumura M**, Yamamoto M, Sakuma H, Kojima T, Maruyama T, Jamali M, Cooper DR, Yasuda K. Leptin and high glucose stimulate cell proliferation in MCF-7 human breast cancer cells: reciprocal involvement of PKC-alpha and PPAR expression. *Biochim Biophys Acta* 2002; **1592**: 107-116 [PMID: 12379472 DOI: 10.1016/S0167-4889(02)00276-8]
33 **Yamamoto M**, Patel NA, Taggart J, Sridhar R, Cooper DR. A shift from normal to high glucose levels stimulates cell proliferation in drug sensitive MCF-7 human breast cancer cells but not in multidrug resistant MCF-7/ADR cells which overproduce PKC-betaII. *Int J Cancer* 1999; **83**: 98-106 [PMID: 10449615]
34 **Nishikawa T**, Edelstein D, Du XL, Yamagishi S, Matsumura T, Kaneda Y, Yorek MA, Beebe D, Oates PJ, Hammes HP, Giardino I, Brownlee M. Normalizing mitochondrial superoxide production blocks three pathways of hyperglycaemic damage. *Nature* 2000; **404**: 787-790 [PMID: 10783895 DOI: 10.1038/35008121]
35 **Takahashi H**, Mizuta T, Eguchi Y, Kawaguchi Y, Kuwashiro T, Oeda S, Isoda H, Oza N, Iwane S, Izumi K, Anzai K, Ozaki I, Fujimoto K. Post-challenge hyperglycemia is a significant risk factor for the development of hepatocellular carcinoma in patients with chronic hepatitis C. *J Gastroenterol* 2011; **46**: 790-798 [PMID: 21331763 DOI: 10.1007/s00535-011-0381-2]
36 **Monnier L**, Mas E, Ginet C, Michel F, Villon L, Cristol JP, Colette C. Activation of oxidative stress by acute glucose fluctuations compared with sustained chronic hyperglycemia in patients with type 2 diabetes. *JAMA* 2006; **295**: 1681-1687 [PMID: 16609090 DOI: 10.1001/jama.295.14.1681]
37 **Fisher WE**, Boros LG, Schirmer WJ. Insulin promotes pancreatic cancer: evidence for endocrine influence on exocrine pancreatic tumors. *J Surg Res* 1996; **63**: 310-313 [PMID: 8661216 DOI: 10.1006/jsre.1996.0266]
38 **Renehan A**, Smith U, Kirkman MS. Linking diabetes and cancer: a consensus on complexity. *Lancet* 2010; **375**: 2201-2202 [PMID: 20609959 DOI: 10.1016/S0140-6736(10)60706-4]
39 **Giovannucci E**, Michaud D. The role of obesity and related metabolic disturbances in cancers of the colon, prostate, and pancreas. *Gastroenterology* 2007; **132**: 2208-2225 [PMID: 17498513 DOI: 10.1053/j.gastro.2007.03.050]
40 **Giovannucci E**, Harlan DM, Archer MC, Bergenstal RM, Gapstur SM, Habel LA, Pollak M, Regensteiner JG, Yee D. Diabetes and cancer: a consensus report. *Diabetes Care* 2010; **33**: 1674-1685 [PMID: 20587728 DOI: 10.3322/caac.20078]
41 **Kawaguchi T**, Taniguchi E, Morita Y, Shirachi M, Tateishi I, Nagata E, Sata M. Association of exogenous insulin or sulphonylurea treatment with an increased incidence of hepatoma in patients with hepatitis C virus infection. *Liver Int* 2010; **30**: 479-486 [PMID: 20040053 DOI: 10.1111/j.1478-3231.2009.02191.x]
42 **Currie CJ**, Poole CD, Gale EA. The influence of glucose-lowering therapies on cancer risk in type 2 diabetes. *Diabetologia* 2009; **52**: 1766-1777 [PMID: 19572116 DOI: 10.1007/s00125-009-1440-6]

P- Reviewer Rana SV **S- Editor** Gou SX
L- Editor Stewart GJ **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i2.258

World J Gastroenterol 2013 January 14; 19(2): 258-264
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Gastrointestinal tuberculosis is not associated with proton pump inhibitors: A retrospective cohort study

Kyoung Sup Hong, Seung Joo Kang, Jong Kyoung Choi, Ju Han Kim, Heewon Seo, Suehyun Lee, Jae-Woo Jung, Hye-Ryun Kang, Sang-Heon Cho, Joo Sung Kim

Kyoung Sup Hong, Jae-Woo Jung, Hye-Ryun Kang, Sang-Heon Cho, Department of Internal Medicine, Drug Safety Monitoring Center, Seoul National University Hospital, Seoul National University College of Medicine, Seoul 110799, South Korea
Seung Joo Kang, Jong Kyoung Choi, Joo Sung Kim, Department of Internal Medicine, Seoul National University Hospital, Seoul National University College of Medicine, Seoul 110799, South Korea
Ju Han Kim, Heewon Seo, Suehyun Lee, Division of Biomedical Informatics, Systems Biomedical Informatics Research Center, Seoul National University College of Medicine, Seoul 110799, South Korea
Author contributions: Hong KS and Kim JS designed the study and wrote the manuscript; Jung JW, Kang HR, and Cho SH contributed to the conception and design; Kang SJ contributed to the data analysis and interpretation; Kim JH contributed to the conception and data acquisition; and Choi JK, Seo H, and Lee S contributed to the data acquisition.
Supported by Basic Science Research Program through a National Research Foundation of Korea Grant Funded by the Ministry of Education, Science, and Technology, No. 2011-0018257; Systems Biomedical Informatics National Core Research Center
Correspondence to: Dr. Joo Sung Kim, Department of Internal Medicine, Seoul National University Hospital, Seoul National University College of Medicine, Seoul 110799, South Korea. jooskim@snu.ac.kr
Telephone: +82-2-20722228 Fax: +82-2-7629662
Received: June 29, 2012 Revised: September 28, 2012
Accepted: October 22, 2012
Published online: January 14, 2013

Abstract

AIM: To evaluate the effect of proton pump inhibitors (PPIs) on the development of gastrointestinal tuberculosis.

METHODS: All patients who were more than 20 years old and who had received a prescription for PPIs among those who visited Seoul National University Hospital from January 1, 2005 to December 31, 2009 were identified. Due to the low sensitivity of the microbiologic test and the nonspecific pathologic findings, the diagnosis of gastrointestinal tuberculosis was confirmed through the presence of active ulcerations and the responses to anti-tuberculosis medications. The patients were divided into two groups according to treatment duration (group 1: ⩽ 3 mo; group 2: > 3 mo) and were followed up from the time they took the first prescription of PPIs until their last visit. Logistic regression analysis was used to calculate the relative risks (RR) and 95%CI, adjusting for covariates.

RESULTS: Among the 61 834 patients exposed to PPIs (50 534 in group 1; 11 300 in group 2), 21 patients were diagnosed with PPI-associated gastrointestinal tuberculosis during 124 274 person-years of follow-up. Of 21 patients, the 12 who revealed only scar changes in the colonoscopy were excluded from the statistical analyses. Of those who remained, 2 were excluded because they underwent gastrointestinal endoscopy within 4 wk of the first prescription for PPIs. Longer exposure to PPI was associated with a higher mean age (55.0 ± 14.5 in group 1 *vs* 58.2 ± 13.3 in group 2, $P < 0.001$) and a higher Charlson co-morbidity index (0.50 ± 0.93 in group 1 *vs* 0.77 ± 1.14 in group 2, $P < 0.001$). The true incidence of active gastrointestinal tuberculosis was 0.65 per 1000 person-years in group 1 and 0.03 per 1 000 person-years in group 2. Like the less-than-three-month PPI treatment period in group 1, the over-three-month PPI therapy period in group 2 was not associated with increased risk of acquiring gastrointestinal tuberculosis, after adjusting for age and co-morbidities, whereas the Charlson co-morbidity index was associated with increased risk of acquiring gastrointestinal tuberculosis based on the score [RR: (reference 1) in group 1 *vs* 1.518 in group 2; 95% CI: 1.040-2.216, $P = 0.03$].

CONCLUSION: Long-term PPI therapy does not seem to be associated with increased risk of acquiring gas-

trointestinal tuberculosis, but a higher Charlson comorbidity index is associated with such.

© 2013 Baishideng. All rights reserved.

Key words: Proton pump inhibitor; Acid suppression; Tuberculosis; Gastrointestinal tuberculosis; Tuberculous colitis

Hong KS, Kang SJ, Choi JK, Kim JH, Seo H, Lee S, Jung JW, Kang HR, Cho SH, Kim JS. Gastrointestinal tuberculosis is not associated with proton pump inhibitors: A retrospective cohort study. *World J Gastroenterol* 2013; 19(2): 258-264 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i2/258.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i2.258

INTRODUCTION

Gut flora is closely related to human health and disease[1]. The intestinal microflora is assumed to be affected by a series of factors that determine the intraluminal environment: the pH in the gastrointestinal tract, oxygen tension, nutrient availability, colonic physiology, bacterial interference, *etc.*[2]. One of the major factors controlling the bacterial distribution in the gastrointestinal tract is the gastric acid barrier, which may be affected by the use of inhibitors of gastric acid secretion, gastrectomy, and dietary indiscretion and stress[3]. The establishment of enteric infection has been considered directly related to gastric acidity reduction[4,5]. Several recent studies associated proton pump inhibitors (PPIs) with a two- to three-fold increase in the risk of *Clostridium difficile* infection[6-8].

PPIs are currently the most powerful gastric acid suppressants and the drug of choice for the treatment of gastroesophageal reflux disease and peptic ulcer. PPIs are very powerful acid suppressants whose effects mean the percentage time intragastric pH > 4 may increase from 20% at baseline to over 60% within a week[9]. Today's life expectancy is longer than ever before, and the number of patients who take antiplatelet agents to prevent the onset of vascular diseases is increasing. Along with this, more and more patients are obliged to take prophylactic gastric acid suppressants, including PPIs, to prevent severe complications, such as peptic ulcer bleeding.

Tuberculosis is still an important health problem in many developing countries, including South Korea[10]. Unlike in developing countries, the disease used to be uncommon in developed countries, but it has re-emerged in the Western countries as a result of the acquired immunodeficiency syndrome (AIDS) epidemic therein as well as the influx of immigrants from developing countries[11-13]. Gastrectomy has been known to be a potential risk factor for tuberculosis for decades[14-16]. To these authors' knowledge, however, there has been no report about a possible association between PPI use and gastrointestinal tuberculosis. This study was thus conducted to evaluate the effect of PPIs on the development of gastrointestinal tuberculosis.

MATERIALS AND METHODS

Setting and design

This study is part of a hospital-based longitudinal cohort study entitled "Seoul National University Hospital (SNUH) PPI Safety Study," in which these authors analyzed the data regarding patients who visited SNUH and who were treated with PPI between January 1, 2005, and December 31, 2009. SNUH is a large urban tertiary care center in Seoul, South Korea. The hospital's institutional review board approved the study with a waiver of informed consent.

Data sources

Data were obtained from a clinical data warehouse fully synchronized with the electronic medical recording (EMR) system created as part of the usual care. SNUH Clinical Data Warehouse (CDW) contains all the information from each visit, not only routine clinical data such as the demographics, diagnosis, medication profiles, laboratory results, and lengths of stay of inpatients since 2001 but also the electronic charts since 2004.

Patients and case definitions

All patients who were at least 20 years old at their first visit and who ingested PPI on prescription during the five-year screening period were included in the study. According to a large retrospective study including 225 Korean patients, histological examination of the colonoscopic biopsy specimens revealed caseous necrosis in only 11.1% of the patients, and acid-fast bacilli (AFB) in 17.3% of the patients. *Mycobacterium tuberculosis* was isolated from the culture of biopsy specimens in 29.3% of the patients[17]. Even though granulomas were observed in 72.4% of the patients, granuloma is not a specific finding in intestinal tuberculosis. Due to the low sensitivity of the microbiologic test and the nonspecific pathologic findings, the diagnosis of active gastrointestinal tuberculosis was confirmed through both an endoscopic finding of active transverse ulcerations and a good response to anti-tuberculosis treatment, which was confirmed through a follow-up colonoscopy within three months from the initiation of the treatment. The definition of good therapeutic response was complete or near-complete healing of all the ulcerations in the follow-up colonoscopy.

The patients in whom colonoscopy revealed only scar changes were excluded from the statistical analysis for two reasons: (1) the exact temporal relationship between PPI ingestion and the development of gastrointestinal tuberculosis could not be verified; and (2) the diagnosis of tuberculosis could not be confirmed as there was no need for anti-tuberculosis treatment and due to the low sensitivity of the histological evaluation of the biopsy specimens. As tuberculosis is a chronic inflammatory disease, the patients who were diagnosed with gastrointestinal tuberculosis within four weeks of their first prescription for PPIs were also excluded from the statistical analyses.

The primary exposure of interest was receipt of PPIs. PPI

Table 1 Clinical information of the patients who were endoscopically diagnosed with suspicious tuberculosis

	Gender/ age	Group[1]	PPI duration before diagnosis (d)	Reason for endoscopy	Endoscopy findings	Pathology findings	Decision for analysis	Note
1	F/65	2	639	Diarrhea	Scar, cecum	No biopsy	Excluded	
2	F/73	1	844	Abdominal pain	Scar, cecum	No biopsy	Excluded	
3	F/65	2	1177	Routine check	Scar, cecum	No biopsy	Excluded	
4	F/60	1	415	Routine check	Scar, terminal ileum	No biopsy	Excluded	Small bowel resection due to tuberculosis 20 years ago
5	F/64	1	616	Anemia	Scar, descending colon	No biopsy	Excluded	
6	F/67	2	56	Abdominal pain	Scar, cecum	No biopsy	Excluded	
7	F/55	2	87	Routine check	Scar, cecum	No biopsy	Excluded	
8	F/82	2	523	Constipation	Scar, cecum	No biopsy	Excluded	
9	F/58	1	65	Lower abdominal pain	Scar, cecum	No biopsy	Excluded	
10	F/55	1	612	Routine check	Scar, cecum	No biopsy	Excluded	
11	M/52	1	894	Blood-tinged stool	Scar, cecum	No biopsy	Excluded	
12	F/61	2	29	Bloating	Scar, cecum	No biopsy	Excluded	
13	F/72	1	125	Hematochezia	Ulcers, cecum	Chronic active colitis	Included	
14	M/44	1	28	Anemia	Ulcers, cecum/ transverse colon	Non-caseating granuloma, negative PCR[2]	Included	
15	F/63	2	55	Epigastric pain	Ulcer, gastric cardia	Non-caseating granuloma, positive PCR[2]	Included	Palpable right supraclavicular lymph node
16	M/49	1	7	Melena	Ulcers, terminal ileum	Chronic active ileitis	Excluded	
17	M/41	1	574	Loose stool	Ulcers, cecum	Non-caseating granuloma, positive PCR[2]	Included	
18	M/20	1	49	Lower abdominal pain	Ulcers, ileocecal valve	Chronic active colitis	Included	
19	F/66	1	1	Melena	Ulcers, terminal ileum	Chronic active ileitis	Excluded	
20	F/59	1	224	Lower abdominal pain	Ulcers, ileocecal valve	Chronic active colitis,	Included	
21	F/46	1	28	Epigastric pain	Ulcers, mid-esophagus	Non-caseating granuloma, positive PCR[2]	Included	

[1]Patients with PPI treatment for three months or less as group 1, and patients with more-than-three-month PPI treatment as group 2; [2]Polymerase chain reaction (PCR) for *Mycobacterium tuberculosis*. PPI: Proton pump inhibitor; M: Male; F: Female.

exposure was classified by overall dosing period before the first endoscopy showing suspicious gastrointestinal tuberculosis or the last prescription date in patients without gastrointestinal tuberculosis. The patients with less than three months of PPI treatment were defined as group 1, and those with three or more months of PPI treatment were defined as group 2. To normalize the different acid-suppressive capacities among the PPI regimens, omeprazole 20 mg, lansoprazole 30 mg, rabeprazole 20 mg, pantoprazole 40 mg, and esomeprazole 40 mg were defined as standard daily doses of PPI. To calculate the adjusted dosing period, the period of the half-dose regimen was multiplied by 0.5, and the period for the double-dose regimen was multiplied by 2.

Statistical analysis

The covariates that may influence the risk of acquiring gastrointestinal tuberculosis and those that may influence the exposure to PPIs were included in the analysis. These variables were age, sex, and co-morbidities. The co-morbidities were determined from the registered diagnosis by the attending physician using the International Classification of Diseases 10th Revision code. Quan's algorithm was used to define the 17 Charlson co-morbidities, and the Charlson index was calculated[18,19].

Unadjusted comparisons were performed using the *t*-test, ANOVA test, Mann-Whitney test, χ^2 test, or Fisher exact test, as appropriate. Logistic regression modeling was used to estimate the relative risk of acquiring gastrointestinal tuberculosis in multivariate analyses. Statistical analysis was performed using the SAS software, version 9.2 (SAS Institute Inc., Cary, North Carolina).

RESULTS

Among the patients who visited SNUH during the five-year study period, 61 834 patients received PPIs and 7 gastrointestinal tuberculosis cases were identified. After excluding the patients who were diagnosed with suspicious gastrointestinal tuberculosis through endoscopy prior to the first prescription of PPIs, a total of 21 patients were screened. Of these, 12 patients who revealed only scar changes in colonoscopy were excluded from the statistical analyses. Of those who remained, 2 were excluded because they underwent gastrointestinal endoscopy within 4 wk of the first prescription of PPIs (Table

Table 2 Summary of descriptive statistical analysis *n* (%)

	Group 1	Group 2
Patients (*n*)	50 534	11 300
Cases	6 (0.012)	1 (0.009)
Esophageal tuberculosis	1	0
Gastric tuberculosis	0	1
Colonic tuberculosis	5	0
Total follow-up (person-mo)	1 102 947	388 345
Average follow-up (mo)	21.8	34.4
Incidence per 1000 person/yr	0.65	0.03

1). Anti-tuberculosis medications (isoniazid, rifampicin, ethambutol, and pyrazinamide) were prescribed to the finally selected 7 patients. Follow-up endoscopy was performed 2 or 3 mo after the start of the anti-tuberculosis treatment, and revealed complete healing or almost-healed ulcerations in all 7 patients.

The true incidence of gastrointestinal tuberculosis was 0.65 per 1000 person-years in group 1 and 0.03 per 1000 person-years in group 2 (Table 2). The characteristics of each group are shown in Table 3. The mean age (± SD) was 55.0 ± 14.5 years in group 1 and 58.2 ± 13.3 years in group 2. Longer exposure to PPI was associated with a higher Charlson co-morbidity index and a higher age (Table 3).

Table 4 presents the demographic and clinical characteristics of the active-gastrointestinal-tuberculosis and non-tuberculosis groups. Due to the small number of patients in the active-tuberculosis group, there was no significant difference between the two groups.

Table 5 shows the results of the multivariable analysis using logistic regression. A longer PPI treatment period (over three months) was not associated with increased risk of acquiring active gastrointestinal tuberculosis. The Charlson index was associated with significantly increased risk of acquiring active gastrointestinal tuberculosis by over 50% per score 1.

Table 3 Demographic and clinical characteristics of each group (mean ± SD)

	Group 1	Group 2	*P*-value
No. of patients	50 534	11 300	
Age, yr	54.96 ± 14.50	58.18 ± 13.31	< 0.001
Gender, male (%)	52.5	46.7	< 0.001
PPI duration, d	25.17 ± 21.51	285.78 ± 437.02	< 0.001
Co-morbidities, *n* (%)			
AIDS	75 (0.15)	6 (0.05)	0.011
Cerebrovascular disease	3655 (7.23)	1201 (10.63)	< 0.001
Congestive heart failure	196 (0.39)	71 (0.63)	< 0.001
Chronic pulmonary disease	184 (0.36)	64 (0.57)	0.002
Dementia	332 (0.66)	159 (1.41)	< 0.001
DM	4438 (8.78)	1600 (14.16)	< 0.001
DM without chronic complication	4280 (8.47)	1532 (13.56)	< 0.001
DM with chronic complication	158 (0.31)	68 (0.60)	< 0.001
Liver disease, mild	3182 (6.30)	1475 (13.05)	< 0.001
Hemiplegia or paraplegia	57 (0.11)	8 (0.07)	0.213
Liver disease, moderate or severe	488 (0.97)	205 (1.81)	< 0.001
Any malignancy	62 (0.12)	13 (0.12)	0.833
Metastatic solid tumor	310 (0.61)	69 (0.61)	0.972
Myocardial infarction	598 (1.18)	121 (1.07)	0.313
Peripheral vascular disease	368 (0.73)	114 (1.01)	0.002
Peptic ulcer	7011 (13.88)	1569 (13.88)	0.977
Rheumatologic disease	284 (0.56)	421 (3.73)	< 0.001
Renal disease	498 (0.99)	342 (3.03)	< 0.001
Charlson index	0.50 ± 0.93	0.77 ± 1.14	< 0.001
Score = 0 *n* (%)	33297 (65.89)	6167 (54.58)	< 0.001
Score = 1 *n* (%)	15536 (30.75)	4363 (38.61)	
Score ⩾ 3 *n* (%)	1699 (3.36)	770 (6.81)	
History of admission (%)	29138 (57.66)	6692 (59.22)	0.002
History of ICU admission (%)	1668 (3.30)	352 (3.12)	0.315

PPI: Proton pump inhibitor; AIDS: Acquired immune deficiency syndrome; ICU: Intensive care unit; DM: Diabetes mellitus.

DISCUSSION

This cohort study was conducted not only to evaluate the possible role of more-than-three-month PPI treatment but also to calculate the incidence rate of, and to find the risk factors for, acquiring active gastrointestinal tuberculosis in all the at-least-20-year-old patients who visited SNUH and who were treated with PPI between January 1, 2005 and December 31, 2009. As a result, more-than-three-month PPI treatment was found not to be associated with increased risk of acquiring active gastrointestinal tuberculosis. The annual incidence rate of tuberculosis was reported to be 97 per 100 000 in 2010 in the South Korean general population[10]. The calculated incidence rate of active gastrointestinal tuberculosis seems to be much lower in PPI-treated patients in the present study (Table 2), even considering the reportedly small proportion of gastrointestinal tuberculosis in the whole tuberculosis population[20].

A diagnosis of gastrointestinal tuberculosis can be confirmed if a characteristic caseous granuloma, positive smear of AFB, or positive culture of mycobacterium is observed in the biopsy specimen[12]. Compared to pulmonary tuberculosis, however, there is a relatively small absolute number of AFB in gastrointestinal tuberculosis[21], and caseous granuloma is infrequently observed in patients with an early-stage disease or who have been treated with anti-tuberculosis medications[22]. Many studies have been performed to assess the diagnostic accuracy of such histological markers, and there have been a number of reports showing granuloma in 41%-48%[23-25], caseous granuloma in 8%-18%[24-26], positive smear of AFB in 0%-100%[24,25,27,28], and positive culture of AFB in 0%-69%[24-26,28] of gastrointestinal-tuberculosis patients. As there have been many reports showing variable results, it seems impossible to set universal standards for diagnosing gastrointestinal tuberculosis. There are adjunct diagnostic modalities, such as polymerase chain reaction (PCR) for *Mycobacterium tuberculosis*, and endoscopic findings. Although PCR is a method that shows over 50% diagnostic sensitivity, its substantial false positivity limits its role to that of an adjunctive test for the diagnosis of gastrointestinal tuberculosis[29]. In the present study, both non-caseating granuloma and positive PCR were observed in two patients with gastroesophageal

Table 4 Demographic and clinical characteristics of the active-tuberculosis and non-tuberculosis groups (mean ± SD)

	Active tuberculosis	Non-tuberculosis[1]
No. of patients	7	61 790
Age, yr	50.6 ± 17.7	55.6 ± 14.3
Gender, male (%)	3 (43)	31 803 (51)
PPI duration	47.7 ± 97.9	72.8 ± 213.1
Charlson index	1.29 ± 2.36	0.55 ± 0.97
Score = 0 *n* (%)	5 (71)	39 459 (64)
Score = 1, 2 *n* (%)	0 (0)	19 899 (32)
Score ⩾ 3 *n* (%)	2 (29)	2467 (4)
History of admission	6 (86)	35 824 (58)

[1]Patients without gastrointestinal tuberculosis irrespective of the temporal relationship with proton pump inhibitor treatment or disease activity. PPI: Proton pump inhibitor.

Table 5 Results of the multivariable analysis using logistic regression

Factor	Relative risk (95%CI)	*P*-value
PPI exposure		
Group 1	Reference 1	
Group 2	0.697 (0.083-5.891)	0.74
Age, increase by year	0.972 (0.923-1.023)	0.27
Charlson index, increase by score 1	1.518 (1.040-2.216)	0.03
Sex	1.697 (0.374-7.690)	0.49
History of admission	4.317 (0.501-37.220)	0.18

PPI: Proton pump inhibitor.

tuberculosis, but non-caseating granuloma was observed in only 2 of the 7 patients with intestinal tuberculosis, and PCR was positive in 1 of the 2 patients who showed granuloma.

The endoscopic findings of gastrointestinal tuberculosis are often nonspecific[12,30]. Intestinal tuberculosis and Crohn's disease are chronic inflammatory bowel disorders that are difficult to differentiate from each other[31,32]. A study on colonoscopic findings reported that four parameters (involvement of fewer than 4 segments, a patulous ileocecal valve, transverse ulcers, and scars or pseudopolyps) were more frequently observed in intestinal tuberculosis patients than in Crohn's disease patients. Four parameters (anorectal lesions, longitudinal ulcers, aphthous ulcers, and cobblestone appearance) were significantly more common in Crohn's disease patients than in intestinal tuberculosis patients. A systematic analysis of the 8 parameters of colonoscopy was very useful in the differential diagnosis as it could differentiate between intestinal tuberculosis and Crohn's disease with 87.5% accuracy[33]. In the endemic areas of tuberculosis, it seems reasonable to prescribe anti-tuberculosis medications for patients with endoscopic findings favoring intestinal tuberculosis even if there is no specific histological finding from the biopsy specimens[12]. In the present study, anti-tuberculosis medications were also prescribed for 5 patients with only nonspecific chronic inflammations found through biopsy. Intestinal tuberculosis was confirmed in all 5 patients through follow-up colonoscopy, which revealed good therapeutic responses as well as symptom relief.

This study has a number of strengths. First, to these authors' knowledge, this study is the first study that evaluated PPI's role in the development of gastrointestinal tuberculosis. Second, all the data in this study were extracted from the SNUH CDW system that is fully synchronized with the EMR system and is optimized for research. Using the SNUH CDW system, 61 834 patients among the over one million patients who visited SNUH during the 5-year study period were rapidly screened, and the patients with gastrointestinal tuberculosis were sensitively sought out *via* browsing endoscopy and pathology reports. Most of all, compared to cohort studies using a public database, through which only the cumulative incidence rate could be estimated, it is notable that the true incidence rate of gastrointestinal tuberculosis in PPI-treated patients was calculated. The calculated incidence rate was as low as 0.65 per 1000 person-years in group 1 and 0.03 per 1000 person-years in group 2, and anti-tuberculosis medications were shown to be effective in all the patients with active gastrointestinal tuberculosis. Therefore, the risk of acquiring gastrointestinal tuberculosis does not seem to be clinically significant in PPI-treated patients.

This study, however, has several key limitations. First, the study was performed with patients from a single urbanized tertiary care hospital. In general, a hospital cohort is vulnerable to selection bias, and the results have poor generalizability. In South Korea, a single compulsory medical insurance takes effect. Due to the open medical delivery system, all the patients are practically free to visit tertiary care hospitals. In this study, 64% of the patients fall under Charlson index 0. Therefore, their possible difference from primary care patients in the aspect of clinical severity does not seem substantial. Another limitation of a hospital cohort is its dynamic nature, which enables the members to easily join or drop out. Fortunately, even if this study was performed in PPI-treated patients during a 5-year period, the patients could be followed up for 21.8 mo in group 1 and for 34.4 mo in group 2. Therefore, the adherence of the patients in this study seems to have been good.

The second limitation is the potentially different diagnostic sensitivity. This study is an observational study. Therefore, the patients did not necessarily undergo gastrointestinal endoscopy. The implementation of endoscopy was determined according to the patient's symptoms/signs, patient's will, and doctor's decision. There might have been differences in these factors between group 1 and group 2. In this study, most of the 12 patients with only ileo-colonic scars were females and underwent colonoscopy due to mild symptoms or as a routine check. Half of them belonged to group 2, showing a relatively higher incidence in group 2 compared to 1 in the patients with active gastrointestinal tuberculosis (Table 1). Presumably, this may be due to the more frequent requests for routine check-up from or the

stricter adherence to the hospital of female patients than male patients. In the 9 patients diagnosed with active gastrointestinal tuberculosis, however, there were nearly equal numbers of male and female patients. Endoscopy was performed to assess bloody diarrhea, anemia, and severe pain, which seemed to be symptoms suggesting active gastrointestinal tuberculosis (Table 1). In this study, statistical analysis was performed in the patients with active gastrointestinal tuberculosis, which could suggest that the results of this study were not highly biased by the patients' will or adherence.

In conclusion, long-term PPI therapy does not seem to be associated with increased risk of acquiring gastrointestinal tuberculosis whereas a higher Charlson comorbidity index is associated with such. These results, however, may not exempt further monitoring due to the small case number.

COMMENTS

Background

Proton pump inhibitors (PPIs) are currently the most powerful gastric acid suppressants. Several studies have associated acid suppression with increased risk of acquiring gastrointestinal infectious diseases, such as *Clostridium difficile* infection.

Research frontiers

Gastrectomy has been known to be a potential risk factor for tuberculosis, and gastric-acid suppression may play a role in its pathogenesis. There has been no report about a possible association between PPI use and gastrointestinal tuberculosis.

Innovations and breakthroughs

Seoul National University Hospital Clinical Data Warehouse is a database that is fully synchronized with an electronic medical recording system. Using the high-quality dataset, gastrointestinal-tuberculosis patients were efficiently screened, and the true incidence and relative risk of longer PPI treatment were calculated.

Applications

Long-term PPI therapy does not seem to be associated with increased risk of acquiring gastrointestinal tuberculosis.

Peer review

The response to therapy must be evaluated very strictly as this was adopted as a diagnostic criterion.

REFERENCES

1 **Guarner F**, Malagelada JR. Gut flora in health and disease. *Lancet* 2003; **361**: 512-519 [PMID: 12583961 DOI: S0140-6736(03)12489-0]

2 **Hill MJ**. Diet and the human intestinal bacterial flora. *Cancer Res* 1981; **41**: 3778-3780 [PMID: 7260945]

3 **Husebye E**. The pathogenesis of gastrointestinal bacterial overgrowth. *Chemotherapy* 2005; **51** Suppl 1: 1-22 [PMID: 15855746 DOI: 81988]

4 **Howden CW**, Hunt RH. Relationship between gastric secretion and infection. *Gut* 1987; **28**: 96-107 [PMID: 3546004]

5 **Simon GL**, Gorbach SL. Intestinal flora in health and disease. *Gastroenterology* 1984; **86**: 174-193 [PMID: 6357937]

6 **Leonard J**, Marshall JK, Moayyedi P. Systematic review of the risk of enteric infection in patients taking acid suppression. *Am J Gastroenterol* 2007; **102**: 2047-256; quiz 2057 [PMID: 17509031 DOI: AJG1275]

7 **Dial S**, Delaney JA, Schneider V, Suissa S. Proton pump inhibitor use and risk of community-acquired Clostridium difficile-associated disease defined by prescription for oral vancomycin therapy. *CMAJ* 2006; **175**: 745-748 [PMID: 17001054 DOI: 175/7/745]

8 **Dial S**, Delaney JA, Barkun AN, Suissa S. Use of gastric acid-suppressive agents and the risk of community-acquired Clostridium difficile-associated disease. *JAMA* 2005; **294**: 2989-2995 [PMID: 16414946 DOI: 294/23/2989]

9 **Miner PB**, Allgood LD, Grender JM. Comparison of gastric pH with omeprazole magnesium 20.6 mg (Prilosec OTC) o.m. famotidine 10 mg (Pepcid AC) b.d. and famotidine 20 mg b.d. over 14 days of treatment. *Aliment Pharmacol Ther* 2007; **25**: 103-109 [PMID: 17229225 DOI: APT3129]

10 WHO global tuberculosis control report 2010. Summary. *Cent Eur J Public Health* 2010; **18**: 237 [PMID: 21361110]

11 **Horvath KD**, Whelan RL. Intestinal tuberculosis: return of an old disease. *Am J Gastroenterol* 1998; **93**: 692-696 [PMID: 9625110 DOI: S0002927098000872]

12 **Marshall JB**. Tuberculosis of the gastrointestinal tract and peritoneum. *Am J Gastroenterol* 1993; **88**: 989-999 [PMID: 8317433]

13 **Snider DE**, Roper WL. The new tuberculosis. *N Engl J Med* 1992; **326**: 703-705 [PMID: 1736110 DOI: 10.1056/NEJM199203053261011]

14 **Snider DE**. Tuberculosis and gastrectomy. *Chest* 1985; **87**: 414-415 [PMID: 3979126]

15 **Steiger Z**, Nickel WO, Shannon GJ, Nedwicki EG, Higgins RF. Pulmonary tuberculosis after gastric resection. *Am J Surg* 1976; **131**: 668-671 [PMID: 937642]

16 **Allison ST**. Pulmonary tuberculosis after subtotal gastrectomy. *N Engl J Med* 1955; **252**: 862-863 [PMID: 14370443 DOI: 10.1056/NEJM195505192522006]

17 **Lee YJ**, Yang SK, Myung SJ, Byeon JS, Park IG, Kim JS, Lee GH, Jung HY, Hong WS, Kim JH, Min YI. [The usefulness of colonoscopic biopsy in the diagnosis of intestinal tuberculosis and pattern of concomitant extra-intestinal tuberculosis]. *Korean J Gastroenterol* 2004; **44**: 153-159 [PMID: 15385724 DOI: 200409153]

18 **Sundararajan V**, Quan H, Halfon P, Fushimi K, Luthi JC, Burnand B, Ghali WA. Cross-national comparative performance of three versions of the ICD-10 Charlson index. *Med Care* 2007; **45**: 1210-1215 [PMID: 18007172 DOI: 10.1097/MLR.0b013e3181484347]

19 **Charlson ME**, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis* 1987; **40**: 373-383 [PMID: 3558716]

20 **Al Karawi MA**, Mohamed AE, Yasawy MI, Graham DY, Shariq S, Ahmed AM, al Jumah A, Ghandour Z. Protean manifestation of gastrointestinal tuberculosis: report on 130 patients. *J Clin Gastroenterol* 1995; **20**: 225-232 [PMID: 7797832]

21 **Kalvaria I**, Kottler RE, Marks IN. The role of colonoscopy in the diagnosis of tuberculosis. *J Clin Gastroenterol* 1988; **10**: 516-523 [PMID: 3053873]

22 **Tandon HD**, Prakash A. Pathology of intestinal tuberculosis and its distinction from Crohn's disease. *Gut* 1972; **13**: 260-269 [PMID: 5033841]

23 **Bhargava DK**, Kushwaha AK, Dasarathy S, Shriniwas P. Endoscopic diagnosis of segmental colonic tuberculosis. *Gastrointest Endosc* 1992; **38**: 571-574 [PMID: 1397913]

24 **Singh V**, Kumar P, Kamal J, Prakash V, Vaiphei K, Singh K. Clinicocolonoscopic profile of colonic tuberculosis. *Am J Gastroenterol* 1996; **91**: 565-568 [PMID: 8633510]

25 **Shah S**, Thomas V, Mathan M, Chacko A, Chandy G, Ramakrishna BS, Rolston DD. Colonoscopic study of 50 patients with colonic tuberculosis. *Gut* 1992; **33**: 347-351 [PMID: 1568653]

26 **Bhargava DK**, Tandon HD, Chawla TC, Shriniwas BN, Kapur BM. Diagnosis of ileocecal and colonic tuberculosis by colonoscopy. *Gastrointest Endosc* 1985; **31**: 68-70 [PMID: 3922847]

27 **Pettengell KE**, Larsen C, Garb M, Mayet FG, Simjee AE, Pirie D. Gastrointestinal tuberculosis in patients with pulmonary tuberculosis. *Q J Med* 1990; **74**: 303-308 [PMID: 2385737]
28 **Sakai Y**. Colonoscopic diagnosis of the intestinal tuberculosis. *Mater Med Pol* 1979; **11**: 275-278 [PMID: 548670]
29 **Anand BS**, Schneider FE, El-Zaatari FA, Shawar RM, Clarridge JE, Graham DY. Diagnosis of intestinal tuberculosis by polymerase chain reaction on endoscopic biopsy specimens. *Am J Gastroenterol* 1994; **89**: 2248-2249 [PMID: 7977255]
30 **Breiter JR**, Hajjar JJ. Segmental tuberculosis of the colon diagnosed by colonoscopy. *Am J Gastroenterol* 1981; **76**: 369-373 [PMID: 7325151]
31 **Yang XS**, Zhang L, Shi XY, Zhang YL, Lv YM. [Current diagnostic status, clinical and pathologic manifestation for a Crohn's disease]. *Beijing Da Xue Xue Bao* 2006; **38**: 407-410 [PMID: 16892148]
32 **Liu TH**, Pan GZ, Chen MZ. Crohn's disease. Clinicopathologic manifestations and differential diagnosis from enterocolonic tuberculosis. *Chin Med J (Engl)* 1981; **94**: 431-440 [PMID: 6796347]
33 **Lee YJ**, Yang SK, Byeon JS, Myung SJ, Chang HS, Hong SS, Kim KJ, Lee GH, Jung HY, Hong WS, Kim JH, Min YI, Chang SJ, Yu CS. Analysis of colonoscopic findings in the differential diagnosis between intestinal tuberculosis and Crohn's disease. *Endoscopy* 2006; **38**: 592-597 [PMID: 16673312 DOI: 10.1055/s-2006-924996]

P- Reviewer Misra SP **S- Editor** Gou SX **L- Editor** O'Neill M
E- Editor Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i2.265

World J Gastroenterol 2013 January 14; 19(2): 265-273
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Clinical outcomes and predictive factors in oral corticosteroid-refractory active ulcerative colitis

Han Ho Jeon, Hyun Jung Lee, Hui Won Jang, Jin Young Yoon, Yoon Suk Jung, Soo Jung Park, Sung Pil Hong, Tae Il Kim, Won Ho Kim, Jae Hee Cheon

Han Ho Jeon, Hyun Jung Lee, Hui Won Jang, Jin Young Yoon, Yoon Suk Jung, Soo Jung Park, Sung Pil Hong, Tae Il Kim, Won Ho Kim, Jae Hee Cheon, Department of Internal Medicine, Institute of Gastroenterology, Yonsei University College of Medicine, Seoul 120-752, South Korea

Won Ho Kim, Jae Hee Cheon, Brain Korea 21 Project for Medical Science, Yonsei University College of Medicine, Seoul 120-752, South Korea

Author contributions: Jeon HH performed the data analysis and wrote the manuscript; Cheon JH had original idea on this subject, performed colonoscopy, performed the data analysis, and wrote the manuscript; Lee HJ, Jang HW, Yoon JY, Jung YS, Park SJ, Hong SP, Kim TI, and Kim WH performed colonoscopy and reviewed the manuscript critically.

Correspondence to: Jae Hee Cheon, MD, PhD, Department of Internal Medicine, Yonsei University College of Medicine, 50 Yonsei-ro, Seodaemun-gu, Seoul 120-752, South Korea. geniushee@yuhs.ac

Telephone: +82-2-22281990 Fax: +82-2-3936884

Received: September 14, 2012 Revised: November 13, 2012
Accepted: November 24, 2012
Published online: January 14, 2013

Abstract

AIM: To evaluate the clinical outcomes and prognostic factors after intravenous corticosteroids following oral corticosteroid failure in active ulcerative colitis patients.

METHODS: Consecutive patients with moderate to severe ulcerative colitis who had been treated with a course of intravenous corticosteroids after oral corticosteroid therapy failure between January 1996 and July 2010 were recruited at Severance Hospital, Seoul, South Korea. The disease activity was measured by the Mayo score, which consists of stool frequency, rectal bleeding, mucosal appearance at flexible sigmoidoscopy, and Physician Global Assessment. We retrospectively evaluated clinical outcomes at two weeks, one month, three months, and one year after the initiation of intravenous corticosteroid therapy. Two weeks outcomes were classified as responders or non-responders. One month, three month and one year outcomes were classified into prolonged response, steroid dependency, and refractoriness.

RESULTS: Our study included a total of 67 eligible patients. At two weeks, 56 (83.6%) patients responded to intravenous corticosteroids. At one month, complete remission was documented in 18 (32.1%) patients and partial remission in 26 (46.4%). Eleven patients (19.7%) were refractory to the treatment. At three months and one year, we found 37 (67.3%) and 25 (46.3%) patients in prolonged response, ten (18.2%) and 23 (42.6%) patients in corticosteroid dependency, 8 (14.5%) and 6 (11.1%) patients with no response, respectively. Total 9 patients were underwent elective proctocolectomy within 1 year. The duration of oral corticosteroid therapy (> 14 d *vs* ⩽ 14 d, $P = 0.049$) and lower hemoglobin level (⩽ 11.0 mg/dL *vs* >11.0 mg/dL, $P = 0.02$) were found to be poor prognostic factors for response at two weeks. For one year outcome, univariate analysis revealed that only a partial Mayo score (⩾ 6 *vs* <6, $P = 0.057$) was found to be associated with a poor response.

CONCLUSION: The duration of oral corticosteroid therapy and lower hemoglobin level were strongly associated with poor outcome.

© 2013 Baishideng. All rights reserved.

Key words: Clinical outcome; Prognosis; Corticosteroid; Ulcerative colitis

Jeon HH, Lee HJ, Jang HW, Yoon JY, Jung YS, Park SJ, Hong SP, Kim TI, Kim WH, Cheon JH. Clinical outcomes and predictive factors in oral corticosteroid-refractory active ulcerative colitis. *World J Gastroenterol* 2013; 19(2): 265-273 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i2/265.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i2.265

INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory disease of the colon with unknown etiology and is characterized by a typical natural course with recurrent flares of mucosal inflammation. About 15% of patients with UC have been reported to have an overall chance of acute exacerbation and require admission to hospital and treatment with systemic corticosteroids[1,2]. Systemic corticosteroids remain as the gold standard treatment of acute moderate to severe UC. However, about 15%-57% of UC patients remain steroid-dependent or refractory even after steroid treatment[3]. Before the mid-1990s, proctocolectomy was the only treatment in steroid-unresponsive UC patients. Recently, intensive drug therapies have been shown to be effective in reducing and delaying the need for colectomy[4,5].

For UC patients who fail to improve with a maximal dosage of 5-aminosalicylic acids, oral steroid therapy should be considered. Moreover, for those who also do not respond to orally administered steroids, hospital admission is usually required for intensive intravenous treatment[6,7]. However, to the best of our knowledge, there has been no published study describing the clinical course of patients with acute attack of UC who were treated first with oral corticosteroids and subsequently with intravenous corticosteroids due to oral steroid failure. Before demonstrating the efficacy of second line drugs, the response and the clinical outcomes of intravenous corticosteroid therapy after oral administration need to be clarified. In addition, identification of early predictors of intravenous corticosteroid responsiveness could facilitate appropriate patient selection and well-timed administration of second line therapies in such UC patients. Clinically, earlier detection of response is important to help estimate risks, benefits, and duration of treatment, as this will enable the use of alternative drugs before complications of long term use of systemic corticosteroids can develop. However, data on predictive response factors of intravenous corticosteroids following oral corticosteroid therapy are lacking.

Here we sought to identify predictive clinical or biological factors associated with intravenous corticosteroid responsiveness in UC patients that initially did not respond to oral corticosteroids, as well as two week, one month, three month, and one year clinical outcomes of such treated patients with acute attack of UC.

MATERIALS AND METHODS

Patients

The clinical records of patients with active UC who were treated with intravenous corticosteroids immediately after treatment failure of oral corticosteroids at the Yonsei University College of Medicine, Seoul, South Korea between January 1996 and July 2010 were retrospectively evaluated. The diagnosis of ulcerative colitis was based on the accepted clinical, endoscopic, and histopathological criteria[8]. The criteria for eligibility were male or female patients with a diagnosis of UC followed regularly for at least 1 year. The exclusion criteria were patients with a history of corticosteroid therapy at other hospitals, corticosteroid use for diseases other than UC and a follow-up duration of less than 1 year. All enrolled patients were initiated intravenous corticosteroid therapy after admission after failure of oral steroid therapy which was done at outpatient clinic.

Intravenous corticosteroid therapy was initiated with intravenous administration of 100 mg of hydrocortisone every eight hours. Intravenous corticosteroid therapy was continued for 1-2 wk, with the treatment duration depending on the individual patient conditions, followed by gradual tapering of corticosteroids. After clinical improvement of UC, the dose of intravenous hydrocortisone was reduced to 200 mg daily. If the patients had no clinical exacerbation of UC, they were administered 30 mg/d of oral corticosteroid therapy before discharge. Our oral corticosteroid tapering policy was to reduce prednisolone by 5 or 10 mg weekly for patients with improved clinical symptoms but to sustain the current dose of prednisolone for one week for patients with lasting clinical symptoms[9,10]. All patients who were concomitantly taking sulfasalazine (2-4 g/d) or mesalamine (1.5-4 g/d) at the time of flare up continued therapy. Patients who took immuomodulators at the time of acute flare up also maintained their initial immuomodulator therapy while taking corticosteroids.

Our study is a retrospective study from a prospectively collected database. The data are stored in a form of Assess file as well as paper form. After then, the questionnaire including Mayo or partial Mayo scores including Physician Global Assessment (PGA) is updated every visit of the patient to outpatient clinics.

The disease activity was measured by the Mayo score, which consists of stool frequency, rectal bleeding, mucosal appearance at flexible sigmoidoscopy, and PGA[11,12]. Each component was scored 0 to 3 points, and the total score ranged from 0 to 12 points. However, a measurement of this score necessarily requires invasive flexible sigmoidoscopy, which limits repeated measurement. Therefore, most actual disease activity was measured on a nine-point partial Mayo score, which excluded the mucosal appearance at endoscopy[13]. At the initiation of oral corticosteroid therapy, demographic data including age, age at diagnosis, duration, gender, number of acute attacks, extent of disease, concomitant medications, duration of oral corticosteroid therapy, Mayo score, and partial Mayo score were collected. Partial Mayo score and laboratory parameters were also recorded at two weeks, one month, three months, and one year after the time of initiation of intravenous corticosteroid therapy. This study was approved by the institutional board of Severance Hospital.

Definitions

Clinical outcome was measured at two weeks, one month,

three months, and one year after the initiation of intravenous corticosteroids. The classification of response to intravenous corticosteroid therapy was adopted from previous studies with minor modification[3,11,14,15]. Patients at two weeks were classified as responders or non-responders. Non-responder of intravenous therapy after oral corticosteroid therapy was defined as persistent active disease despite administration of intravenous corticosteroids over two weeks, death due to UC attack before day 14, proctocolectomy before day 14, or secondary alternative drug use such as cyclosporine and infliximab before day 14. Proctocolecctomy was performed when the patient had intractable bloody diarrhea, a continued PGA score of 3, severe anemia, persistent abdominal pain, or severe malnutrition despite intensive medical treatment. One month outcomes were classified as complete remission, partial remission, or refractoriness. Complete remission was defined as a stool frequency ≤ 2/d with no rectal bleeding, stool urgency, fever, or any other systemic symptoms, and a PGA score of 0. Partial remission was defined as stool frequency ≤ 4/d or ≤ 50% of initial stool frequency with regression of other clinical symptoms and a PGA score of 1 or 2. Refractoriness was defined as persistent active status despite administration of prednisolone up to 30 mg/d or the equivalent dose over the period of four weeks[14]. Three month outcomes were classified into prolonged response, steroid dependency, and refractoriness. Prolonged response was defined as sustained complete or partial remission during the planned dose reduction or after the completion of corticosteroids[3]. Steroid dependency was defined as need for the same corticosteroid dose for more than two weeks despite clinical improvement, requiring an increased dose, or restarting corticosteroid therapy within two weeks because of exacerbation of symptoms[11,14]. Refractoriness was defined as no improvement of clinical symptoms despite continued corticosteroid use. Similarly, outcomes at one year were subdivided into three groups. Prolonged response was characterized by two conditions: maintaining complete or partial remission after discontinuation of corticosteroid therapy and requiring the same dose for more than two weeks or an increasing dose of corticosteroids only in the first three months. Steroid dependency was defined as restart of corticosteroid therapy due to recurrent flare-up of UC after the first three months or being unable to reduce prednisolone to 10 mg/day within three months. Non-response was defined in the same way as for the intermediate outcomes.

Statistical analysis

Continuous variables were presented as the mean ± SD or median (range) and were compared using two-sample *t* tests. Categorical variables were compared by χ^2 tests or Fisher's exact test. Logistic regression analysis was performed to identify predictive variables of clinical outcomes. *P* values less than 0.05 were considered statistically significant. All the statistical analyses were performed using the statistical software package SPSS 12.0 for Windows (SPSS Inc., Chicago, IL).

Table 1 Baseline characteristics of patients with active ulcerative colitis receiving intravenous corticosteroids after oral corticosteroid therapy failure

	Total patients (*n* = 67)
Gender (male/female)	39/28 (58.2/41.8)
Age (yr)	35 (13-78)
Age at diagnosis (yr)	31 (11-78)
Disease duration (mo)	24 (0-132)
Disease extent	
Proctitis	5 (7.5)
Left-sided colitis	21 (31.3)
Extensive colitis	41 (61.2)
First attack of UC	32 (47.8)
Number of previous flares	2 (1-4)
Initial disease activity	
Full Mayo score	9 (5-10)
Partial Mayo score	6 (3-7)
Initial prednisolone dose (mg)	
≥ 30 and < 40	35 (52.2)
≥ 40	32 (47.8)
Duration of oral corticosteroid use (d)	13 (3-50)
Maintenance before flare up	
None/salicylates/azathioprine	6/47/14
Concomitant medications	
Salicylates	53
Azathioprine	14
CRP (mg/dL)	10.9 (0.1-153.0)
ESR (mm/h)	40.0 (3.0-120.0)
Hemoglobin (mg/dL)	11.7 (6.4-16.5)
Albumin (mg/dL)	3.6 (2.3-5.0)

Data are expressed as absolute numbers (percentage) or median. UC: Ulcerative colitis; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate.

RESULTS

Patient characteristics

A total of 67 patients, 39 men and 28 women, with active UC were included in this study (Table 1). The median age at diagnosis was 31 years (range: 11-78 years), and median disease duration at the time of oral corticosteroid therapy was 24 mo (range: 0-132 mo). The median length of oral corticosteroid use before intravenous therapy was 13 d (range: 3-50 d). Forty-one (61.2%) patients had extensive disease, and 32 (47.8%) had their first attack of active UC.

Clinical outcomes after intravenous corticosteroid therapy

Clinical outcomes of patients were organized in a flow chart (Figure 1). At two weeks after treatment, 56 (83.6%) patients responded to intravenous systemic corticosteroids. Seven of the 11 non-responders underwent proctocolectomy before day 14. Three patients were treated with an intravenous tumor necrosis factor-α blocker, and one patient was treated with intravenous cyclosporine. At one month after treatment, 18 (32.1%) patients were in complete remission, 26 (46.4%) in partial remission, and 12 (21.5%) had no response. Collectively, 21.5% of

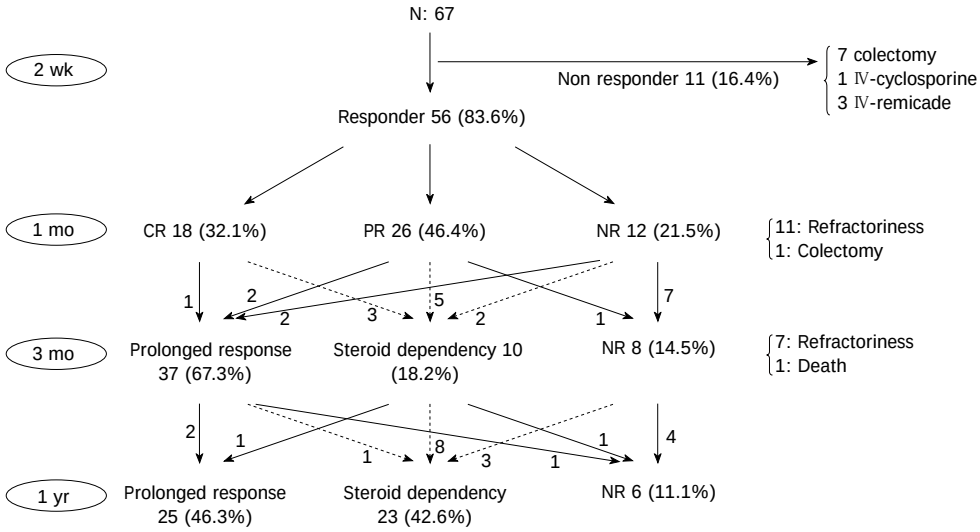


Figure 1 Clinical outcomes of patients with active ulcerative colitis treated with intravenous corticosteroids in oral corticosteroid refractory patients. CR: Complete remission; PR: Partial remission; NR: Non-response.

patients showed treatment failure to systemic corticosteroid therapy, and the other 78.5% of patients showed a partial or complete response to intravenous corticosteroid therapy. At three months, 37 patients had prolonged response, ten had steroid dependency, and seven showed no response. Of the 11 patients who were refractory to corticosteroid therapy at one month, two were in partial remission, two were steroid-dependent, seven had persistent refractoriness at three months, and one patient, a 59-year-old man who underwent proctocolectomy, died of pneumonia and sepsis. At one year, 25 of the 67 patients (37.3%) were categorized with prolonged response, 23 as steroid-dependent (34.3%), and six as non-responders (9.0%).

In our study, cytomegalovirus infection was detected in 5 patients. All were diagnosed by histologic examinations and were treated with ganciclovir. Of these, one patient underwent proctocolectomy within 14 d after the treatment. The rest of them responded to ganciclovir treatment. Finally, three patients were in partial remission and one patient was steroid dependent at one year.

Predictive factors for favorable outcomes

We performed univariate and multivariate analyses to detect clinical or laboratory factors capable of predicting poor outcomes of intravenous corticosteroid therapy after oral corticosteroid therapy failure. For the evaluation of intravenous corticosteroids at two weeks, we divided all patients into two groups: responders and non-responders who experienced death, proctocolectomy, or secondary medical treatment before two weeks. Patients were also divided into good responders and poor responders at one month, three months, and at one year after the corticosteroids therapy: good responders were those who showed a prolonged response, and poor responders were steroid-dependent or non-responsive. Multivariate analysis was carried out using the factors that were found to be statistically significant by univariate analysis.

For two weeks outcomes, univariate analysis of predictors for non-responders showed that disease duration (> 24 mo), duration of oral corticosteroids use (> 14 days), and lower hemoglobin level (≤ 11 mg/dL) were associated with poor prognosis. According to multivariate analysis, the duration of oral corticosteroids use (> 14 d *vs* ≤ 14 d, $P = 0.049$) and lower hemoglobin level (≤ 11.0 mg/dL *vs* > 11.0 mg/dL, $P = 0.02$) remained predictive factors for non-responders (Table 2). No predictive factors for poor responders at one month or three months were identified (Tables 3 and 4). For one year outcome, univariate analysis revealed that only a partial Mayo score (≥ 6 *vs* < 6, $P = 0.057$) was found to be associated with a poor response (Table 5).

DISCUSSION

We analyzed the clinical outcomes and identified predictive factors associated with corticosteroid responsiveness of patients who had an acute attack of UC and had been administered intravenous corticosteroids after a previous course of systemic oral corticosteroid therapy. There have been many previous studies that have reported the predictors of clinical response to systemic corticosteroid treatment in moderate to severe active UC patients[16-19]. However, there are few reports of outcomes of a conservative approach of intravenous steroid treatment in moderate to severe active UC patients after oral corticosteroid therapy failure.

In our study, intravenous corticosteroid therapy following a failure after oral corticosteroid therapy was successful at inducing response at two weeks of treatment in 83.6% of patients. This result implies that intravenous corticosteroids could be administered when a lack of response to oral corticosteroid therapy is shown. This

Table 2 Comparison of clinical factors in immediate outcomes of the two groups of active ulcerative colitis patients receiving intravenous corticosteroids after oral corticosteroid therapy failure

	Responders (n = 56)	Non-responders[1] (n = 11)	P-value		Odds ratio (95%CI)
			Univariate	Multivariate	
Gender (M/F)	32/24 (57.1/42.9)	7/4 (63.6/36.4)	0.690		
Age (yr)	37 (13-78)	39 (29-50)	0.458		
Disease duration (mo)					
≤ 24	34 (60.7)	3 (27.3)	0.041	0.123	3.38 (0.72-15.88)
> 24	22 (39.3)	8 (72.7)			
First attack of UC	26 (46.4)	6 (54.5)	0.273		
Disease extent			0.884		
Proctitis	4 (7.1)	1 (9.1)			
Left-sided colitis	17 (30.4)	4 (36.4)			
Extensive colitis	35 (62.5)	6 (54.5)			
Disease activity					
Full Mayo score (< 9/≥ 9)	27/24 (52.9/47.1)	3/8 (27.3/72.7)	0.122		
Partial Mayo score (< 6/≥ 6)	14/42 (25/75)	1/10 (9.1/90.9)	0.227		
Initial prednisolone dose (mg)			0.622		
≥ 30 and < 40	29 (51.8)	6 (54.5)			
≥ 40	27 (48.2)	5 (45.5)			
Duration of oral corticosteroid use (d)					
≤ 14	33 (58.9)	3 (27.3)	0.054	0.049	4.9 (1.01-23.81)
> 14	23 (41.1)	8 (72.7)			
Concomitant medications			0.809		
Salicylates	44 (78.6)	9 (81.8)			
Azathioprine	12 (21.4)	2 (18.2)			
CRP (mg/dL)					
≤ 8	37 (66.1)	11 (84.6)	0.303		
> 8	19 (33.9)	2 (15.4)			
ESR (mm/h)	40 (3-120)	40 (12-83)	0.999		
Hemoglobin (mg/dL)					
≤ 11	18 (32.1)	8 (72.7)	0.012	0.02	0.16 (0.03-0.75)
> 11	38 (67.9)	3 (27.3)			
Albumin (mg/dL)	3.63 (2.3-5.0)	3.56 (2.8-4.8)	0.331		

[1]Non-responder group included patients that had secondary alternative drug use, proctocolectomy and death before day 14. M/F: Male/female; UC: Ulcerative colitis; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate.

result was partly explained by previous pharmacokinetic studies, which demonstrated delayed prednisolone absorption after an oral dose in patients with acute colitis as compared to healthy controls and higher plasma levels after intravenous steroid administration[20,21]. In addition, intravenous steroid administration in the hospital ensures good treatment compliance.

The rate of failure of intravenous corticosteroid therapy in moderate to severe UC attacks was quite high, accounting for 30%-40% in recent studies investigating Western populations[16-19,22,23]. Moreover, Meyers *et al*[24] showed a better clinical improvement of intravenous corticotrophin therapy for acute UC in steroid-naïve patients than in patients who had already received steroid treatment. Considering that our enrolled patients were an oral corticosteroid failure group and approximately half of them were non steroid-naïve patients, our results at two weeks showed a much higher response rate to intravenous corticosteroid therapy compared with previous studies[16-19,22,23]. Moreover, In our study, 13.4% of patients underwent proctocolectomy or experienced death within one year, while the incidence of cumulative proctocolectomy was reported to be about one-fifth that of exacerbation of UC[25-27]. This difference in the results of steroid treatment reaffirms the previous report that UC patients in the Korean population have a lower cumulative probability of proctocolectomy compared to those in Western countries[28,29]. Taken together, these results suggest that Korean patients with UC might have a more favorable prognosis compared to Western counterparts. In order to investigate the clinical application of our results in the treatment of UC patients, further prospective studies are warranted.

One month after the initiation of intravenous corticosteroid therapy, 78.5% of patients (32.1% with complete response and 46.4% with partial response) showed clinical improvement, whereas 42.6% were dependent on steroids at one year. It has been previously reported that 22% of patients in a western study became steroid-dependent[3]. Recently, Yoon *et al*[10] reported that a steroid-naïve Korean UC patient showed a good response to steroid treatment, while 40% of UC patients eventually became steroid-dependent or refractory. Our study showed that the responses to intravenous corticosteroids following a failure after oral corticosteroid treatment were similarly favorable in the short term period. However, more than half of patients eventually became steroid-dependent or refractory in the long term.

Table 3 Comparison of clinical factors in one month outcomes of the two groups of active ulcerative colitis patients receiving intravenous corticosteroids after oral corticosteroid therapy failure

	Good responders[1] (*n* = 44)	Poor responders[2] (*n* = 12)	*P*-value univariate
Gender (M/F)	24/20 (54.5/45.5)	8/4 (66.7/33.3)	0.956
Age (yr)	37 (11-78)	38 (29-59)	0.342
Disease duration (mo)			
≤ 24	25 (56.8)	9 (75)	0.253
> 24	19 (43.2)	3 (3)	
First attack of UC	18 (40.9)	8 (66.7)	0.113
Disease extent			0.943
Proctitis	3 (6.8)	1 (8.3)	
Left-sided colitis	13 (29.5)	4 (33.3)	
Extensive colitis	28 (63.6)	7 (58.3)	
Disease activity			
Full Mayo score (< 9/≥ 9)	23/19 (54.8/45.2)	4/5 (44.4/55.6)	0.574
Partial Mayo score (< 6/≥ 6)	12/32 (27.3/72.7)	2/10 (16.7/83.3)	0.452
Initial prednisolone dose (mg)			0.429
≥ 30 and < 40	24 (54.5)	5 (41.7)	
≥ 40	20 (45.5)	7 (58.3)	
Duration of oral corticosteroid use (d)			
≤ 14	26 (59.1)	7 (58.3)	0.962
> 14	18 (40.9)	5 (41.7)	
Concomitant medications			0.212
Salicylates	33 (75)	11 (91.7)	
Azathioprine	11 (25)	1 (8.3)	
CRP (mg/dL)			
≤ 8	28 (63.6)	9 (75)	0.461
> 8	16 (36.4)	3 (25)	
ESR (mm/h)	40.7 (3-120)	37.2 (12-83)	0.464
Hemoglobin (mg/dL)			
≤ 11	15 (34.1)	3 (25)	0.550
> 11	29 (65.9)	9 (75)	
Albumin (mg/dL)	3.6 (2.3-5.0)	3.45 (2.6-4.8)	0.295

[1]Good responders included patients with prolonged response that maintained complete remission or partial remission after completion of corticosteroid therapy; [2]Poor responders included patients with steroid dependence and non-response. M/F: Male/female; UC: Ulcerative colitis; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate.

Table 4 Comparison of clinical factors in three month outcomes of the two groups of active ulcerative colitis patients receiving intravenous corticosteroids after oral corticosteroid therapy failure

	Good responders[1] (*n* = 37)	Poor responders[2] (*n* = 18)	*P*-value univariate
Gender (M/F)	21/16 (56.8/43.2)	10/8 (55.6/44.4)	0.933
Age (yr)	35 (13-78)	41 (20-62)	0.146
Disease duration (mo)			
≤ 24	22 (59.5)	12 (66.7)	0.606
> 24	15 (40.5)	6 (33.3)	
First attack of UC	15 (40.5)	10 (55.6)	0.294
Disease extent			0.317
Proctitis	3 (8.1)	1 (5.6)	
Left-sided colitis	9 (24.3)	8 (44.4)	
Extensive colitis	25 (67.6)	9 (50)	
Disease activity			
Full Mayo score (< 9/≥ 9)	20/14 (58.8/41.2)	7/9 (43.8/56.3)	0.318
Partial Mayo score (< 6/≥ 6)	11/26 (29.7/70.3)	3/15 (16.7/83.3)	0.297
Initial prednisolone dose (mg)			0.925
≥ 30 and < 40	19 (51.4)	9 (50)	
≥ 40	18 (48.6)	9 (50)	
Duration of oral corticosteroid use (d)			0.639
≤ 14	23 (62.2)	10 (55.6)	
> 14	14 (37.8)	8 (44.4)	
Concomitant medications			0.180
Salicylates	27 (73)	16 (88.9)	
Azathioprine	10 (27)	2 (11.1)	
CRP (mg/dL)			
≤ 8	24 (64.9)	12 (66.7)	0.895
> 8	13 (35.1)	6 (33.3)	
ESR (mm/h)	36.9 (3-120)	47.6 (6-120)	0.251
Hemoglobin (mg/dL)			
≤ 11	14 (37.8)	4 (22.2)	0.247
> 11	23 (62.2)	14 (77.8)	
Albumin (mg/dL)	3.73 (2.7-5.0)	3.44 (2.3-4.6)	0.082

[1]Good responders included patients with prolonged response that maintained complete remission or partial remission after completion of corticosteroid therapy; [2]Poor responders included patients with steroid dependence and non-response. M/F: Male/female; UC: Ulcerative colitis; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate.

Reports evaluating predictive factors for poor clinical outcomes have shown different and controversial results. Some found no predictive factors[15,24,30] while others showed several factors related to clinical outcomes[16-19,22,23]. In our study, the UC duration, the duration of oral corticosteroid use, and lower hemoglobin level were associated with failure of steroid treatment on univariate analysis. Of these variables, only duration of oral corticosteroid therapy and lower hemoglobin level were found to be independently associated with failure of corticosteroid therapy on multivariate analysis. Knowledge of the clinical factors associated with non-response to intravenous corticosteroid therapy after oral therapy may be useful in clinical decision-making. Our study was such an attempt to explain this situation. Our results showing the relationships between simple clinical factors, such as lower hemoglobin or duration of oral steroid use, and intravenous treatment outcome might help in predicting poor responders. Lower hemoglobin level as a risk factor for poor response in our study is in accordance with an earlier reports, which reflects that initial severity of disease might be a significant predictor of poor clinical outcome after steroid treatment[19,31,32]. Also, anemia is a common and important complication of IBD with a prevalence rate ranging from 8.8% to 66.6% in UC patients[33,34]. The quality of life, an ability of work, and cognitive function can be impaired because of anemia in IBD patients[35,36]. Impaired quality of life by anemia in UC patients could influence patient well-being sense and PGA. For this reason, lower hemoglobin level could be a risk factor for poor response in our study. However, none of the previous studies have evaluated duration of oral corticosteroid use before intravenous corticosteroids to predict outcome of corticosteroid therapy in patients with UC. We found that the duration of oral corticosteroid administration was an independent predictor of non-response to intravenous corticosteroid therapy on multivariate analysis in patients with acute exacerbation

Table 5 Comparison of clinical factors in one year outcomes of the two groups of active ulcerative colitis patients receiving intravenous corticosteroids after oral corticosteroid therapy failure

	Good responders[1] (*n* = 25)	Poor responders[2] (*n* = 29)	*P*-value univariate
Gender (M/F)	15/10 (60/40)	16/13 (55/45)	0.721
Age (yr)	35 (16-78)	39 (13-64)	0.445
Disease duration (mo)	30 (0-84)	29 (0-120)	0.325
≤ 24	14 (56)	20 (69)	
> 24	11 (44)	9 (31)	
First attack of UC	9 (36)	15 (51.7)	0.248
Disease extent			0.304
Proctitis	1 (4)	3 (10.3)	
Left-sided colitis	6 (24)	11 (37.9)	
Extensive colitis	18 (72)	15 (51.7)	
Disease activity			
Full Mayo score (< 9/≥ 9)	15/9 (62.5/37.5)	11/14 (44/56)	0.195
Partial Mayo score (< 6/≥ 6)	9/16 (36/64)	4/25 (13.8/86.2)	0.057
Initial prednisolone dose (mg)			0.785
≥ 30 and < 40	13 (52)	14 (48.3)	
≥ 40	12 (48)	15 (51.7)	
Duration of oral corticosteroid use (d)			0.337
≤ 14	17 (68)	16 (55.2)	
> 14	8 (32)	13 (44.8)	
Concomitant medications			0.771
Salicylates	19 (76)	23 (79.3)	
Azathioprine	6 (24)	6 (20.7)	
CRP (mg/dL)			
≤ 8	16 (64%)	19 (65.5)	0.907
> 8	9 (36%)	10 (34.5)	
ESR (mm/h)	34.1 (4-81)	46.3 (3-120)	0.171
Hemoglobin (mg/dL)			
≤ 11	9 (36)	8 (27.6)	0.507
> 11	16 (64)	21 (72.4)	
Albumin (mg/dL)	3.8 (2.7-4.7)	3.57 (2.3-5.0)	0.230

[1]Good responders included patients with prolonged response that maintained complete remission or partial remission after corticosteroid therapy had finished; [2]Poor responders included patients with steroid dependence and non-response. M/F: Male/female; UC: Ulcerative colitis; CRP: C-reactive protein; ESR: Erythrocyte sedimentation rate.

of UC. Patients with UC with more severe disease activity achieved remission less often than who tended to achieve remission earlier[37]. The duration of oral corticosteroid therapy was likely to reflect the severity nature of UC in these non-responding patients. Additionally, prolonged oral corticosteroid therapy might be associated with mortality and morbidity such as infection and sepsis in UC patients undergoing surgery. With regard to the duration of intravenous corticosteroid treatment, the limit of 7-10 d for certifying the criteria of steroid resistance was based on historical series, which showed that the median time of remission of UC was 7.5 d and that prolonged treatment beyond 10 d did not increase the remission rate[37]. In contrast with this point of view, a large retrospective study of single experienced hospital was in favor of more conservative approach, which entered into remission within the 21 d of treatment[38]. Therefore it is difficult to define resistance to corticosteroid which day after treatment is used as a limit marker. None of the previous studies have shown duration of oral corticosteroid administration was an independent predictor of non-response to intravenous corticosteroid therapy. This result demonstrates that optimal timing of intravenous corticosteroids after oral therapy was an important clinical factor of medical treatment response. In other words, use of oral corticosteroids for more than two weeks appears to be less effective in terms of clinical outcome. Additionally, long term oral corticosteroids use could lead to worse clinical outcomes and complications. An extended pre-operative use of steroids might increase the risk of surgical complications[39,40]. UC patients undergoing an elective surgery have been shown to be at an increased risk of postoperative infectious complications in patients treated with corticosteroids[40]. In our study, a total of 9 patients underwent elective proctocolectomy within 1 year. Among of them, one patient died of pneumonia and sepsis after proctocolectomy. Therefore, in the management of UC, the optimal timing of administration of intravenous corticosteroids after oral corticosteroid failure should be determined within two weeks after oral corticosteroid therapy. However, the optimal time limit with oral corticosteroid therapy in the face of response has not been clearly defined by randomized controlled trials. Therefore, large scaled, prospective studies are needed to confirm this result.

Yoon *et al*[10] reported that partial Mayo score was a predictive factor of steroid dependency in steroid-naïve patients with UC. In our study, we also showed that initial higher partial Mayo score might be associated with long term poor prognosis of corticosteroid therapy. Considered overall, the partial Mayo scores could help to predict long term potential corticosteroid dependency or refractoriness in active UC patients who are treated with systemic corticosteroid therapy.

Our study on clinical outcomes and factors for response prediction after intravenous therapy after oral corticosteroids failure in active UC patient has clear clinical significance. There have been no previously published studies describing the clinical course of patients with acute attack of UC who were treated first with oral corticosteroids and subsequently intravenous corticosteroids. This study may provide valuable clinical data that can be used in the management of UC patients receiving oral corticosteroids therapy. Such studies could also be used to suggest optimal timing for administration of intravenous corticosteroids in UC patients who are treated with oral corticosteroids. Moreover, our enrolled UC patients possessed the quality of comparatively homogenous corticosteroid therapy indication and same dose of corticosteroids. These facts might be advantageous in terms of eliminating confounding factors.

There were several limitations in our study. Our study was a retrospective study in a single tertiary hospital. Mucosal healing has emerged as an important treatment goal in UC because evidence is accumulating that it can alter the clinical course of UC. However, evidence of cortico-

steroid's ability to promote mucosal healing is limited. A considerable portion of a period in this study was at moment before mucosal healing has emerged as an emerging parameter in UC. Then we could not evaluate the mucosal healing as a parameter of clinical outcomes in this study. Moreover, our study was not a placebo-controlled comparative study. Finally, the sample size was relatively small.

In conclusion, our study showed that most Korean patients with active UC responded well to intravenous corticosteroid therapy after oral corticosteroid therapy failure. However, a considerable number of patients turned out to be refractory to or dependent on this therapy. The duration of oral corticosteroid therapy and lower hemoglobin level were strongly associated with poor outcome. Further prospective studies are warranted to confirm these results and to determine the optimal timing and dose of corticosteroids.

COMMENTS

Background

Ulcerative colitis (UC) is a chronic inflammatory disease of the colon with unknown etiology and is characterized by a typical natural course with recurrent flares of mucosal inflammation. Systemic corticosteroids remain as the gold standard treatment of acute moderate to severe UC. For those who also do not respond to orally administered steroids, hospital admission is usually required for intensive intravenous treatment. However, there has been no published study describing the clinical course of patients with acute attack of UC who were treated first with oral corticosteroids and subsequently with intravenous corticosteroids due to oral steroid failure.

Research frontiers

This study showed that the duration of oral corticosteroids use (> 14 d *vs* ⩽ 14 d, $P = 0.049$) and lower hemoglobin level (⩽ 11.0 mg/dL *vs* > 11.0 mg/dL, $P = 0.02$) was predictive factors for non-responders. Lower hemoglobin level and duration of corticosteroids may be useful in clinical decision-making as the clinical factors associated with non-response to intravenous corticosteroid therapy after oral therapy.

Innovations and breakthroughs

These results showing the relationships between lower hemoglobin or duration of oral steroid use, and intravenous treatment outcome might help in predicting poor responders. Low hemoglobin level as a risk factor for poor response is in accordance with an earlier report which reflects that initial severity of disease might be a significant predictor of poor clinical outcome. However, none of the previous studies have evaluated duration of oral corticosteroid use before intravenous corticosteroids to predict outcome of corticosteroid therapy in patients with UC. This result demonstrates that optimal timing of intravenous corticosteroids after oral therapy was an important clinical factor of medical treatment response.

Peer review

This paper is well written and the authors highlight the limitation of the study appropriately in their discussion: Retrospective design, not strictly comparative in terms of therapies, and small numbers. Nonetheless, there is an important observation in terms of better management of patients with UC. Some of the findings have been previously reported (low hemoglobin as a risk factor for refractoriness) but it is valuable to see the principles applied to a different population. The comparative data is compelling and statistically significant using appropriate methods. Duration of oral steroid administration may be a very useful predictor of outcome in these cases. The authors are right to emphasize the need for further study in this area.

REFERENCES

1 **Edwards FC**, Truelove SC. The course and prognosis of ulcerative colitis. *Gut* 1963; **4**: 299-315 [PMID: 14084741 DOI: 10.1136/gut.4.4.299]

2 **Abu-Suboh Abadía M**, Casellas F, Vilaseca J, Malagelada JR. Response of first attack of inflammatory bowel disease requiring hospital admission to steroid therapy. *Rev Esp Enferm Dig* 2004; **96**: 539-44; 544-7 [PMID: 15449985 DOI: 10.4321/S1130-01082004000800003]

3 **Faubion WA**, Loftus EV, Harmsen WS, Zinsmeister AR, Sandborn WJ. The natural history of corticosteroid therapy for inflammatory bowel disease: a population-based study. *Gastroenterology* 2001; **121**: 255-260 [PMID: 11487534 DOI: 10.1053/gast.2001.26279]

4 **Domènech E**, Garcia-Planella E, Bernal I, Rosinach M, Cabré E, Fluvià L, Boix J, Gassull MA. Azathioprine without oral ciclosporin in the long-term maintenance of remission induced by intravenous ciclosporin in severe, steroid-refractory ulcerative colitis. *Aliment Pharmacol Ther* 2002; **16**: 2061-2065 [PMID: 12452938 DOI: 10.1046/j.1365-2036.2002.01385.x]

5 **Kohn A**, Daperno M, Armuzzi A, Cappello M, Biancone L, Orlando A, Viscido A, Annese V, Riegler G, Meucci G, Marrollo M, Sostegni R, Gasbarrini A, Peralta S, Prantera C. Infliximab in severe ulcerative colitis: short-term results of different infusion regimens and long-term follow-up. *Aliment Pharmacol Ther* 2007; **26**: 747-756 [PMID: 17697208 DOI: 10.1111/j.1365-2036.2007.03415.x]

6 **Truelove SC**, Witts LJ. Cortisone in ulcerative colitis; final report on a therapeutic trial. *Br Med J* 1955; **2**: 1041-1048 [PMID: 13260656 DOI: 10.1136/bmj.2.4947.1041]

7 **Seo M**, Okada M, Yao T, Ueki M, Arima S, Okumura M. An index of disease activity in patients with ulcerative colitis. *Am J Gastroenterol* 1992; **87**: 971-976 [PMID: 1642220]

8 **Lennard-Jones JE**. Classification of inflammatory bowel disease. *Scand J Gastroenterol Suppl* 1989; **170**: 2-6; discussion 16-19 [PMID: 2617184 DOI: 10.3109/00365528909091339]

9 **Panaccione R**, Rutgeerts P, Sandborn WJ, Feagan B, Schreiber S, Ghosh S. Review article: treatment algorithms to maximize remission and minimize corticosteroid dependence in patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2008; **28**: 674-688 [PMID: 18532990 DOI: 10.1111/j.1365-2036.2008.03753.x]

10 **Yoon JY**, Cheon JH, Park JJ, Hong SP, Kim TI, Kim WH. Clinical outcomes and factors for response prediction after the first course of corticosteroid therapy in patients with active ulcerative colitis. *J Gastroenterol Hepatol* 2011; **26**: 1114-1122 [PMID: 21299620 DOI: 10.1111/j.1440-1746.2011.06688.x]

11 **D'Haens G**, Sandborn WJ, Feagan BG, Geboes K, Hanauer SB, Irvine EJ, Lémann M, Marteau P, Rutgeerts P, Schölmerich J, Sutherland LR. A review of activity indices and efficacy end points for clinical trials of medical therapy in adults with ulcerative colitis. *Gastroenterology* 2007; **132**: 763-786 [PMID: 17258735 DOI: 10.1053/j.gastro.2006.12.038]

12 **Schroeder KW**, Tremaine WJ, Ilstrup DM. Coated oral 5-aminosalicylic acid therapy for mildly to moderately active ulcerative colitis. A randomized study. *N Engl J Med* 1987; **317**: 1625-1629 [PMID: 3317057 DOI: 10.1056/NEJM198712243172603]

13 **Lewis JD**, Chuai S, Nessel L, Lichtenstein GR, Aberra FN, Ellenberg JH. Use of the noninvasive components of the Mayo score to assess clinical response in ulcerative colitis. *Inflamm Bowel Dis* 2008; **14**: 1660-1666 [PMID: 18623174 DOI: 10.1002/ibd.20520]

14 **Stange EF**, Travis SP, Vermeire S, Reinisch W, Geboes K, Barakauskiene A, Feakins R, Fléjou JF, Herfarth H, Hommes DW, Kupcinskas L, Lakatos PL, Mantzaris GJ, Schreiber S, Villanacci V, Warren BF. European evidence-based Consensus on the diagnosis and management of ulcerative colitis: Definitions and diagnosis. *J Crohns Colitis* 2008; **2**: 1-23 [PMID: 21172194 DOI: 10.1016/j.crohns.2007.11.001]

15 **Munkholm P**, Langholz E, Davidsen M, Binder V. Frequency of glucocorticoid resistance and dependency in Crohn's disease. *Gut* 1994; **35**: 360-362 [PMID: 8150347 DOI: 10.1136/gut.35.3.360]

16 **Ho GT**, Mowat C, Goddard CJ, Fennell JM, Shah NB,

Prescott RJ, Satsangi J. Predicting the outcome of severe ulcerative colitis: development of a novel risk score to aid early selection of patients for second-line medical therapy or surgery. *Aliment Pharmacol Ther* 2004; **19**: 1079-1087 [PMID: 15142197 DOI: 10.1111/j.1365-2036.2004.01945.x]

17 **Bernal I**, Mañosa M, Domènech E, Garcia-Planella E, Navarro M, Lorenzo-Zúñiga V, Cabré E, Gassull MA. Predictors of clinical response to systemic steroids in active ulcerative colitis. *Dig Dis Sci* 2006; **51**: 1434-1438 [PMID: 16868820 DOI: 10.1007/s10620-006-9103-7]

18 **Kumar S**, Ghoshal UC, Aggarwal R, Saraswat VA, Choudhuri G. Severe ulcerative colitis: prospective study of parameters determining outcome. *J Gastroenterol Hepatol* 2004; **19**: 1247-1252 [PMID: 15482530 DOI: 10.1111/j.1440-1746.2004.03486.x]

19 **Carbonnel F**, Gargouri D, Lémann M, Beaugerie L, Cattan S, Cosnes J, Gendre JP. Predictive factors of outcome of intensive intravenous treatment for attacks of ulcerative colitis. *Aliment Pharmacol Ther* 2000; **14**: 273-279 [PMID: 10735919 DOI: 10.1046/j.1365-2036.2000.00705.x]

20 **Elliott PR**, Powell-Tuck J, Gillespie PE, Laidlow JM, Lennard-Jones JE, English J, Chakraborty J, Marks V. Prednisolone absorption in acute colitis. *Gut* 1980; **21**: 49-51 [PMID: 7364320 DOI: 10.1136/gut.21.1.49]

21 **Berghouse LM**, Elliott PR, Lennard-Jones JE, English J, Marks V. Plasma prednisolone levels during intravenous therapy in acute colitis. *Gut* 1982; **23**: 980-983 [PMID: 7129207 DOI: 10.1136/gut.23.11.980]

22 **Travis SP**, Farrant JM, Ricketts C, Nolan DJ, Mortensen NM, Kettlewell MG, Jewell DP. Predicting outcome in severe ulcerative colitis. *Gut* 1996; **38**: 905-910 [PMID: 8984031 DOI: 10.1136/gut.38.6.905]

23 **Benazzato L**, D'Incà R, Grigoletto F, Perissinotto E, Medici V, Angriman I, Sturniolo GC. Prognosis of severe attacks in ulcerative colitis: effect of intensive medical treatment. *Dig Liver Dis* 2004; **36**: 461-466 [PMID: 15285525 DOI: 10.1016/j.dld.2003.12.017]

24 **Meyers S**, Sachar DB, Goldberg JD, Janowitz HD. Corticotropin versus hydrocortisone in the intravenous treatment of ulcerative colitis. A prospective, randomized, double-blind clinical trial. *Gastroenterology* 1983; **85**: 351-357 [PMID: 6305758]

25 **Chakravarty BJ**. Predictors and the rate of medical treatment failure in ulcerative colitis. *Am J Gastroenterol* 1993; **88**: 852-855 [PMID: 8503379]

26 **Mantzaris GJ**, Petraki K, Archavlis E, Amberiadis P, Kourtessas D, Christidou A, Triantafyllou G. A prospective randomized controlled trial of intravenous ciprofloxacin as an adjunct to corticosteroids in acute, severe ulcerative colitis. *Scand J Gastroenterol* 2001; **36**: 971-974 [PMID: 11521989 DOI: 10.1080/003655201750305503]

27 **Seo M**, Okada M, Yao T, Matake H, Maeda K. Evaluation of the clinical course of acute attacks in patients with ulcerative colitis through the use of an activity index. *J Gastroenterol* 2002; **37**: 29-34 [PMID: 11824797 DOI: 10.1007/s535-002-8129-2]

28 **Park SH**, Kim YM, Yang SK, Kim SH, Byeon JS, Myung SJ, Cho YK, Yu CS, Choi KS, Chung JW, Kim B, Choi KD, Kim JH. Clinical features and natural history of ulcerative colitis in Korea. *Inflamm Bowel Dis* 2007; **13**: 278-283 [PMID: 17206722 DOI: 10.1002/ibd.20015]

29 **Kim ES**, Kim WH. Inflammatory bowel disease in Korea: epidemiological, genomic, clinical, and therapeutic characteristics. *Gut Liver* 2010; **4**: 1-14 [PMID: 20479907 DOI: 10.5009/gnl.2010.4.1.1]

30 **Lee JH**, Cheon JH, Moon CM, Park JJ, Hong SP, Kim TI, Kim WH. Do patients with ulcerative colitis diagnosed at a young age have more severe disease activity than patients diagnosed when older? *Digestion* 2010; **81**: 237-243 [PMID: 20110709 DOI: 10.1159/000253850]

31 **Park BJ**, Lee KJ, Hwang JC, Sin SJ, Chung JY, Cho SW. [Relapse rates of ulcerative colitis in remission and factors related to relapse]. *Korean J Gastroenterol* 2008; **52**: 21-26 [PMID: 19077487]

32 **Park SM**, Han DS, Yang SK, Hong WS, Min YI. Clinical features of ulcerative colitis in Korea. *Korean J Intern Med* 1996; **11**: 9-17 [PMID: 8882472]

33 **Wilson A**, Reyes E, Ofman J. Prevalence and outcomes of anemia in inflammatory bowel disease: a systematic review of the literature. *Am J Med* 2004; **116** Suppl 7A: 44S-49S [PMID: 15050885 DOI: 10.1016/j.amjmed.2003.12.011]

34 **Gasche C**, Lomer MC, Cavill I, Weiss G. Iron, anaemia, and inflammatory bowel diseases. *Gut* 2004; **53**: 1190-1197 [PMID: 15247190 DOI: 10.1136/gut.2003.035758]

35 **Wells CW**, Lewis S, Barton JR, Corbett S. Effects of changes in hemoglobin level on quality of life and cognitive function in inflammatory bowel disease patients. *Inflamm Bowel Dis* 2006; **12**: 123-130 [PMID: 16432377 DOI: 10.1097/01.MIB.0000196646.64615.db]

36 **Gisbert JP**, Bermejo F, Pajares R, Pérez-Calle JL, Rodríguez M, Algaba A, Mancenido N, de la Morena F, Carneros JA, McNicholl AG, González-Lama Y, Maté J. Oral and intravenous iron treatment in inflammatory bowel disease: hematological response and quality of life improvement. *Inflamm Bowel Dis* 2009; **15**: 1485-1491 [PMID: 19408339 DOI: 10.1002/ibd.20925]

37 **Meyers S**, Lerer PK, Feuer EJ, Johnson JW, Janowitz HD. Predicting the outcome of corticoid therapy for acute ulcerative colitis. Results of a prospective, randomized, double-blind clinical trial. *J Clin Gastroenterol* 1987; **9**: 50-54 [PMID: 3031150 DOI: 10.1097/00004836-198702000-00013]

38 **Daperno M**, Sostegni R, Scaglione N, Ercole E, Rigazio C, Rocca R, Pera A. Outcome of a conservative approach in severe ulcerative colitis. *Dig Liver Dis* 2004; **36**: 21-28 [PMID: 14971812 DOI: 10.1016/j.dld.2003.04.001]

39 **Faiz O**, Warusavitarne J, Bottle A, Tekkis PP, Clark SK, Darzi AW, Aylin P. Nonelective excisional colorectal surgery in English National Health Service Trusts: a study of outcomes from Hospital Episode Statistics Data between 1996 and 2007. *J Am Coll Surg* 2010; **210**: 390-401 [PMID: 20347730 DOI: 10.1016/j.jamcollsurg.2009.11.017]

40 **Aberra FN**, Lewis JD, Hass D, Rombeau JL, Osborne B, Lichtenstein GR. Corticosteroids and immunomodulators: postoperative infectious complication risk in inflammatory bowel disease patients. *Gastroenterology* 2003; **125**: 320-327 [PMID: 12891531 DOI: 10.1016/S0016-5085(03)00883-7]

P- Reviewer Goll R **S- Editor** Gou SX **L- Editor** A
E- Editor Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i2.274

World J Gastroenterol 2013 January 14; 19(2): 274-283
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Dual probiotic strains suppress high fructose-induced metabolic syndrome

Do-Young Park, Young-Tae Ahn, Chul-Sung Huh, Robin A McGregor, Myung-Sook Choi

Do-Young Park, Young-Tae Ahn, Chul-Sung Huh, Korea Yakult Co., Ltd., Yongin, Gyeonggi 449-901, South Korea
Robin A McGregor, Myung-Sook Choi, Department of Food Science and Nutrition, Center for Food and Nutritional Genomics, Kyungpook National University, Daegu 702-701, South Korea
Author contributions: Park DY, Ahn YT, Huh CS and Choi MS contributed to the conception, design and acquisition of data; Park DY and McGregor RA contributed to the analysis and interpretation of data; Park DY, McGregor RA and Choi MS contributed to drafting the article or revising it critically for important intellectual content; all authors gave final approval of the version to be published.
Supported by The Basic Science Research Program, Center for Food and Nutritional Genomics, the National Research Foundation (NRF) of Korea funded by the Ministry of Education, Science and Technology, No. 2011-0000912 and Korea Yakult Co., Ltd.
Correspondence to: Myung-Sook Choi, PhD, Professor, Department of Food Science and Nutrition, Center for Food and Nutritional Genomics, Kyungpook National University, 1370 Sankyuk Dong, Buk-gu, Daegu 702-701, South Korea. mschoi@knu.ac.kr
Telephone: +82-53-9506232 Fax: +82-53-9506229
Received: July 7, 2012 Revised: September 16, 2012
Accepted: November 14, 2012
Published online: January 14, 2013

Abstract

AIM: To investigate the effect of novel probiotics on the clinical characteristics of high-fructose induced metabolic syndrome.

METHODS: Male Wistar rats aged 4 wk were fed a 70% w/w high-fructose diet (n = 27) or chow diet (n = 9) for 3 wk to induce metabolic syndrome, the rats were then randomized into groups and administered probiotic [*Lactobacillus curvatus* (*L. curvatus*) HY7601 and *Lactobacillus plantarum* (*L. plantarum*) KY1032] at 10^9 cfu/d or 10^{10} cfu/d or placebo by oral gavage for 3 wk. Food intake and body weight were measured once a week. After 6 wk, the rats were fasted for 12 h, then anesthetized with diethyl ether and sacrificed. Blood samples were taken from the inferior vena cava for plasma analysis of glucose, insulin, C-peptide, total-cholesterol, triglycerides and thiobarbituric acid-reacting substances. Real-time polymerase chain reaction was performed using mouse-specific Taqman probe sets to assess genes related to fatty acid β-oxidation, lipogenesis and cholesterol metabolism in the liver. Target gene expression was normalized to the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase.

RESULTS: Rodents fed a high-fructose diet developed clinical characteristics of the metabolic syndrome including increased plasma glucose, insulin, triglycerides, total cholesterol and oxidative stress levels, as well as increased liver mass and liver lipids compared to chow fed controls. Probiotic treatment (*L. curvatus* HY7601 and *L. plantarum* KY1032) at high (10^{10} cfu/d) or low dosage (10^9 cfu/d) lowered plasma glucose, insulin, triglycerides and oxidative stress levels. Only high-dose probiotic treatment reduced liver mass and liver cholesterol. Probiotic treatment reduced lipogenesis *via* down-regulation of SREBP1, FAS and SCD1 mRNA levels and increased β-oxidation *via* up-regulation of PPARα and CPT2 mRNA levels.

CONCLUSION: Probiotic *L. curvatus* HY7601 and *L. plantarum* KY1032 combined suppressed the clinical characteristics of high-fructose-induced metabolic syndrome, therefore, may provide a natural alternative for the treatment of diet-induced metabolic syndrome.

© 2013 Baishideng. All rights reserved.

Key words: Dyslipidemia; Fasting glucose; Gut microbiota; High-fructose diet; Inflammation; Insulin resistance; Lactobacillus; Metabolic syndrome; Oxidative stress; Probiotic

Park DY, Ahn YT, Huh CS, McGregor RA, Choi MS. Dual probiotic strains suppress high fructose-induced metabolic syndrome.

World J Gastroenterol 2013; 19(2): 274-283 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i2/274.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i2.274

INTRODUCTION

Metabolic syndrome is a rapidly growing worldwide pandemic, which is associated with a greater risk of multiple chronic pathologies including cardiovascular disease and Type 2 diabetes. Metabolic syndrome is characterised by a cluster of metabolic abnormalities including insulin resistance, elevated fasting glucose, elevated plasma triglycerides, elevated blood pressure, low-grade inflammation, abdominal obesity and reduced high-density lipoprotein (HDL)-cholesterol[1,2]. There is no universal cause of metabolic syndrome, although major underlying risk factors include abdominal obesity, physical inactivity or diabetogenic diets[1].

Diabetogenic diets with high fructose content have been strongly implicated in the development of metabolic syndrome, cardiovascular disease and Type 2 diabetes[3]. Food manufacturers are increasingly using fructose corn syrup to increase sweetness, as well as the palatability of food and beverages. However, there is growing experimental evidence that excessive fructose intake can lead to metabolic abnormalities, including insulin resistance, dyslipidemia as well as abdominal adiposity in both animals and humans[4]. Animals fed a high-fructose diet develop clinical characteristics of metabolic syndrome, therefore, high-fructose fed animals are particularly useful for assessing potential therapeutic interventions against metabolic syndrome[5]. Excess dietary fructose can be converted to triglycerides through *de novo* lipogenesis, resulting in increased lipid accumulation in the liver and elevated blood lipid levels. Over a period of time prolonged high intracellular and systemic lipid levels can cause increased oxidative stress and inflammation, both of which can trigger insulin resistance, leading to increased blood glucose levels. Due to the lack of effective drugs to treat metabolic syndrome, there is growing interest in natural therapeutics to prevent or manage metabolic syndrome.

Over the past five years, probiotics have rapidly emerged as natural therapeutics with potential to target key risk factors associated with metabolic syndrome[6,7]. Probiotics consist of single or multiple live bacterial species, which may directly or indirectly modulate gut microbial activity and improve host health. The human gut harbours between 10^{14} bacterial species collectively forming the gut microbiota[8]. Gut microbial communities are proposed to provide the host with the ability to harvest otherwise inaccessible energy from the diet[9-11] and modulate host genes associated with energy storage in adipose tissue[2,12]. Probiotics have been widely assessed *in-vivo* in diet-induced obesity models[13-17], however, different probiotic species even from the same family can exert variable effects on lipid accumulation and obesity[18], therefore, it remains essential to assess the effectiveness of probiotic strains in different animal disease models *in-vivo*. Probiotic yogurt containing multiple *Lactobacillus* strains has been reported to alleviate fasting blood glucose, plasma insulin and triglyceride in high-fructose fed rats[19]. However, few other studies have considered the impact of probiotics on high-fructose diet-induced metabolic syndrome. To our knowledge, the dose-dependent and metabolic effects of probiotic treatment in high-fructose-induced metabolic syndrome remain to be established.

The aim of this study was to assess the dose-dependent effects of a probiotic consisting of *Lactobacillus curvatus* (*L. curvatus*) HY7601 and *Lactobacillus plantarum* (*L. plantarum*) KY1032 on hyperlipidemia, hyperglycemia, insulin resistance, oxidative stress and hepatic metabolism related gene expression in high-fructose fed rats with metabolic syndrome. We hypothesized that probiotic treatment may protect against dysregulated metabolism induced by a high-fructose diet in a dose-dependent manner.

MATERIALS AND METHODS

Animals, diets and experimental design

Male Wistar rats (n = 36) aged 4 wk were purchased from Jackson Laboratories (Bar Harbor, United States). All rats were individually housed under a constant temperature and humidity (22 ± 1 ℃, 55% ± 10%) with a 12 h light/12 h dark cycle. The experimental design consisted of a pretreatment phase (0-3 wk) and a treatment phase (3-6 wk). During the pretreatment phase, male Wistar rats were fed a 70% w/w high-fructose diet (n = 27) to induce metabolic abnormalities or a chow diet (n = 9) for 3 wk. The composition of the high-fructose diet was formulated according to Table 1. During the treatment phase, the placebo (HF) group (n = 9), low dose probiotic (LP) group (n = 9) and high dose probiotic (HP) group (n = 9) were fed the same high-fructose diet with placebo, 10^9 cfu probiotics or 10^{10} cfu probiotics, respectively, administered orally each day for a further 3 wk. The chow control group was fed the same chow diet with placebo administered orally each day for a further 3 wk. Freeze-dried *Lactobacillus* strains were produced by Culture Systems Inc. (United States), and packed with lactose according to Table 2. Each pack was resuspended in 500 μL distilled water prior to administration. Food intake and body weight were measured once a week. Before sacrifice, rats were fasted for 12 h and anesthetized with diethyl ether. Blood samples were taken from the inferior vena cava for plasma analysis. Epididymal adipose tissue and liver tissue were removed, rinsed with phosphate buffered saline, weighed and immediately frozen at -70 ℃. The experimental design was approved by the Ethics Committee of Korea Yakult Company Limited R and D Center.

Blood analysis

Plasma glucose, insulin and C-peptide concentrations

Table 1 Composition of high fructose diet

Ingredient	High fructose diet (g)
Casein	200.0
L-cystine	3.0
Fructose	700.0
Cellulose powder	50.0
Corn oil	25.0
Lard	20.0
Mineral Mix S10026	10.0
DiCalcium Phosphate	13.0
Calcium Carbonate	5.5
Potassium Citrate	16.5
Vitamin Mix V10001	10.0
Choline Bitartrate	2.0
Total	1000.0

Table 2 The composition of each supplement pack

	Placebo (mg)	10^9 probiotics (mg)	10^{10} probiotics (mg)
Lactobacillus curvatus HY7601	-	5.0 (5×10^8)	50.0 (5×10^9)
Lactobacillus plantarum KY1032	-	2.5 (5×10^8)	25.0 (5×10^9)
Lactose	100.0	92.5	25.0
Total	100.0	100.0	100.0

Freeze-dried *Lactobacillus curvatus* HY7601 and *Lactobacillus plantarum* KY1032 concentration was 1×10^8 and 2×10^8 cfu/mg, respectively.

were determined using the glucose assay kit (Cayman, United States), insulin enzyme-linked immunosorbent assay (ELISA) kit (Millipore, United States) and rat C-peptide ELISA kit (EIAab, China), respectively, according to the manufacturer's instructions. Insulin resistance was assessed based on homeostasis model assessment of insulin resistance (HOMA-IR), calculated as the product of fasting plasma glucose (FPG) and insulin (FPI), divided by a constant[20]. The equation was [FPG (mg/dL) × FPI (μU/mL)]/2430. Plasma total-cholesterol and triglyceride concentrations were determined using commercial kits (AsanPharm, South Korea). Plasma thiobarbituric acid-reacting substances (TBARS) were measured to assess oxidative stress as described previously[21].

Hepatic lipid profile analysis

Hepatic lipids were extracted as previously reported[22]. The dried lipid residues were dissolved in 1 mL of isopropanol for the triglyceride and cholesterol assays. Hepatic triglyceride and cholesterol concentrations were measured using the same commercial kits (AsanPharm, South Korea) used for the plasma analysis.

RT-qPCR

Total RNA was extracted from liver (15 mg) tissue using an RNAqueous kit (Ambion, United States). DNA was removed with a Turbo DNA-free kit (Ambion, United States). RNA integrity was verified and RNA quantified using a GeneQuant Pro spectrophotometer (GE Healthcare, United States). Total RNA (2 μg) was reverse-transcribed into cDNA with a high-capacity RNA-to-cDNA kit (Applied Biosystems Inc., United States). Then cDNA was amplified on a 7500 Real Time PCR System (Applied Biosystems Inc., United States) using mouse-specific Taqman probe sets (Table 3) under the following conditions: 95 ℃ for 10 min, followed by 40 cycles at 95 ℃ for 15 s, and 60 ℃ for 1 min. Target gene expression was normalized to the housekeeping gene, glyceraldehyde-3-phosphate dehydrogenase (catalog number Rn01775763_g1). Data was analyzed using the ABI 7500 System Sequence Detection software (Applied Biosystems Inc., United States) and presented as mean ± SE.

Table 3 Catalog numbers of Taqman probes

Taqman probe	Catalog number
Peroxisome proliferative activated receptor a	Rn00566193_m1
Carnitine palmitoyl transferase 1	Rn00580702_m1
Carnitine palmitoyl transferase 2	Rn00563995_m1
Acyl-CoA oxidase 1	Rn01460628_m1
Sterol regulatory element binding protein-1	Rn01495769_m1
Fatty acid synthase	Rn00569117_m1
Stearoyl-CoA desaturase 1	Rn00594894_g1
Cholesterol 7 alpha-hydroxylase	Rn00564065_m1
Low density lipoprotein receptor	Rn00598442_m1

Statistical analysis

All data were presented as mean ± SE. Statistical analysis was performed using SPSS software (SPSS Inc., United States). Data were analyzed by one way analysis of variance, and the differences between experimental groups were evaluated using Duncan's multiple range test at the $P < 0.05$ level. Significant differences between chow control and HF control groups were determined using unpaired Student's *t*-test. Significant differences between week 3 and week 6 in each parameter were determined using the paired Student's *t*-test, and the values were considered statistically significant when $P < 0.05$.

RESULTS

Effects of probiotic treatment on food intake, body weight and tissue mass

Metabolic syndrome was induced in rodents by feeding them a high-fructose diet over 3 wk, while a control group was fed a chow diet. Rodents with metabolic syndrome were then randomized into three treatment groups and fed a high-fructose diet for a further 3 wk, with daily treatment of either probiotic (HP) at a dose of 1×10^{10} cfu/d, probiotic (LP) at a dose of 1×10^9 cfu/d or placebo administered by oral gavage. The chow control group were fed the same diet for the same period and administered placebo by oral gavage. Average food intake was suppressed in the high-fructose diet fed rodents compared to the chow fed controls (Figure 1A). However, low or high dose probiotic treatment had no significant effect on food intake (Figure 1A). Body weight gain and epididymal fat mass were not significantly affected by high-fructose feeding or probiotic treatment regardless of dosage (Figure 1B and C). Importantly, average liver

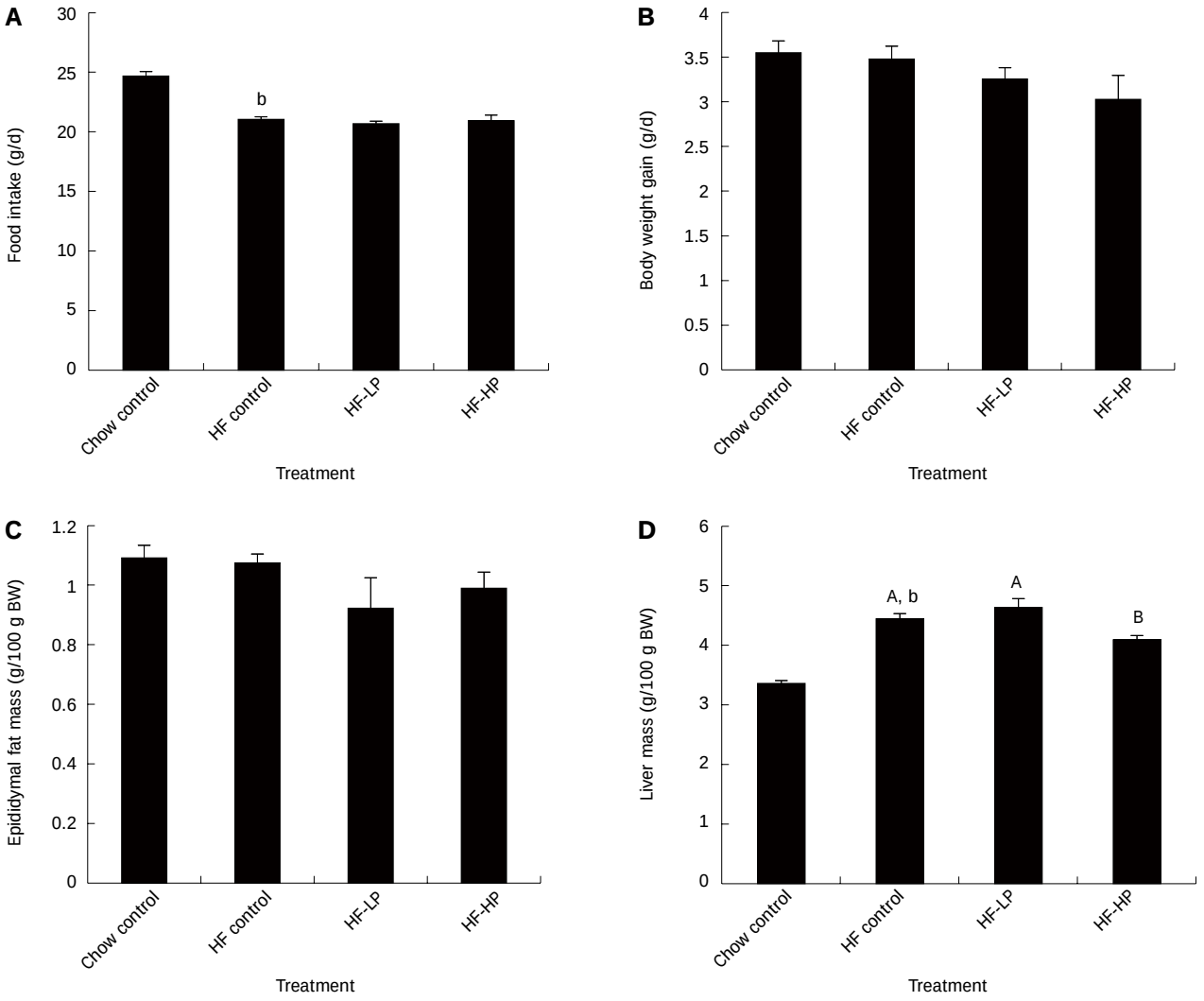


Figure 1 Effects of probiotic treatment on food intake (A), body weight gain (B), epididymal fat mass (C) and liver mass (D) in high-fructose diet-fed rats. Results are expressed as mean ± SE. $^{b}P < 0.01$ *vs* chow control by unpaired Student's *t*-test; ^{AB}Bars with different capital letters are significantly different at $P < 0.05$ by Duncan's multiple range tests. HP: High dose probiotic; HF: High fructose diet; LP: Low dose probiotic; BW: Body weight.

mass, which was significantly increased by high-fructose diet, was significantly lower following HP treatment by 10% ($P < 0.01$) compared to high-fructose fed controls (Figure 1D).

Effects of probiotic treatment on dyslipidemia

Hypertriglyceridemia was effectively induced by a high-fructose diet within 3 wk (163.91 ± 6.50 mg/dL *vs* 49.26 ± 3.99 mg/dL, HF and chow group, respectively). However, subsequently 3-wk of probiotic treatment significantly reduced average plasma triglyceride levels by 46% compared to placebo treatment in the high-fructose fed rodents (Figure 2A). The probiotic treatment had no influence on either total cholesterol or HDL cholesterol levels, which were increased by high-fructose diet intake (Figure 2B and C).

Effects of probiotic treatment on hyperglycemia, insulin resistance and oxidative stress

The average plasma glucose levels of the high-fructose fed rats were significantly higher than that of the chow fed controls at week 6, but were effectively reduced following LP and HP treatment by 24% ($P < 0.05$) and 14% ($P < 0.05$), respectively, (Figure 3A). The average plasma insulin levels of the high-fructose fed rats were significantly higher at both weeks 3 and 6 compared to the chow fed controls. However, after HP treatment plasma, insulin levels were substantially lower (31%, $P < 0.05$) compared to the high-fructose fed rats. Moreover, the plasma insulin levels following LP and HP treatment were reduced by 33% ($P < 0.01$) and 29% ($P < 0.05$), respectively, compared to before treatment (Figure 3B). The HOMA-IR, a representative index of insulin resistance, was significantly higher in the high-fructose fed rats compared to the chow fed controls at week 6, but was significantly reduced by LP (35%) and HP (34%) treatment (Figure 3C). Furthermore, HOMA-IR following LP treatment was 25% ($P < 0.05$) lower compared to before treatment (Figure 3C). Plasma C-peptide is another biomarker associated with insulin resistance.

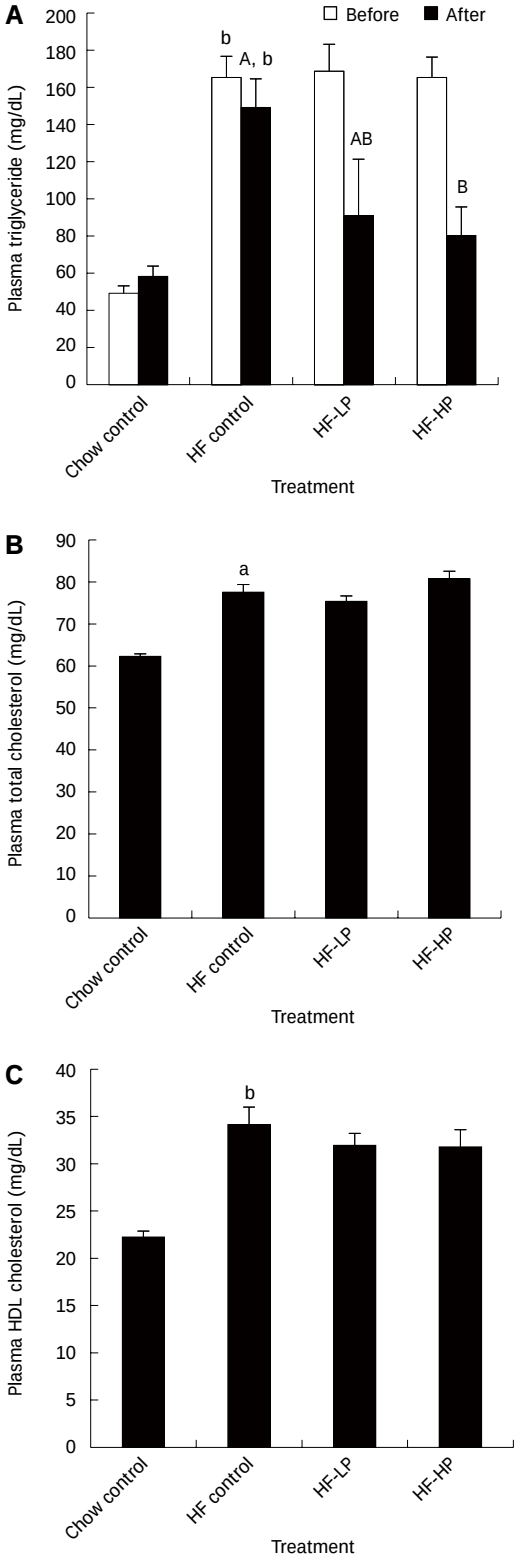


Figure 2 Effects of probiotic treatment on plasma triglyceride (A), plasma total cholesterol (B) and plasma high-density lipoprotein cholesterol (C) in high-fructose diet-fed rats. Results are expressed as mean ± SE. $^{a}P < 0.05$ and $^{b}P < 0.01$ *vs* chow control by unpaired Student's *t*-test; ^{AB}Bars with different capital letters are significantly different at $P < 0.05$ by Duncan's multiple range tests. HF: High fructose diet; HP: High dose probiotic; LP: Low dose probiotic; HDL: High-density lipoprotein.

Plasma C-peptide level was 24% ($P < 0.05$) lower following HP treatment compared to placebo treatment in high-fructose fed rats (Figure 3D). Moreover, plasma C-peptide levels after HP treatment were 28% ($P < 0.05$) lower compared to before treatment (Figure 3D). In addition, probiotic treatment of either LP or HP decreased plasma TBARS levels by 37% ($P < 0.01$) and 50% ($P < 0.001$), respectively (Figure 3E).

Effects of probiotic treatment on hepatic lipid content and gene expression

Both hepatic triglyceride and cholesterol levels in the high-fructose fed rats were significantly higher than those in the chow fed controls. Probiotic treatment with either LP or HP tended to reduce hepatic triglyceride levels (Figure 4A), and HP significantly reduced (27%, $P < 0.05$) hepatic cholesterol levels compared to the high-fructose fed controls (Figure 4B).

In order to determine the mechanisms underlying the probiotic effect on hepatic lipid homeostasis, we examined the mRNA levels of hepatic genes associated with lipid metabolism (Figure 5A-C). High-fructose diet intake altered gene expression compared to chow diet intake. The changes in gene expression in the high-fructose group indicated decreases in fatty acid β-oxidation (PPARα, CPT1, CPT2 and ACOX1), cholesterol uptake (LDLR) and bile acid synthesis (CYP7A1), and increases in fatty acid synthesis (SREBP1, FAS, SCD1). Importantly, HP treatment significantly reversed high-fructose-induced gene expression changes including up-regulation of PPARα (+76%), CPT2 (+66%) and CYP7A1 (+71%), and down-regulation of SREBP1 (-30%), FAS (-54%) and SCD1 (-23%), although LP treatment did not cause any significant changes in hepatic lipid metabolism gene expression.

DISCUSSION

Recent studies indicate that the gut microbiota plays an important role in host lipid and glucose metabolism. Therefore, therapeutic probiotics which can manipulate the gut microbiota may also prevent some of the risk factors underlying the development of metabolic syndrome including dyslipidemia, elevated fasting glucose levels and insulin resistance[23]. In the present study, we used a novel probiotic consisting of *L. curvatus* HY7601 and *L. plantarum* KY1302 isolated from Korean fermented cabbage. We showed that probiotic administered to high-fructose fed mice reversed the risk factors underlying the metabolic syndrome. Previous evidence from high-fructose diet-fed rat studies indicated that a probiotic-cultured yogurt called Dahi can also improve metabolic abnormalities[19,24]. Importantly, in the present study, we established the dose-dependent effects of a novel combination of probiotic strains on metabolic syndrome.

High-fructose intake is reported to promote lipogenesis and suppress glucose intake[25-27]. Consistent with other studies of high-fructose fed animals, hepatic gene expression analysis indicated that lipogenesis was increased *via*

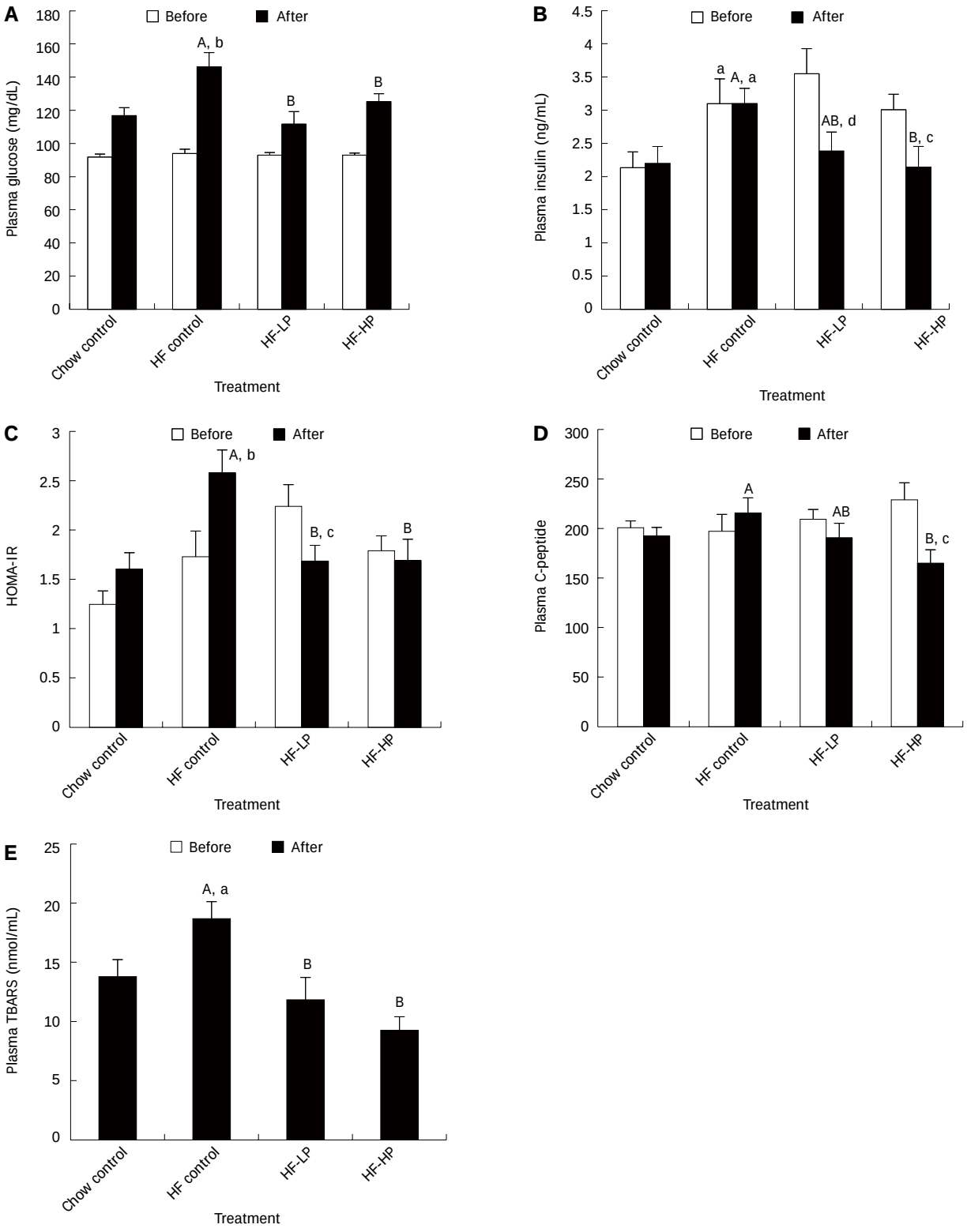


Figure 3 Effects of probiotic treatment on plasma glucose, plasma insulin, insulin resistance, plasma C-peptide and plasma thiobarbituric acid-reacting substances in high-fructose diet-fed rats. Results are expressed as mean ± SE. [a]*P* < 0.05 and [b]*P* < 0.01 HF control *vs* chow control by unpaired Student's *t*-test; [c]*P* < 0.05 and [d]*P* < 0.01 week 6 *vs* week 3 by paired Student's *t*-test; [AB]Bars with different capital letters are significantly different at *P* < 0.05 by Duncan's multiple range tests. HF: High fructose diet; LP: Low dose probiotic; HP: High dose probiotic; TBARS: Thiobarbituric acid-reacting substances.

upregulation of SREBP1, FAS and SCD1, conversely β-oxidation was decreased *via* downregulation of PPARα and PPARα-regulated CPT1, CPT2 and ACOX1 expression[25-27]. Increased lipogenesis and decreased β-oxidation lead to excess accumulation of cellular lipids, as shown by the liver enlargement and hypertriglyceridemia in the high-fructose fed rats in the present study. Hypertriglyceridemia is known to be an important predictor of car-

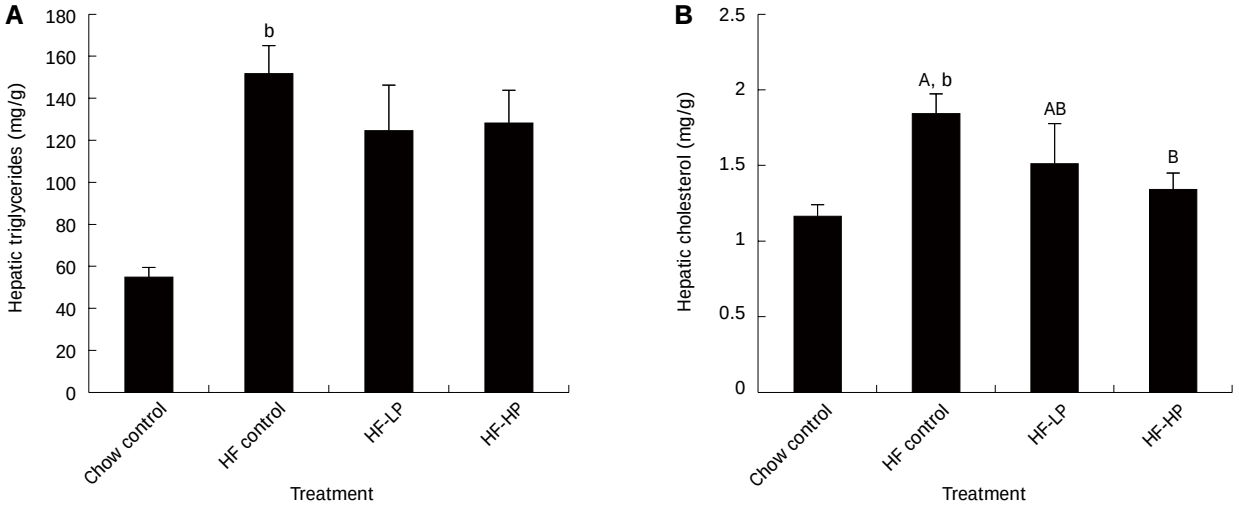


Figure 4 Effects of probiotic treatment on hepatic triglyceride and hepatic total cholesterol in high-fructose diet-fed rats. Results are expressed as mean ± SE. $^{b}P < 0.01$ HF control *vs* chow control by unpaired Student's *t*-test; ^{AB}Bars with different capital letters are significantly different at $P < 0.05$ by Duncan's multiple range tests. HF: High fructose diet; LP: Low dose probiotic; HP: High dose probiotic.

diovascular disease mortality in subjects with diabetes or impaired glucose tolerance[28], therefore, reducing plasma triglyceride levels may improve long-term health.

Previous studies have shown that probiotics can alter the gut microbiota[29], and direct gut microbiota manipulations in germ-free mice significantly affect host lipid metabolism[2,12]. Here we showed that 10^{10} cfu/d probiotic treatment led to upregulated PPARα and CPT2 expression reflecting activation of β-oxidation, and downregulated SREBP1, FAS and SCD1 expression reflecting suppression of lipogenesis. Moreover, these probiotics induced transcriptional changes which resulted in a significant reduction in liver mass and plasma triglyceride levels. These findings are consistent with a previous report which showed that *L. plantarum* KY1032 inhibits lipid droplet accumulation during adipocyte differentiation. In contrast to plasma triglyceride levels, hepatic triglyceride levels were only slightly reduced by probiotic treatment. We hypothesized that the probiotic-induced increase in hepatic β-oxidation related gene expression was partly to clear excess hepatic triglycerides generated through high-fructose-induced *de novo* lipogenesis.

Contrary to hepatic triglyceride levels, cholesterol levels were significantly reduced by probiotic treatment, whereas plasma cholesterol levels were unchanged. We assessed whether the reduction in hepatic cholesterol following probiotic treatment was due to altered hepatic cholesterol metabolism related gene expression. High-fructose diet suppressed CYP7A1 expression, which encodes cholesterol 7 α-hydroxylase, the rate-limiting enzyme involved in the formation of bile from cholesterol. However, 10^{10} cfu/d probiotic treatment upregulated CYP7A1 expression indicating increased bile synthesis activity. Some probiotics are also reported to increase bile salt hydrolase activity[30]. Hepatic LDLR expression remained unchanged, suggesting that probiotic treatment did not enhance uptake of plasma cholesterol into the liver, which is consistent with the absence of changes in plasma cholesterol levels. However, the effect of probiotics on liver cholesterol metabolism may be partly dependent on the diet regime used, because in high-fat or high-cholesterol diet fed animals, probiotics are reported to decrease plasma and hepatic cholesterol levels together.

The probiotic effect on host lipid levels may also contribute to the observed improvement in glucose homeostasis. Excess intracellular lipids can inhibit insulin signalling, hence reduce insulin-stimulated glucose uptake leading to hyperglycemia and hyperinsulinemia as shown in this study. We observed that probiotic treatment at 10^{9} or 10^{10} cfu/d significantly lowered plasma glucose, insulin and C-peptide concentrations, and reduced insulin resistance indicated by the reduced HOMA-IR index[20,31]. Excess fructose intake is also reported to promote lipid peroxidation and oxidative stress is implicated in the pathogenesis of insulin resistance[32,33]. In the present study, probiotic treatment at 10^{9} or 10^{10} cfu/d significantly reversed the oxidative stress present in the high-fructose fed rats with metabolic syndrome, indicated by lower plasma TBARS levels. Some *Lactobacillus* strains are also reported to possess anti-oxidative activity. For example, probiotics have been reported to reduce exercise-induced oxidative stress, *via* increases in anti-oxidative activity, which helps neutralize reactive oxygen species[34]. Furthermore, studies in human Type 2 diabetes show probiotic supplementation can increase superoxide dismutase and glutathione peroxidase activities[35], which are anti-oxidants that help protect against oxidative stress.

While evidence is growing on the effects of probiotic treatment on many chronic pathologies, some issues remain to be addressed. Probiotics are widely assumed to modulate the gut microbiota, and exert health benefits *via* direct modification of gut microbial communities[8]. Studies on diet-induced mice indicate that probiotics alter the gut microbiota[29], similar to our recent experience with the same probiotic used in this study (unpublished observations). However, in another study using germ-

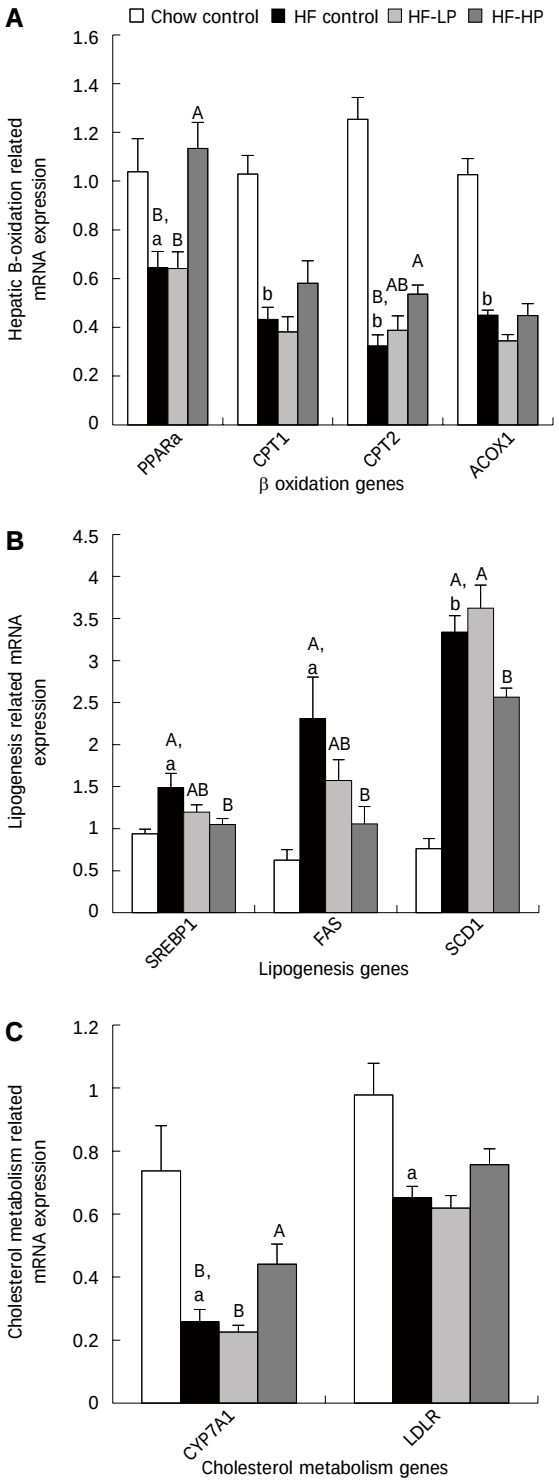


Figure 5 Effects of probiotic treatment on hepatic B-oxidation, lipogenesis and cholesterol metabolism related gene expression in high-fructose diet-fed rats. Results are expressed as mean ± SE. $^{a}P < 0.05$ and $^{b}P < 0.01$ HF control *vs* chow control by unpaired Student's *t*-test; ^{AB}Bars with different capital letters are significantly different at $P < 0.05$ by Duncan's multiple range tests. HF: High fructose diet; LP: Low dose probiotic; HP: High dose probiotic; FAS: Fatty acid synthase; SREBP1: Sterol regulatory element-binding protein-1; PPARα: Peroxisome proliferator-activated receptor α; CPT: Carnitine palmitoyltransferase; ACOX: Acyl-coenzyme A oxidase; SCD: Steaoryl-CoA desaturase; CYP7A1: Cholesterol 7α-hydroxylase gene; LDLR: Low-density lipoprotein receptor.

free mice transplanted with a small artificial gut microbial community, a multi-species probiotic failed to change the gut microbiota[36]. Whether multi-species probiotics are more effective than single-species probiotics is not clear, because few studies make this comparison. We used a two species probiotic in the present study, and based on our preliminary data the results suggest that combined species are more effective than either species alone. Finally, whether probiotics exert dose-dependent effects against metabolic syndrome has not been evaluated, nor has the minimum amount of live bacteria that is necessary for functional health benefits. Evidence from the present study indicates that probiotics containing 10^{10} cfu/d showed the greatest effectiveness against high-fructose-induced metabolic syndrome over a relatively short period, without any adverse effects. In contrast, probiotic treatment at 10^{9} cfu/d exerted minimal effects on hypertriglyceridemia, but effectively improved hyperglycaemia and insulin resistance. In the case of hepatic gene expression, 10^{9} cfu/d probiotic treatment had no modulatory effect on any of the genes tested, but probiotic treatment at 10^{10} cfu/d modulated lipogenesis and β-oxidation related genes.

In conclusion, probiotic *L. curvatus* HY7601 and *L. plantarum* KY1032 combined can suppress the clinical characteristics of high-fructose-induced metabolic syndrome, therefore, may provide a natural alternative for the treatment of diet-induced metabolic syndrome.

ACKNOWLEDGMENTS

Choi MS and McGregor RA declare no conflicts of interest. Park DY, Ahn YT and Huh CS are current or past employees of Korea Yakult Co., Ltd.

COMMENTS

Background

Metabolic syndrome is a growing health problem characterised by elevated blood sugar and blood lipid levels, insulin resistance, high blood pressure and abdominal obesity. Poor diet consisting of high sugar or high fat content is a major risk factor for metabolic syndrome. There is no universal treatment for metabolic syndrome. Probiotics found in fermented foods have emerged as a natural way of protecting against metabolic syndrome, but many probiotic strains have varying metabolic effects. Therefore, a major challenge for scientists is discovering probiotic strains, which may help protect against metabolic syndrome.

Research frontiers

Probiotic strains identified in kimchi, a traditional Korean fermented cabbage consumed regularly in South Asian countries, are reported to have various beneficial properties. Current research aims to determine whether these probiotic strains are effective in different animal models of disease including metabolic syndrome, Type 2 diabetes and obesity.

Innovations and breakthroughs

The authors showed that two probiotic strains, *Lactobacillus curvatus* (*L. curvatus*) HY7601 and *Lactobacillus plantarum* (*L. plantarum*) KY1032, can suppress metabolic abnormalities such as hypertriglyceridemia, hyperglycemia and insulin resistance in high-fructose-induced metabolic syndrome. These probiotic health benefits were associated with decreased lipogenesis and increased β-oxidation-related gene expression in the liver.

Applications

Probiotic with *L. curvatus* HY7601 and *L. plantarum* KY1032 may provide a natural supplement for the management of the underlying risk factors of metabolic syndrome.

Terminology

Probiotics consist of live micro-organisms which confer beneficial effects on host health. Hypertriglyceridemia is prolonged elevated triglyceride levels in blood. Hyperglycemia is prolonged elevated glucose levels in the blood. Insulin resistance is the inability of insulin to stimulate glucose uptake pathways in fat, skeletal muscle and the liver.

Peer review

This paper investigated the effect of novel probiotics on the clinical characteristics of high-fructose induced metabolic syndrome. The authors were suggesting a mechanism. One novel thing of this paper is that no one has looked at these specific microbes in this combination. It is well written.

REFERENCES

1 **Grundy SM**, Cleeman JI, Daniels SR, Donato KA, Eckel RH, Franklin BA, Gordon DJ, Krauss RM, Savage PJ, Smith SC, Spertus JA. Diagnosis and management of the metabolic syndrome: an American Heart Association/National Heart, Lung, and Blood Institute scientific statement: Executive Summary. *Crit Pathw Cardiol* 2005; **4**: 198-203 [PMID: 18340209]

2 **Bäckhed F**, Ding H, Wang T, Hooper LV, Koh GY, Nagy A, Semenkovich CF, Gordon JI. The gut microbiota as an environmental factor that regulates fat storage. *Proc Natl Acad Sci USA* 2004; **101**: 15718-15723 [PMID: 15505215]

3 **Malik VS**, Popkin BM, Bray GA, Després JP, Hu FB. Sugar-sweetened beverages, obesity, type 2 diabetes mellitus, and cardiovascular disease risk. *Circulation* 2010; **121**: 1356-1364 [PMID: 20308626]

4 **Stanhope KL**, Schwarz JM, Keim NL, Griffen SC, Bremer AA, Graham JL, Hatcher B, Cox CL, Dyachenko A, Zhang W, McGahan JP, Seibert A, Krauss RM, Chiu S, Schaefer EJ, Ai M, Otokozawa S, Nakajima K, Nakano T, Beysen C, Hellerstein MK, Berglund L, Havel PJ. Consuming fructose-sweetened, not glucose-sweetened, beverages increases visceral adiposity and lipids and decreases insulin sensitivity in overweight/obese humans. *J Clin Invest* 2009; **119**: 1322-1334 [PMID: 19381015]

5 **de Moura RF**, Ribeiro C, de Oliveira JA, Stevanato E, de Mello MA. Metabolic syndrome signs in Wistar rats submitted to different high-fructose ingestion protocols. *Br J Nutr* 2009; **101**: 1178-1184 [PMID: 19007450]

6 **Everard A**, Lazarevic V, Derrien M, Girard M, Muccioli GG, Neyrinck AM, Possemiers S, Van Holle A, François P, de Vos WM, Delzenne NM, Schrenzel J, Cani PD. Responses of gut microbiota and glucose and lipid metabolism to prebiotics in genetic obese and diet-induced leptin-resistant mice. *Diabetes* 2011; **60**: 2775-2786 [PMID: 21933985]

7 **Cani PD**, Amar J, Iglesias MA, Poggi M, Knauf C, Bastelica D, Neyrinck AM, Fava F, Tuohy KM, Chabo C, Waget A, Delmée E, Cousin B, Sulpice T, Chamontin B, Ferrières J, Tanti JF, Gibson GR, Casteilla L, Delzenne NM, Alessi MC, Burcelin R. Metabolic endotoxemia initiates obesity and insulin resistance. *Diabetes* 2007; **56**: 1761-1772 [PMID: 17456850]

8 **Sekirov I**, Russell SL, Antunes LC, Finlay BB. Gut microbiota in health and disease. *Physiol Rev* 2010; **90**: 859-904 [PMID: 20664075]

9 **Turnbaugh PJ**, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE, Sogin ML, Jones WJ, Roe BA, Affourtit JP, Egholm M, Henrissat B, Heath AC, Knight R, Gordon JI. A core gut microbiome in obese and lean twins. *Nature* 2009; **457**: 480-484 [PMID: 19043404]

10 **Turnbaugh PJ**, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006; **444**: 1027-1031 [PMID: 17183312]

11 **Bird AR**, Brown IL, Topping DL. Starches, resistant starches, the gut microflora and human health. *Curr Issues Intest Microbiol* 2000; **1**: 25-37 [PMID: 11709851]

12 **Bäckhed F**, Manchester JK, Semenkovich CF, Gordon JI. Mechanisms underlying the resistance to diet-induced obesity in germ-free mice. *Proc Natl Acad Sci USA* 2007; **104**: 979-984 [PMID: 17210919]

13 **Lee K**, Paek K, Lee HY, Park JH, Lee Y. Antiobesity effect of trans-10,cis-12-conjugated linoleic acid-producing Lactobacillus plantarum PL62 on diet-induced obese mice. *J Appl Microbiol* 2007; **103**: 1140-1146 [PMID: 17897219 DOI: 10.1111/j.1365-2672.2007.03336.x]

14 **Lee HY**, Park JH, Seok SH, Baek MW, Kim DJ, Lee KE, Paek KS, Lee Y, Park JH. Human originated bacteria, Lactobacillus rhamnosus PL60, produce conjugated linoleic acid and show anti-obesity effects in diet-induced obese mice. *Biochim Biophys Acta* 2006; **1761**: 736-744 [PMID: 16807088 DOI: 10.1016/j.bbalip.2006.05.007]

15 **Kondo S**, Xiao JZ, Satoh T, Odamaki T, Takahashi S, Sugahara H, Yaeshima T, Iwatsuki K, Kamei A, Abe K. Antiobesity effects of Bifidobacterium breve strain B-3 supplementation in a mouse model with high-fat diet-induced obesity. *Biosci Biotechnol Biochem* 2010; **74**: 1656-1661 [PMID: 20699581]

16 **Kang JH**, Yun SI, Park HO. Effects of Lactobacillus gasseri BNR17 on body weight and adipose tissue mass in diet-induced overweight rats. *J Microbiol* 2010; **48**: 712-714 [PMID: 21046354 DOI: 10.1007/s12275-010-0363-8]

17 **Chen JJ**, Wang R, Li XF, Wang RL. Bifidobacterium longum supplementation improved high-fat-fed-induced metabolic syndrome and promoted intestinal Reg I gene expression. *Exp Biol Med (Maywood)* 2011; **236**: 823-831 [PMID: 21685239 DOI: 10.1258/ebm.2011.010399]

18 **Yin YN**, Yu QF, Fu N, Liu XW, Lu FG. Effects of four Bifidobacteria on obesity in high-fat diet induced rats. *World J Gastroenterol* 2010; **16**: 3394-3401 [PMID: 20632441]

19 **Yadav H**, Jain S, Sinha PR. Antidiabetic effect of probiotic dahi containing Lactobacillus acidophilus and Lactobacillus casei in high fructose fed rats. *Nutrition* 2007; **23**: 62-68 [PMID: 17084593]

20 **Cacho J**, Sevillano J, de Castro J, Herrera E, Ramos MP. Validation of simple indexes to assess insulin sensitivity during pregnancy in Wistar and Sprague-Dawley rats. *Am J Physiol Endocrinol Metab* 2008; **295**: E1269-E1276 [PMID: 18796548]

21 **Do GM**, Oh HY, Kwon EY, Cho YY, Shin SK, Park HJ, Jeon SM, Kim E, Hur CG, Park TS, Sung MK, McGregor RA, Choi MS. Long-term adaptation of global transcription and metabolism in the liver of high-fat diet-fed C57BL/6J mice. *Mol Nutr Food Res* 2011; **55** Suppl 2: S173-S185 [PMID: 21618427 DOI: 10.1002/mnfr.201100064]

22 **Park DY**, Ahn YT, Huh CS, Jeon SM, Choi MS. The inhibitory effect of Lactobacillus plantarum KY1032 cell extract on the adipogenesis of 3T3-L1 Cells. *J Med Food* 2011; **14**: 670-675 [PMID: 21554138 DOI: 10.1089/jmf.2010.1355]

23 **Kootte RS**, Vrieze A, Holleman F, Dallinga-Thie GM, Zoetendal EG, de Vos WM, Groen AK, Hoekstra JB, Stroes ES, Nieuwdorp M. The therapeutic potential of manipulating gut microbiota in obesity and type 2 diabetes mellitus. *Diabetes Obes Metab* 2012; **14**: 112-120 [PMID: 21812894]

24 **Yadav H**, Jain S, Sinha PR. Effect of skim milk and dahi (yogurt) on blood glucose, insulin, and lipid profile in rats fed with high fructose diet. *J Med Food* 2006; **9**: 328-335 [PMID: 17004894]

25 **Basciano H**, Federico L, Adeli K. Fructose, insulin resistance, and metabolic dyslipidemia. *Nutr Metab (Lond)* 2005; **2**: 5 [PMID: 15723702]

26 **Miyazaki M**, Dobrzyn A, Man WC, Chu K, Sampath H, Kim HJ, Ntambi JM. Stearoyl-CoA desaturase 1 gene expression is necessary for fructose-mediated induction of lipogenic gene expression by sterol regulatory element-binding protein-1c-dependent and -independent mechanisms. *J Biol Chem* 2004; **279**: 25164-25171 [PMID: 15066988]

27 **Nagai Y**, Nishio Y, Nakamura T, Maegawa H, Kikkawa R, Kashiwagi A. Amelioration of high fructose-induced meta-

bolic derangements by activation of PPARalpha. *Am J Physiol Endocrinol Metab* 2002; **282**: E1180-E1190 [PMID: 11934685]
28 **Fontbonne A**, Eschwège E, Cambien F, Richard JL, Ducimetière P, Thibult N, Warnet JM, Claude JR, Rosselin GE. Hypertriglyceridaemia as a risk factor of coronary heart disease mortality in subjects with impaired glucose tolerance or diabetes. Results from the 11-year follow-up of the Paris Prospective Study. *Diabetologia* 1989; **32**: 300-304 [PMID: 2666216]
29 **Murphy EF**, Cotter PD, Hogan A, O'Sullivan O, Joyce A, Fouhy F, Clarke SF, Marques TM, O'Toole PW, Stanton C, Quigley EM, Daly C, Ross PR, O'Doherty RM, Shanahan F. Divergent metabolic outcomes arising from targeted manipulation of the gut microbiota in diet-induced obesity. *Gut* 2012; Epub ahead of print [PMID: 22345653 DOI: 10.1136/gutjnl-2011-300705]
30 **Begley M**, Hill C, Gahan CG. Bile salt hydrolase activity in probiotics. *Appl Environ Microbiol* 2006; **72**: 1729-1738 [PMID: 16517616]
31 **Wu T**, Giovannucci E, Pischon T, Hankinson SE, Ma J, Rifai N, Rimm EB. Fructose, glycemic load, and quantity and quality of carbohydrate in relation to plasma C-peptide concentrations in US women. *Am J Clin Nutr* 2004; **80**: 1043-1049 [PMID: 15447918]
32 **Kelley GL**, Allan G, Azhar S. High dietary fructose induces a hepatic stress response resulting in cholesterol and lipid dysregulation. *Endocrinology* 2004; **145**: 548-555 [PMID: 14576175]
33 **Busserolles J**, Rock E, Gueux E, Mazur A, Grolier P, Rayssiguier Y. Short-term consumption of a high-sucrose diet has a pro-oxidant effect in rats. *Br J Nutr* 2002; **87**: 337-342 [PMID: 12064343]
34 **Martarelli D**, Verdenelli MC, Scuri S, Cocchioni M, Silvi S, Cecchini C, Pompei P. Effect of a probiotic intake on oxidant and antioxidant parameters in plasma of athletes during intense exercise training. *Curr Microbiol* 2011; **62**: 1689-1696 [PMID: 21400082]
35 **Ejtahed HS**, Mohtadi-Nia J, Homayouni-Rad A, Niafar M, Asghari-Jafarabadi M, Mofid V. Probiotic yogurt improves antioxidant status in type 2 diabetic patients. *Nutrition* 2012; **28**: 539-543 [PMID: 22129852]
36 **McNulty NP**, Yatsunenko T, Hsiao A, Faith JJ, Muegge BD, Goodman AL, Henrissat B, Oozeer R, Cools-Portier S, Gobert G, Chervaux C, Knights D, Lozupone CA, Knight R, Duncan AE, Bain JR, Muehlbauer MJ, Newgard CB, Heath AC, Gordon JI. The impact of a consortium of fermented milk strains on the gut microbiome of gnotobiotic mice and monozygotic twins. *Sci Transl Med* 2011; **3**: 106ra106 [PMID: 22030749]

P- Reviewer Gibson DL **S- Editor** Xiong L
L- Editor Webster JR **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i2.284

World J Gastroenterol 2013 January 14; 19(2): 284-289
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Clinical significance and risk factors of postembolization fever in patients with hepatocellular carcinoma

Chung Hwan Jun, Ho Seok Ki, Hoon Ki Lee, Kang Jin Park, Seon Young Park, Sung Bum Cho, Chang Hwan Park, Young Eun Joo, Hyun Soo Kim, Sung Kyu Choi, Jong Sun Rew

Chung Hwan Jun, Ho Seok Ki, Hoon Ki Lee, Kang Jin Park, Seon Young Park, Sung Bum Cho, Chang Hwan Park, Young Eun Joo, Hyun Soo Kim, Sung Kyu Choi, Jong Sun Rew, Division of Gastroenterology, Department of Internal Medicine, Chonnam National University Medical School, Gwangju 501-757, South Korea

Author contributions: Jun CH and Choi SK performed the majority of the study and wrote the manuscript; Cho SB, Park SY, and Park CH were involved in editing the manuscript; Ki HS, Lee GH, Park KJ were involved in data acquisition, analysis or interpretation; all authors were involved in revising and approving the final version for publication.

Correspondence to: Sung Kyu Choi, MD, Division of Gastroenterology, Department of Internal Medicine, Chonnam National University Medical School, 42 Jaebong-ro, Dong-Ku, Gwangju 501-757, South Korea. choisk@jnu.ac.kr

Telephone: +82-62-2206296 Fax: +82-62-2258578

Received: September 14, 2012 Revised: November 14, 2012
Accepted: November 24, 2012
Published online: January 14, 2013

Abstract

AIM: To investigate tumor response and survival in patients with postembolization fever (PEF) and to determine the risk factors for PEF.

METHODS: Four hundred forty-three hepatocellular carcinoma (HCC) patients who underwent the first session of transcatheter arterial chemoembolization (TACE) between January 2005 and December 2009 were analyzed retrospectively. PEF was defined as a body temperature greater than 38.0 ℃ that developed within 3 d of TACE without evidence of infection. The tumor progression-free interval was defined as the interval from the first TACE to the second TACE based on mRECIST criteria. Clinical staging was based on the American Joint Committee on Cancer tumor, node, metastases (TNM) classification of malignant tumors. All patients were admitted before their 1st TACE treatment, and blood samples were obtained from all patients before and after treatment. Clinicoradiological variables and host-related variables were compared between two groups: patients with PEF *vs* patients without PEF. Additionally, variables related to 20-mo mortality and tumor progression-free survival were analyzed.

RESULTS: The study population comprised 370 (85.4%) men and 73 (14.6%) women with a mean age of 62.29 ± 10.35 years. A total of 1836 TACE sessions were conducted in 443 patients, and each patient received between 1 and 27 (mean: 4.14 ± 3.57) TACE sessions. The mean follow-up duration was 22.23 ± 19.6 mo (range: 0-81 mo). PEF developed in 117 patients (26.41%) at the time of the first TACE session. PEF was not associated with 20-mo survival (P = 0.524) or computed tomography (CT) response (P = 0.413) in a univariate analysis. A univariate analysis further indicated that diffuse-type HCC (P = 0.021), large tumor size (≥ 5 cm) (P = 0.046), lipiodol dose (≥ 7 mL, P = 0.001), poor blood glucose control (P = 0.034), alanine aminotransferase (ALT) value after TACE (P = 0.004) and C-reactive protein (CRP) value after TACE (P = 0.036) served as possible risk factors correlated with PEF. The ALT value after TACE (P = 0.021) and lipiodol dose over 7 mL (P = 0.011) were independent risk factors for PEF in the multivariate analysis. For the 20-mo survival, poor blood sugar control (P < 0.001), portal vein thrombosis (P = 0.001), favorable CT response after TACE (P < 0.001), initial aspartate aminotransferase (P = 0.02), initial CRP (P = 0.042), tumor size (P < 0.001), TNM stage (P < 0.001) and lipiodol dose (P < 0.001) were possible risk factors in the univariate analysis. Tumor size (P = 0.03), poor blood sugar control (P = 0.043), and portal vein thrombosis (P = 0.031) were significant predictors of survival in the multivariate analysis. Furthermore, the tumor progression-free interval was closely associated with CRP > 1 mg/dL (P = 0.003), tumor size > 5 cm (P < 0.001), tumor type (poorly defined) (P < 0.001), and lipiodol dose (> 7 mL, P < 0.001).

CONCLUSION: PEF has no impact on survival at 20 mo or radiologic response. However, the ALT level after TACE and the lipiodol dose represent significant risk factors for PEF.

© 2013 Baishideng. All rights reserved.

Key words: Chemoembolization; Therapeutic; Fever; Carcinoma; Hepatocellular; Prognosis; Progression-free survival

Jun CH, Ki HS, Lee HK, Park KJ, Park SY, Cho SB, Park CH, Joo YE, Kim HS, Choi SK, Rew JS. Clinical significance and risk factors of postembolization fever in patients with hepatocellular carcinoma. *World J Gastroenterol* 2013; 19(2): 284-289 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i2/284.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i2.284

INTRODUCTION

Hepatocellular carcinoma (HCC) is the seventh most common carcinoma worldwide and the third most common cause of cancer-related mortality[1]. In South Korea, the age-standardized incidence rate of HCC is 46.5 per 100 000 individuals[2]. Recent advances in treatment, including liver transplantation, surgical resection, percutaneous ethanol injection therapy, radiofrequency ablation and transcatheter arterial chemoembolization (TACE) have improved the prognosis for patients with HCC[3,4]. In addition, beneficial therapeutic options that may affect long-term cure include surgical resection, liver transplantation, and percutaneous ablation[5]. However, these curative therapies are feasible only for a small subset of patients with HCC[6]. Among the noncurative therapies, only chemoembolization, the most widely used treatment for unresectable HCC, has demonstrated a positive effect on survival[7]. In contrast, TACE can be employed for any type of HCC irrespective of tumor size, location, or number[8].

However, TACE inevitably results in a hypoxic insult to the HCC and the surrounding liver tissue[9], and postembolization syndrome is common[10]. Postembolization syndrome, which consists of temporary fever, ileus, and abdominal pain, is the most common side effect of chemoembolization, affecting 60% to 80% of patients with HCC[5,11]. Postembolization fever after TACE is the most significant adverse effect, and it frequently affects the duration of hospitalization and causes the needless administration of antibiotics, although the fever is self-limited in most cases. However, few data about postembolization fever have been reported. Therefore, we evaluated the risk factors and prognostic significance of postembolization fever in patients with HCC.

MATERIALS AND METHODS

Patients and methods

Four hundred forty-three HCC patients who underwent the first session of TACE between January 2005 and December 2009 were analyzed retrospectively. The diagnosis of HCC was confirmed histologically or based on consistent findings obtained from at least two imaging techniques: ultrasonography, computed tomography (CT), magnetic resonance imaging, and/or selective hepatic arterial angiography[12,13]. Clinical staging was determined based on the American Joint Committee on Cancer tumor, node, metastases (TNM) classification of malignant tumors[14].

Clinicoradiological variables were compared between two groups (patients with PEF *vs* patients without PEF). The host-related variables included age, sex, viral status, cause of HCC, Child-Pugh score, Eastern Cooperative Oncology Group (ECOG) performance status, white blood cell counts, aspartate aminotransferase (AST), alanine aminotransferase (ALT), α-fetoprotein (AFP), and 20-mo mortality. The tumor-related variables included maximal tumor size, number of tumors, TNM stage, radiological findings (poorly defined or well defined), portal vein thrombosis, and CT response after 1st TACE.

All patients were admitted before their 1st TACE, and blood samples were obtained from all patients before and after treatment. Serum AFP, CRP, blood chemistry and ECOG score at admission were measured. After the 1st TACE, the patients were carefully followed. Dynamic CT was performed after 4 wk and then every 3 to 6 mo (Figure 1).

Our institutional review board did not require approval because the procedures were performed for clinical reasons. Informed consent was obtained from all patients after the nature and purpose of the TACE procedure had been fully explained.

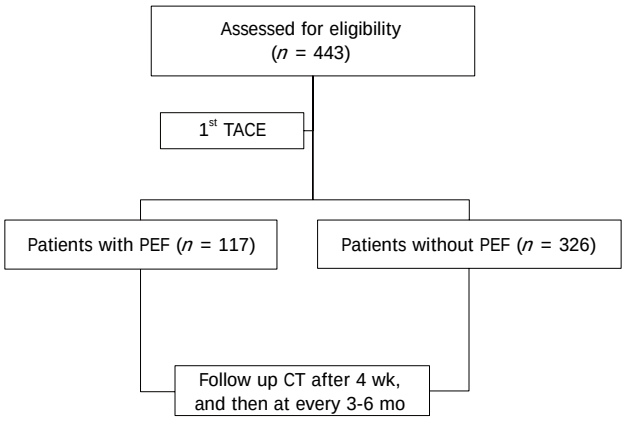


Figure 1 Flowchart of study patient enrollment. PEF: Postembolization fever; TACE: Transcatheter arterial chemoembolization; CT: Computed tomography.

Chemoembolization procedure

An arterial catheter was inserted into the femoral artery using the Seldinger method and placed in the hepatic artery. Tumor-feeding vessels were superselected whenever possible, and a solution containing 10 to 40 mg of doxorubicin hydrochloride (ADM; Dong-A Pharmacy, Seoul, Korea) and 0 to 40 mL of iodized oil (Lipiodol; Guer-

Table 1 Comparison of transcatheter arterial chemoembolization sessions with or without postembolization fever

Variables	Patients with PEF (n = 117)	Patients without PEF (n = 326)	P-value
Sex (M/F)	94/23	276/50	0.309
Age (yr)	62.26 ± 10.79	62.16 ± 9.98	0.971
Cause of HCC (HBV/HCV/alcohol/other)	66/25/14/11	180/44/73/25	0.088
Tumor type (well defined/poorly defined)	57/44	202/90	0.021
Portal vein thrombosis (yes/no)	9/93	39/253	0.292
Poor blood glucose control (yes/no)	29/88	116/210	0.039
Favorable tumor response	61 (52.5%)	155 (48.5%)	0.516
20-mo mortality	60 (51.7%)	172 (53.4%)	0.828
Initial WBC/mm^3	5969 ± 3497	5547 ± 2535	0.194
Initial AST U/L	68.87 ± 45.49	64.11 ± 44.79	0.358
Initial ALT U/L	50.84 ± 41.58	43.89 ± 33.16	0.129
Initial CRP mg/dL	1.23 ± 1.66	0.86 ± 0.93	0.116
Initial AFP IU/mL	2778 ± 8014	2600 ± 8656	0.857
Child-Pugh score	5.66 ± 0.72	5.77 ± 0.97	0.296
TNM stage	1.95 ± 1.05	1.83 ± 1.07	0.305
Interval from 1st TACE to 2nd TACE (d)	157 ± 225	180 ± 270	0.412
Total TACE sessions	1.48 ± 3.45	4.02 ± 3.62	0.230

TACE: Transcatheter arterial chemoembolization; AFP: α-fetoprotein; PEF: Postembolization fever; TNM: Tumor, node, metastases; CRP: C-reactive protein; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; HCC: Hepatocellular carcinoma; M: Male; F: Female; HBV: Hepatitis B virus; HCV: Hepatitis C virus.

bet, Aulnay-sous-Bois, France) with absorbable gelatin particles (Gelfoam; Upjohn, Kalamazoo, Michigan) was injected through the catheter (5F) or microcatheter (2.8 or 3F). The doses of doxorubicin and iodized oil were individually determined according to tumor size, tumor extent, and the patient's underlying liver function.

Monitoring and management of postembolization fever

For the purpose of this study, we defined postembolization fever as a body temperature greater than 38.0 ℃ during the 3 d after TACE. Body temperature was measured *qid* by nurses using an axillary thermometer. Bacterial cultures from blood and urine and chest X-rays were performed for patients who had fevers after TACE to detect any potential infectious agents. Empirical broad-spectrum intravenous antibiotics were used to treat potential infections if there was fever but were discontinued when bacterial cultures did not reveal any causative agent and the fever had subsided. Ultrasonography or CT scans were performed if the fever persisted despite the use of antibiotics to detect the possible formation of an abscess. Acetaminophen or nonsteroidal anti-inflammatory drugs were used for symptom control, if necessary.

Response assessment

The efficacy of TACE was evaluated by comparing the CT scans obtained before and after chemoembolization in terms of iodized oil uptake patterns in the tumor that could be considered necrotic[12] and tumor extent. The iodized oil uptake was considered compact if the oily contrast medium was clearly dispersed through all viable target tumors but was noncompact in all other cases[8]. Tumor response to TACE was defined as a compact uptake of iodized oil or at least a 30% decrease in the sum of the largest diameters of viable tumors, despite noncompact iodized oil uptake.

Definitions

PEF was defined as a body temperature greater than 38.0 ℃ that developed within 3 d of TACE without evidence of infection. Poor blood glucose control was defined as a mean blood glucose level > 200 mg/dL. Poorly defined tumor type was defined as diffuse-type HCC, whereas well-defined tumor type was defined as nodular HCC. Tumor progression-free survival was defined as the interval during and after treatment in which a patient remained alive and the disease did not worsen (in this case, the interval from the 1st TACE to the 2nd TACE).

Statistical analysis

Comparisons were performed using the student's t test for continuous variables and Pearson's χ^2 test. Factors that were significant in the univariate analysis were entered into a stepwise multivariate analysis to identify the most significant risk factors. The hazard function data were estimated using the Kaplan-Meier curve and compared using the log-rank test. Multivariate analyses were performed using the Cox proportional hazards model to identify prognostic factors. We performed statistical analyses using SPSS 17.0 (SPSS Inc., Chicago, United States). A P-value less than 0.05 was considered statistically significant.

RESULTS

Fever after TACE and clinical features

The study population consisted of 370 (85.4%) men and 73 (14.6%) women with a mean age of 62.29 ± 10.35 years. A total 1836 sessions of TACE were conducted in 443 patients between January 2005 and December 2009. Each patient received between 1 and 27 (mean, 4.14 ± 3.57) sessions of TACE. The mean follow-up duration was 22.23 ± 19.6 mo (range: 0-81 mo).

One hundred seventeen episodes of postembolization fever (26.41%) occurred in 443 HCC patients after the 1st TACE session. Most of the postembolization fever episodes peaked within the first two days after TACE. The post-TACE fever was usually self-limiting, with durations ranging from 1 to 10 d (mean: 1.72 ± 1.11 d). The infectious complication rate was 0.16% (3/1836 cases). Two cases of bacteremia and one case of liver abscess developed after 1836 TACE sessions. A comparison of the TACE sessions with and without fever is presented in Table 1.

Association of PEF with clinical variables

A univariate analysis indicated that diffuse-type HCC (P < 0.05), large tumor size (≥ 5 cm) (P < 0.05), lipiodol

Table 2 Multivariate and univariate analysis for postembolization fever, 20-mo mortality, and tumor progression-free survival

Variables	HR	95%CI	*P*-value
Multivariate analysis[1]			
ALT value after TACE	1.002	1.00-1.005	< 0.05
Lipiodol dose (< 7 mL)	0.539	0.329-0.881	< 0.05
Univariate analysis[2]			
Poor BS control	2.673	1.77-4.034	< 0.01
Portal vein thrombosis	3.048	1.536-6.06	< 0.01
Poor CT response	2.638	1.785-3.891	< 0.01
Initial AST	1.006	1.001-1.011	< 0.05
Initial CRP	1.348	1.011-1.796	< 0.05
Tumor size	1.31	1.198-1.433	< 0.01
TNM	1.555	1.26-1.92	< 0.01
Lipiodol dose	1.12	1.058-1.185	< 0.01
Multivariate analysis[3]			
Tumor size	1.252	1.108-1.414	< 0.01
Poor BS control	2.442	1.310-4.55	< 0.01
Portal vein thrombosis	3.344	1.021-10.98	< 0.05
Cox regression analysis[4]			
Poor CT response after TACE	0.302	0.192-0.765	< 0.01
Lipiodol dose (≥ 7 mL)	0.494	0.279-0.874	< 0.05

[1]Predictors for postembolization fever; [2]Risk factors for 20-mo mortality; [3]Predictors for 20-mo mortality; [4]Predictors for tumor progression-free survival. HR: Hazard ratio; CRP: C-reactive protein; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; TNM: Tumor, node, metastases; TACE: Transcatheter arterial chemoembolization; BS: Base of support.

dose (≥ 7 mL) ($P < 0.01$), poor blood glucose control ($P < 0.05$), ALT value after TACE ($P < 0.01$) and CRP value after TACE ($P < 0.05$) were possible risk factors correlated with postembolization fever in patients with HCC.

A multivariate analysis using logistic regression showed that the ALT value after TACE ($P < 0.05$) and the lipiodol dose (≥ 7 mL) ($P < 0.05$) were independent predictive factors of postembolization fever (Table 2).

PEF was not associated with 20-mo survival ($P = 0.754$), 10-mo survival ($P = 0.524$) and CT response ($P = 0.461$) in the univariate analysis.

20-mo mortality

The univariate analysis revealed that poor blood sugar control ($P < 0.01$), portal vein thrombosis ($P < 0.01$), favorable CT response after TACE ($P < 0.01$), initial AST ($P < 0.05$), initial CRP ($P < 0.05$), tumor size ($P < 0.01$), TNM stage ($P < 0.01$) and lipiodol dose ($P < 0.01$) were possible risk factors correlated with 20-mo mortality (Table 2). A multivariate analysis using logistic regression showed that tumor size ($P < 0.01$), poor blood glucose control ($P < 0.01$) and portal vein thrombosis ($P < 0.05$) were independent risk factors for 20-mo mortality (Table 2).

Tumor progression-free survival

The progression-free survival in the poorly defined tumor (diffuse) type group was significantly shorter than in the well-defined (nodular) tumor type group ($P < 0.01$). Additionally, large size (size ≥ 5 cm, $P < 0.01$), no antiviral treatment ($P < 0.05$), poor CT response ($P < 0.01$), lipiodol dose (dose ≥ 7 mL, $P < 0.01$), antibiotic use

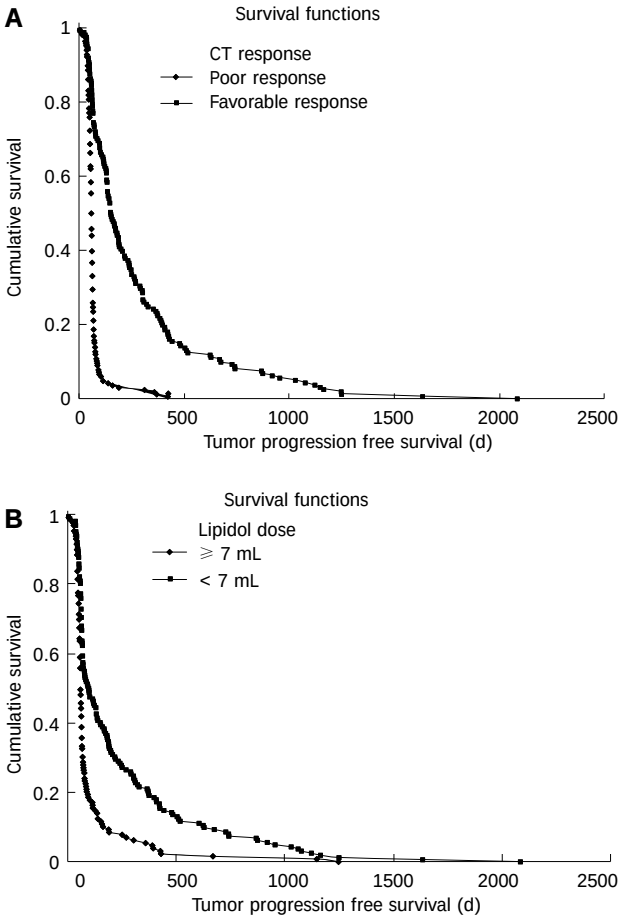


Figure 2 Kaplan-Meier curves of progression-free survival. A: According to computed tomography response (log-rank test, $P < 0.001$); B: According to lipiodol dose (log-rank test, $P < 0.001$).

($P < 0.05$) and CRP (CRP ≥ 1 mg/dL, $P < 0.01$) were statistically significant factors in the univariate analysis (Figure 2A and B). In the multivariate Cox proportional hazard model for progression-free survival, CT response ($P < 0.01$) and lipiodol dose (≥ 7 mL) ($P < 0.05$) were identified as independent factors (Table 2).

DISCUSSION

TACE is one of the major treatment methods for unresectable HCC and has demonstrated survival benefits[4,10-15]. Chemoembolization acts by obstructing the hepatic artery with embolization agents, usually gelatin, and introducing antitumor agents (e.g., cisplatin, doxorubicin, and mitomycin C) emulsified in iodized oil, thereby inducing extensive necrosis in large vascularized HCC tumors[7]. TACE complications can be categorized as hepatic injuries, including deterioration of hepatic function, hepatic infarction, or intrahepatic biloma, and liver abscess; extrahepatic complications, including gastrointestinal bleeding, gallbladder or spleen infarction, and pulmonary embolism; and systemic complications, including postembolization syndrome and septicemia[16]. The most frequent complication of chemoembolization is postembolization fever, which can typically be satisfactorily

alleviated with symptom treatment[11,16]. Nevertheless, PEF frequently troubles patients, family members and physicians, and few data have been published concerning postembolization fever; therefore, we investigated the risk factors and clinical significance of PEF that developed after TACE in patients with HCC.

Infectious complications are very rare because of the standard antiseptic procedures associated with TACE. Another study reported that only 0.26% of HCC patients developed liver abscesses after TACE[17]. Thus, antibiotic prophylaxis is usually not necessary in patients with HCC who are undergoing TACE[10]. Although fevers were common (27%) in this study, they were generally not caused by an infectious process because very few patients had bacterial infections (0.16%). These fevers can often be adequately controlled with antipyretics, and in most cases, antibiotics are not necessary.

The pathogenesis of PEF remains unclear and complicated. The main aspects are as follows: (1) lipiodol-induced embolisms may result in ischemia, hypoxia, and necrosis in some normal hepatic cells; (2) chemotherapeutic drugs themselves have toxicities[18]; (3) the procedure itself can lead to a considerable release of inflammatory factors[19]; and (4) such stimuli as injury and drugs can contribute to stress responses in the human body. In the present study, the occurrence of PEF was closely associated with several clinical and laboratory variables, including poor blood glucose control, large tumor size (> 5 cm), poorly defined tumor type, post-TACE CRP level, lipiodol dose higher than 7 mL, and post-TACE ALT level in a univariate analysis. However, the multiple regression analysis showed that a lipiodol dose over 7 mL and the post-TACE ALT level were independent risk factors, which is similar to the result of another recent study[20]. No difference was found between favorable CT responses and unfavorable CT responses regarding the presence of PEF, which is in concordance with another study showing that post-TACE fever was not associated with an enhanced tumor response in patients with HCC[20-22].

PEF is thought to reflect extensive tumor necrosis, thereby representing the efficacy of chemoembolization[22-24]. However, we observed no other robust association between PEF and survival in this study. Moreover, PEF did not independently affect progression-free survival, which may be an indirect indicator of treatment efficacy. These findings suggest that the previously described correlation between PEF and the extent of tissue necrosis cannot always be justified because the extent of tissue necrosis after chemoembolization is proportional to tumor mass, a factor that is independently associated with PEF.

Raoul *et al*[25] suggested that factors associated with poor TACE outcomes included Child-Pugh score, reduced liver function, AFP level, tumor size, tumor number, tumor type, portal vein thrombosis, multiple TACE sessions and lobar embolization. In our study, large tumor size, poor blood glucose control and portal vein thrombosis were independent risk factors for 20-mo mortality. However, unlike in other studies, the Child-Pugh score was not a significant prognostic factor in our study. It was likely that most of the patients enrolled in our study had Child A (the Child score for patients with PEF was 5.66 *vs* 5.77 for those without PEF), and as a result, liver function did not affect the prognosis. According to Shim *et al*[22], hepatitis B virus infection, modified UICC stage (Stage 1), and response to chemoembolization are independent predictive factors for the tumor progression-free interval. In good agreement with other studies, we found that the TACE response and lipiodol dose (< 7 mL) were closely associated with tumor progression-free survival. Because the lipiodol dose was dependent on tumor size, progression-free survival was closely associated with tumor size and response to TACE.

This study had several limitations, including its retrospective design. Although we performed laboratory and culture studies of blood, urine, and ascites to detect hidden infections, it was impossible to rule out all infective complications in patients with PEF. In addition, the tumor response to chemoembolization may have been overestimated in patients with poorly defined HCC, particularly those with the diffuse infiltrative type of HCC, because of the difficulty of evaluating the degree of viable tumor. Finally, it may be difficult to determine whether the development of PEF after a single session of TACE was exclusively associated with overall survival because the mortality of TACE-treated HCC patients is subject to many other factors.

In conclusion, ALT levels after TACE and lipiodol dose were independent risk factors for postembolization fever in HCC patients. However, postembolization fever after TACE had no impact on survival at 20 mo or on the radiologic response.

COMMENTS

Background

Postembolization fever (PEF) is thought to reflect extensive tumor necrosis and thereby represent the efficacy of transcatheter arterial chemoembolization (TACE) in hepatocellular carcinoma (HCC). The aim of this study was to assess the tumor response and survival of patients with PEF after TACE and to determine the risk factors for PEF.

Research frontiers

TACE has improved the prognosis of patients with HCC. However, TACE inevitably results in a hypoxic insult to the HCC and the surrounding liver tissue, and postembolization fever is common. However, the clinical meaning of and the risks factor for PEF are not known.

Innovations and breakthroughs

PEF has been associated with overall survival in HCC patients. However, there is limited published data about PEF and the risk factors for PEF. Unlike other studies, their demonstrated that PEF was not associated with overall survival or radiological tumor response. Furthermore, this study demonstrated that alanine aminotransferase (ALT) levels after TACE and lipiodol doses were risk factors for PEF.

Applications

The results of this study imply that PEF is associated with lipiodol dose and ALT levels after TACE but does not affect prognosis; therefore, patients undergoing TACE who have risk factors for PEF should receive active or prophylactic antipyretics for PEF control.

Terminology

Postembolization fever was defined as a body temperature greater than 38.0 ℃

that developed within 3 d of TACE without evidence of infection. Poorly defined tumor type was defined as diffuse-type HCC, whereas well-defined tumor type was defined as nodular HCC. Tumor progression-free survival was defined as the interval of time during and after treatment in which a patient remained alive and the disease did not worsen (in the case of this study, the interval from the 1st TACE to the 2nd TACE).

Peer review

Postembolization fever is a clinically relevant problem in the treatment of patients with HCC. The authors addressed potential causes and risk factors and found that the ALT level after TACE and the lipiodol dose served as independent risk factors for PEF after TACE and that PEF had no impact on 20-mo survival in HCC patients. These results were extracted and calculated from a large group of 443 patients who were treated and observed within a 5-year period.

REFERENCES

1 **Yang JD**, Roberts LR. Hepatocellular carcinoma: A global view. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 448-458 [PMID: 20628345 DOI: 10.1038/nrgastro.2010.100]

2 **Park JW**. [Hepatocellular carcinoma in Korea: introduction and overview]. *Korean J Gastroenterol* 2005; **45**: 217-226 [PMID: 15843747 DOI: 200504302]

3 **Mazzaferro V**, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693-699 [PMID: 8594428 DOI: 10.1056/NEJM199603143341104]

4 **Llovet JM**, Real MI, Montaña X, Planas R, Coll S, Aponte J, Ayuso C, Sala M, Muchart J, Solà R, Rodés J, Bruix J. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002; **359**: 1734-1739 [PMID: 12049862 DOI: 10.1016/S0140-6736(02)08649-X]

5 **Bruix J**, Sala M, Llovet JM. Chemoembolization for hepatocellular carcinoma. *Gastroenterology* 2004; **127**: S179-S188 [PMID: 15508083 DOI: S0016508504016117]

6 **Llovet JM**, Bruix J, Gores GJ. Surgical resection versus transplantation for early hepatocellular carcinoma: clues for the best strategy. *Hepatology* 2000; **31**: 1019-1021 [PMID: 10733561 DOI: 10.1053/he.2000.6959]

7 **Llovet JM**, Bruix J. Systematic review of randomized trials for unresectable hepatocellular carcinoma: Chemoembolization improves survival. *Hepatology* 2003; **37**: 429-442 [PMID: 12540794 DOI: 10.1053/jhep.2003.50047]

8 **Okada S**. Transcatheter arterial embolization for advanced hepatocellular carcinoma: the controversy continues. *Hepatology* 1998; **27**: 1743-1744 [PMID: 9620352 DOI: 10.1002/hep.510270639]

9 **Matsuda Y**, Kawata S, Nagase T, Maeda Y, Yamasaki E, Kiso S, Ishiguro H, Matsuzawa Y. Interleukin-6 in transcatheter arterial embolization for patients with hepatocellular carcinoma. Effects of serine protease inhibitor. *Cancer* 1994; **73**: 53-57 [PMID: 8275438]

10 A comparison of lipiodol chemoembolization and conservative treatment for unresectable hepatocellular carcinoma. Groupe d'Etude et de Traitement du Carcinome Hépatocellulaire. *N Engl J Med* 1995; **332**: 1256-1261 [PMID: 7708069 DOI: 10.1056/NEJM199505113321903]

11 **Chan AO**, Yuen MF, Hui CK, Tso WK, Lai CL. A prospective study regarding the complications of transcatheter intraarterial lipiodol chemoembolization in patients with hepatocellular carcinoma. *Cancer* 2002; **94**: 1747-1752 [PMID: 11920537 DOI: 10.1002/cncr.10407]

12 **Takayasu K**, Arii S, Matsuo N, Yoshikawa M, Ryu M, Takasaki K, Sato M, Yamanaka N, Shimamura Y, Ohto M. Comparison of CT findings with resected specimens after chemoembolization with iodized oil for hepatocellular carcinoma. *AJR Am J Roentgenol* 2000; **175**: 699-704 [PMID: 10954453]

13 **Boix J**, Lorenzo-Zúñiga V, Moreno de Vega V, Domènech E, Gassull MA. Endoscopic resection of ampullary tumors: 12-year review of 21 cases. *Surg Endosc* 2009; **23**: 45-49 [PMID: 18398649 DOI: 10.1007/s00464-008-9866-3]

14 **Kanematsu T**, Furuta T, Takenaka K, Matsumata T, Yoshida Y, Nishizaki T, Hasuo K, Sugimachi K. A 5-year experience of lipiodolization: selective regional chemotherapy for 200 patients with hepatocellular carcinoma. *Hepatology* 1989; **10**: 98-102 [PMID: 2544499 DOI: S0270913989001254]

15 **Lo CM**, Ngan H, Tso WK, Liu CL, Lam CM, Poon RT, Fan ST, Wong J. Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. *Hepatology* 2002; **35**: 1164-1171 [PMID: 11981766 DOI: 10.1053/jhep.2002.33156]

16 **Chung JW**, Park JH, Han JK, Choi BI, Han MC, Lee HS, Kim CY. Hepatic tumors: predisposing factors for complications of transcatheter oily chemoembolization. *Radiology* 1996; **198**: 33-40 [PMID: 8539401]

17 **Ong GY**, Changchien CS, Lee CM, Wang JH, Tung HD, Chuah SK, Chiu KW, Chiou SS, Cheng YF, Lu SN. Liver abscess complicating transcatheter arterial embolization: a rare but serious complication. A retrospective study after 3878 procedures. *Eur J Gastroenterol Hepatol* 2004; **16**: 737-742 [PMID: 15256974 DOI: 00042737-200408000-00003]

18 **Nagano M**, Nakamura T, Niimi S, Fujino T, Nishimura T, Murayama N, Ishida S, Ozawa S, Saito Y, Sawada J. Substitution of arginine for cysteine 643 of the glucocorticoid receptor reduces its steroid-binding affinity and transcriptional activity. *Cancer Lett* 2002; **181**: 109-114 [PMID: 12430185]

19 **Distelhorst CW**. Recent insights into the mechanism of glucocorticosteroid-induced apoptosis. *Cell Death Differ* 2002; **9**: 6-19 [PMID: 11803370 DOI: 10.1038/sj.cdd.4400969]

20 **Li CP**, Chao Y, Chen LT, Lee RC, Lee WP, Yuan JN, Yen SH, Lee SD. Fever after transcatheter arterial chemoembolization for hepatocellular carcinoma: incidence and risk factor analysis. *Scand J Gastroenterol* 2008; **43**: 992-999 [PMID: 19086281]

21 **Wigmore SJ**, Redhead DN, Thomson BN, Currie EJ, Parks RW, Madhavan KK, Garden OJ. Postchemoembolisation syndrome--tumour necrosis or hepatocyte injury? *Br J Cancer* 2003; **89**: 1423-1427 [PMID: 14562011 DOI: 10.1038/sj.bjc.6601329]

22 **Shim JH**, Park JW, Choi JI, Kim HB, Lee WJ, Kim CM. Does postembolization fever after chemoembolization have prognostic significance for survival in patients with unresectable hepatocellular carcinoma? *J Vasc Interv Radiol* 2009; **20**: 209-216 [PMID: 19084432 DOI: 10.1016/j.jvir.2008.10.021]

23 **Hemingway AP**, Allison DJ. Complications of embolization: analysis of 410 procedures. *Radiology* 1988; **166**: 669-672 [PMID: 3340761]

24 **Yamada R**, Sato M, Kawabata M, Nakatsuka H, Nakamura K, Takashima S. Hepatic artery embolization in 120 patients with unresectable hepatoma. *Radiology* 1983; **148**: 397-401 [PMID: 6306721]

25 **Raoul JL**, Sangro B, Forner A, Mazzaferro V, Piscaglia F, Bolondi L, Lencioni R. Evolving strategies for the management of intermediate-stage hepatocellular carcinoma: available evidence and expert opinion on the use of transarterial chemoembolization. *Cancer Treat Rev* 2011; **37**: 212-220 [PMID: 20724077 DOI: 10.1016/j.ctrv.2010.07.006]

P- Reviewer Gangl A **S- Editor** Gou SX
L- Editor A **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i2.290

World J Gastroenterol 2013 January 14; 19(2): 290-298
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

IL28B polymorphism and cytomegalovirus predict response to treatment in Egyptian HCV type 4 patients

Mostafa K El Awady, Noha G Bader El Din, Ashraf Tabll, Yaser El Hosary, Ashraf O Abdel Aziz, Hesham El Khayat, Mohsen Salama, Tawfeek H Abdelhafez

Mostafa K El Awady, Noha G Bader El Din, Ashraf Tabll, Tawfeek H Abdelhafez, Department of Microbial Biotechnology, National Research Center, Cairo, Giza 12622, Egypt
Yaser El Hosary, Department of Internal Medicin, National Research Center, Cairo, Giza 12622, Egypt
Ashraf O Abdel Aziz, Department of Tropical Medicine, Cairo University, Giza 12622, Egypt
Hesham El Khayat, Theodor Bilharz Research Institute, Giza 12622, Egypt
Mohsen Salama, National Liver Institute, Shebeen El Kom 32512, Egypt
Author contributions: El Awady MK designed the experiment, designed the new primers, and wrote the manuscript; Bader El Din NG, performed research work (IL28 PCR, CMV PCR, HCV PCR), edited the manuscript and made the final revision; Tabll A helped in the manuscript writing; El Hosary Y, Abdet Aziz AO, El Khayat H, Salama M provided blood samples; Abdelhafez TH participated in designing the new primers, and performed research work (IL28 PCR).
Supported by Misr El-Khair Foundation, Cairo, Egypt
Correspondence to: Dr. Mostafa El Awady, PhD, Department of Microbial Biotechnology, National Research Center, El-Behooth Street 12622, Dokki, Cairo, Giza 12622, Egypt. mkawady@yahoo.com
Telephone: +20-12-3132640 Fax: +20-2-3370931
Received: January 14, 2012 Revised: April 28, 2012
Accepted: May 5, 2012
Published online: January 14, 2013

Abstract

AIM: To test whether the status of positive cytomegalovirus (CMV) DNA detection adds to the predictive value of IL28B and to further categorize C/T allele carriers.

METHODS: This study included 166 chronic hepatitis C (CHC) patients who received combined interferon and ribavirin therapy for 48 wk, 84 spontaneous hepatitis C virus (HCV) resolvers who were positive for IgG anti-HCV antibody and negative for HCV RNA, and 100 healthy subjects who were negative for both HCV antibodies and RNA as controls. Genomic DNA from peripheral blood was used for IL28B rs.12979860 single nucleotide polymorphism (SNP) and CMV DNA detection. A 139 bp fragment containing IL28B SNP was amplified in all subjects by polymerase chain reaction using a specifically designed primer. Then the IL28B rs.12979860 SNP was detected by restriction fragment length polymorphism (RFLP) genotyping. The presence of CMV DNA was tested by amplification of the *gB1* gene using nested polymerase chain reaction. The role of CMV and IL28B rs.12979860 SNP genotypes in determining the response rate to combined interferon therapy and clinical status of patients were statistically analyzed.

RESULTS: Current data showed that 67% of patients carrying the IL28B 12979860 C/C allele had a sustained viral response (SVR) while the genotypes C/T and TT were associated with lower SVR rates, 50% and 48%, respectively. SVR rates for the C/C allele were lower than other HCV genotypes and/or other populations. Genotype CC was associated with the response to interferon (P = 0.025). Genotype C/C was reduced from 48% in controls to 14% in CHC patients suggesting its protective role against progression to chronicity. The majority of spontaneously cleared subjects (86%) were C/C, confirming its protective role. The C/T allele was present in 71% of CHC patients compared with 38% of controls, so the use of IL28B SNP genotyping only in these patients may be of little value as a predictor of response. CMV reactivation occurred in 40% of CHC patients. Co-infection with CMV seriously diminished the response to interferon (IFN) therapy, with SVR rates in C/C genotypes 87.5% in CMV-negative patients and 12.5% in CMV-positive patients (P < 0.0001). SVR rates among C/T carriers were reduced to < 50% in patients with positive CMV DNA while the non-response rate doubled. These data indicate that a supplemental assay for CMV viremia adds to the prognostic value of IL28B genotyping.

CONCLUSION: The results suggest that both genetic (i.e., spontaneous) and therapeutic (IFN-based therapy) arms are complementary in the battle against HCV. CMV DNA testing may be of value to better predict the response to IFN, particularly in IL28B C/T carriers.

© 2013 Baishideng. All rights reserved.

Key words: Hepatitis C; Interleukin 28B; Genetic polymorphisms; Human cytomegalovirus; Spontaneous clearance

El Awady MK, Bader El Din NG, Tabll A, El Hosary Y, Abdel Aziz AO, El Khayat H, Salama M, Abdelhafez TH. IL28B polymorphism and cytomegalovirus predict response to treatment in Egyptian HCV type 4 patients. *World J Gastroenterol* 2013; 19(2): 290-298 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i2/290.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i2.290

INTRODUCTION

The current standard-of-care for chronic hepatitis C virus (HCV) infection includes weekly injections of pegylated interferon-α (peg-IFN) combined with daily oral ribavirin[1,2] for 48 wk[3]. During the first 3 mo of therapy, HCV viral loads usually fall to undetectable levels in response to interferon-α. However, in non-responder (NR) patients, HCV viral loads persist at or near pretreatment levels. Among those patients with an initial response to treatment, known as early virological responders, up to 50% will relapse after treatment is discontinued and are known as relapsed responders, whereas the remainder will have a sustained virological response (SVR) as determined by the absence of detectable viremia 6 mo after treatment has stopped[4]. In Egypt, several reports stated that approximately 50% of patients infected with genotype 4, the most common among Egyptian HCV patients, achieve a SVR to this regimen. Genome-wide association studies have recently revealed that single nucleotide polymorphisms (SNPs) within or adjacent to IL28B (19q13) that codes for interferon-λ3, predict spontaneous resolution of HCV[5,6] and a likely SVR to IFN-based treatment in chronic hepatitis C (CHC) patients infected with genotypes other than type 4[4,5,7-9]. Therefore, IL28B SNPs may have strong predictive value for the outcome of IFN-based therapy in the difficult to treat HCV genotype 4 patients, with the hope that IL28B SNPs will improve future decision-making in the management of CHC in Egypt. Nevertheless, it was estimated that IL28B variations account for about 15% of the inter-individual variability of SVR, thus supporting the necessity for additional predictor(s) of the response to treatment. Recent predictors include vitamin D deficiency, IFN-c-inducible protein-10 serum levels, steatosis/insulin resistance[10-17], or IFN-stimulated genes (ISGs) such as OAS1[18] and MxA[19]. Therefore, biological models for the prediction of SVR should include a variety of parameters besides IL28B genotyping, as this would offer better accuracy compared with IL28B typing alone[13]. Recently two predictors for SVR in genotype 4 patients were reported; first, reactivation of CMV infection[20] and second, SNP at exon 7 splice acceptor site of 2' 5'OAS gene[18]. The role of several genetic factors that influence spontaneous or treatment-induced HCV clearance, such as ISGs and genes encoding the natural killer cell receptor KIR2DL3 and its ligand, and human leukocyte antigen C group 1[21-23] should be re-analyzed parallel with the IL28 genotype. A major difficulty in decision-making for prediction of the response at the start of treatment is the high frequency of heterozygous carriers rs 12979860 C/T among chronic HCV patients, ranging from 45% to 70% of the CHC populations and provide vague predictive results. This means that the predictive value of rs 12979860 SNP will not be useful in almost half the patient population. The search for additional predictive factors for response is mandatory. Co-infection with other pathogens is, in some instances, an interfering factor against host genotype-based prediction. Several studies revealed comparable associations between IL28B variations and treatment-induced HCV clearance in patients co-infected with human immunodeficiency virus (HIV)[5,24-26]. For example, one study in HIV co-infected patients reported 2-3-fold higher SVR rates for HCV genotypes 1 and 4 according to rs12979860 C/C *vs* C/T, T/T, but no difference for HCV genotype 3 according to rs12979860 C/C *vs* C/T, T/T[26]. Recently, active CMV infection was shown by our laboratory[20] to dramatically interfere with SVR rates in genotype 4 CHC patients, probably by inhibiting the JAK-STAT cascade. We, therefore, hypothesize that the role of CMV in the IL28B association with SVR should be a strong candidate for further categorization of C/T genotype carriers.

MATERIALS AND METHODS

Subjects

The present study comprised 350 subjects, including 166 CHC patients, 84 spontaneous HCV resolvers and 100 healthy control subjects.

Chronic HCV patients: All 166 patients had mild liver disease F0-F3 according to the Metavir classification[27]. All subjects had undetectable HBV surface antigen (HBsAg), Anti-schistosoma antibodies (Abs), and autoimmune Abs. None of the patients had a history of long-term drug use, aflatoxins consumption or alcohol intake. All patients were infected with HCV genotype 4. Patients included 105 males and 61 females, with an age range of 19-57 years. None had uncontrolled type II diabetes mellitus, thyroid dysfunction or obesity. All patients received weekly injection of peg-IFN-α plus daily oral ribavirin treatment for 48 wk. Eighty six patients achieved a SVR, namely undetectable HCV RNA 24 wk after the end of

the treatment response that was achieved after 48 wk, i.e., a total of 72 wk. The remaining 80 patients were NR who failed to achieve a SVR, i.e., either did not respond to therapy after 12 wk or achieved undetectable viremia but failed to maintain the state of no viremia till 48 wk (breakthrough) or post 48 wk (viral relapse).

Spontaneous HCV resolvers: Eighty four subjects who tested positive for IgG anti-HCV Abs (3rd generation) and negative for HCV RNA were enrolled in the study. Patients included 53 males and 31 females, with an age range of 18-55 years.

Controls: One hundred healthy subjects who tested negative for both IgG anti HCV Abs and HCV RNA served as controls for the IL28B SNP and CMV reactivation studies. Also, they were negative for HBsAg, anti-schistosoma Abs, autoimmune Abs and CMV DNA. Subjects included 50 males and 50 females, with an age range of 18-64 years. None of subjects had a history of liver insult, either viral, metabolic or drug exposure.

Methods

Extraction of peripheral blood DNA: Peripheral blood was withdrawn from all subjects into EDTA solution and DNA was extracted using genomic DNA extraction kit (Qiagen, Milan Italy). Plasma samples were separated before DNA extraction and utilized for testing HBsAg, anti schistosomiases Abs and autoimmune Abs (ANA, AMA, LKM1, ASMA). Purified genomic DNA was used for IL28B SNP analysis and for testing the presence of CMV DNA.

Detection of IL-28B rs12979860 C/T polymorphism: Genotyping for the IL-28B rs12979860 C/T polymorphism was performed according to Fabris *et al*[24] with minor modification by polymerase chain reaction based restriction fragment length polymorphism (PCR-RFLP). Using purified genomic DNA, a 139 base-pair (bp) product was obtained with the forward primer 5'-CCAGGGCCCCTAACCTCTGCA-3' and the reverse primer 5'-GGGAGCGCGGAGTGCAATTCA-3', newly designed with the aid of NCBI Primer-Blast Tool (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). Amplification was carried out in a total volume of 10 μL containing 10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, Tween-20 0.01%, 0.2 mmol/L deoxyribonucleotides, 2-4 pmol of each primer, 2.0 mmol/L MgCl2, 0.5 units hot-start Taq DNA polymerase (Righ Taq, Euroclone, Milan, Italy) and ~10 ng of genomic DNA. The thermal protocol for amplification included 35 cycles of denaturation (at 95 ℃ for 60 s), annealing (at 64 ℃ for 60 s), and polymerization (at 72 ℃ for 60 s). Ten μL of the amplicons were digested with 1 unit of the BstU-I restriction endonuclease (New England Biolabs, Hitchin, UK) in a total volume of 20 μL at 37 ℃ for 4-6 h or overnight. The fragments were resolved by electrophoresis in 4% agarose gel after staining with ethidium bromide. A band of 139 bp indicated the TT genotype, 109 bp indicated the CC genotype; 139 + 109 bp indicated the CT genotype.

PCR amplification of the CMV *gB* gene: Primers for the first-round PCR were derived from the *gB* region of CMV, and PCR protocols were followed as described previously[28,29] The amplification mixture contained 10 pmol of each primer (CMV1 and CMV2), 0.2 mmol/L from each dNTP (Promega, Madison, WI, United States), 0.1 U Taq polymerase (Promega, Madison, WI, United States), 1 μL Taq buffer, 5 μL template, and distilled water to a final volume of 20 μL. A negative control tube (in which water replaced the DNA sample) was incorporated into each run. A sample from the first PCR product was used as a template in a second-round PCR (nested). Briefly, 1 μL of first round product was added to the reaction mixture, which is the same as the first-round reaction mix except for use of CMV3 and CMV4 as nested primers. The thermal cycling protocol was as follows: 1 min at 94 ℃, 1 min at 55 ℃ and 1 min at 72 ℃ for 30 cycles. Then, the nested PCR products were analyzed with 3% agarose gel electrophoresis and stained with ethidium bromide. A result was considered positive when a clear 100 bp product was visible on the gel.

Primers used for the first and the second-round PCR were CMV1: 5'-GAGGACAACGAAATCCTGTTGGGCA-3'; CMV2: 5'-GTCGACGGTGGAGATACTGCTGAGG-3'; CMV3: 5'-ACCACCGCACTGAGGAATGTCAG-3'; and CMV4: 5'-TCAATCATGCGTTTGAAGAGGTA-3'.

Assessment of SVR status (real-time and RT-PCR of HCV RNA: As reported in our previous study[20,29]. Disappearance of viremia was confirmed by a variety of tests including RT-PCR using nested primers derived from the highly conserved HCV 5'UTR and real-time PCR. These tests were done 3, 6, 12 and 18 mo after the start of IFN therapy. HCV RNA was extracted using the BIOZOL-total RNA extraction reagent kit (Hangzhou Bioer Technology, Hangzhou, China). Nested RT-PCR performed in a 25 μL reaction mixture containing 20 units of avian myeloblastosis virus reverse transcriptase (Clontech, Mountain View, CA, United States), 200-400 ng of total cellular RNA as template, 40 units of RNAsin (Clontech), 0.2 μmol/L from each dNTP (QBIOGENE, Carlsbad, CA, United States), and 50 pmol of the reverse primer P2. The reaction was incubated at 42 ℃ for 60 min and denatured at 98 ℃ for 10 min. Amplification of the highly conserved 5'-UTR sequences was done using two rounds of PCR with two pairs of nested primers. First-round amplification was done in 50 μL of reaction containing 50 pmol from each of the P1 forward primer and P2 reverse primer, 0.2 mmol/L from each dNTP, 10 μL from RT reaction mixture as template and two units of Taq DNA polymerase (Finnzyme, Woburn, MA, Unit-

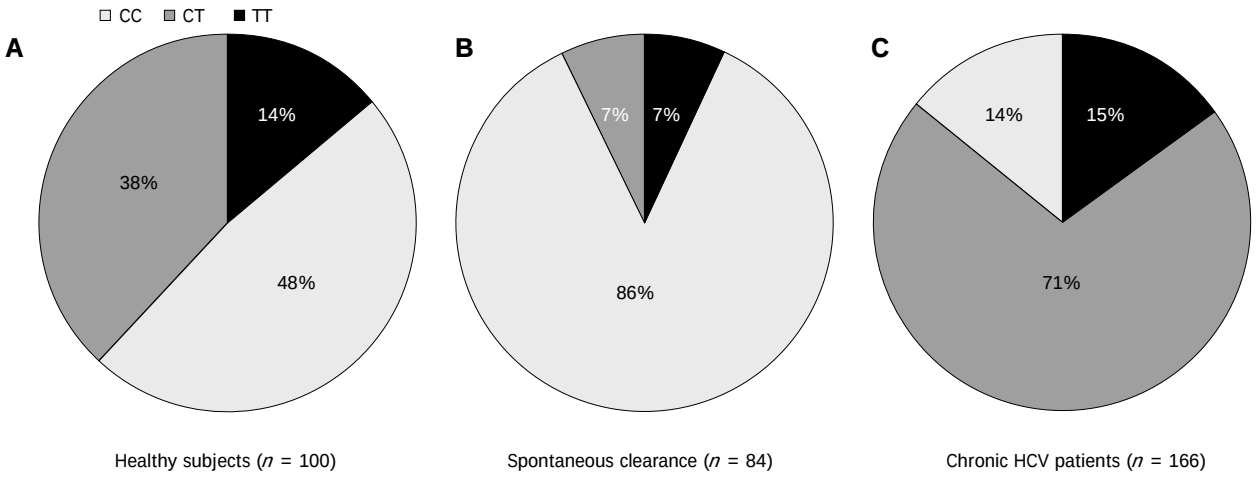


Figure 1 Distribution of LI28B rs12979860 single nucleotide polymorphism alleles among a healthy Egyptian population, spontaneous clearance and chronic hepatitis C patients. A: A hundred healthy subjects representing ethnic backgrounds of the Egyptian population were typed for IL28B single nucleotide polymorphism (SNP) rs 12979860. The distribution of different allele frequencies is depicted as a pie chart; B: Eighty four patients with spontaneous clearance from hepatitis C virus (HCV) infection were typed for IL28B SNP rs 12979860. The distribution of different allele frequencies is depicted as a pie chart; C: A hundred and sixty six chronic hepatitis C (CHC) patients with low F scores (F0-F2) were typed for IL28B SNP rs 12979860. The distribution of different allele frequencies is depicted as a pie chart.

ed States) in a 1 × buffer supplied with the enzyme. The thermal cycling protocol was as follows: 1 min at 94 ℃, 1 min at 55 ℃ and 1 min at 72 ℃ for 30 cycles. The second round of amplification was done similar to the first round, except for use of the nested reverse primer p4 and forward primer p3 at 50 pmol each. A fragment of 171 bp length was identified in positive samples. Primer sequences were as follows: P1: 5'-AACTACTGTCTTCACGCAGAA-3'; P2: 5'-GGTGCACGGTCTACGAGACCTC-3'; P3: 5'-GTGCAGCCTCCAGGACCC-3'; and P4: 5'-ACTCGGCTAGCAGTCTCGCG-3'.

HCV quantitation was performed using the Fluorescence Quantitative Detection kit (Hangzhou Bioer Technology, Hangzhou, China). HCV RNA was reverse transcribed and a specific fragment was amplified with specific primers in a one-step RT-PCR reaction. The products were detected using specific Taqman-MGB Probes. The real-time RT-PCR reaction was done in 50 μL of reaction containing 25 μL RT-PCR mix, 2.5 μL Mn^{2+}, 1.5 μL HCV probe mix, and 1 μL internal control. Then 20 μL of the corresponding HCV RNA template were added. For the standard, 20 μL of the standards control 1-4 were added. All PCR tubes were placed in the real-time PCR Detection Instrument. The thermal cycling protocol was as follows: one cycle (30 s at 90 ℃, 20 min at 61 ℃ and 1 min at 95 ℃) followed by 30 cycles and 50 cycles (15 s at 95 ℃, 1 min at 60 ℃).

Statistical analysis

The significance of the response to IFN therapy was investigated by multivariate logistic regression analysis. CMV significantly affected the HCV response rate in C/T heterozygous carriers. The frequency of rs 12979860 SNP was compared in the control group *vs* the CHC patient group. All statistical analyses were performed using SPSS 9.0 statistical software (SPSS Inc., Chicago, IL, United States). The odds ratio (OR) and 95% confidence interval (CI) were calculated to assess the relative risk confidence. A two tailed *P*-value 0.05 was considered statistically significant.

RESULTS

IL 28 C/C frequency in spontaneous resolvers of HCV infection

To test the hypothesis whether patients who spontaneously cleared HCV infection during or shortly following the acute phase bear the protective C/C allele more frequently than healthy subjects, we typed the IL28 B SNP (rs 12979860) in 84 spontaneous resolvers and 100 healthy subjects. The results of this study were previously described by our laboratory and are shown in Figure 1A and B, which clearly indicate the protective role of the C/C allele in patients who spontaneously cleared the virus, where C/C was present in 86% of spontaneous resolvers *vs* 48% of healthy subjects.

IL28B C/C frequency in chronic HCV patients

To examine the allele frequencies of the C/C genotype according to progression to chronic HCV infection, we compared the IL28B typing data derived from 166 chronic HCV patients with 84 subjects with spontaneous clearance of HCV. The results showed that the frequency of the protective C/C allele was markedly decreased from 86% in spontaneous resolvers to 48% in healthy controls to 14% in CHC patients regardless of their response to IFN therapy (Figure 1A-C). This decline was associated with a comparable increase in the heterozygous C/T from 7% in spontaneous resolvers to 38% in healthy subjects and to 71% in CHC patients.

Table 1 Statistical analyses of the different IL28B genotypes and alleles in non-responder and sustained viral response patients *n* (%)

	Non-responders (NR = 79)	Responders (SVR = 87)	Odds ratio	95%CI	*P* value
CC (*n* = 24)	8 (33.3)	16 (66.7)	4.575	1.376-7.846	0.025
CT (*n* = 117)	58 (49.6)	59 (50.4)	0.858	0.538-1.449	0.589
TT (*n* = 25)	13 (52)	12 (48)	0.978	0.758-1.759	0.464
C (*n* = 165)	74 (44.8)	91 (55.2)	1.636	0.773-3.465	0.198
T (*n* = 167)	84 (50.3)	83 (49.7)	0.798	0.598-1.349	0.604

Frequencies of genotypes and alleles at IL28B SNP were compared between SVR and NR patients. Genotype CC were associated with SVR (*P* = 0.025). SVR: Sustained viral response; NR: Non-responder.

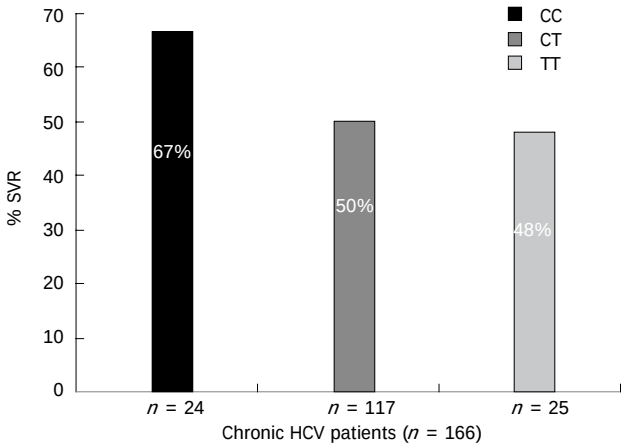


Figure 2 Rate of sustained viral response in IL28B variants. Among 166 chronic hepatitis C (CHC) patients treated with combined interferon (IFN) and ribavirin therapy, 80 patients did not achieve a sustained viral response (SVR) while 86 patients achieved a SVR. Among the 14% (24) of CHC patients bearing the C/C allele, 67% (16 patients) achieved a SVR, while the other two genotypes C/T and TT were associated with lower SVR rates; 50% and 48%, respectively. Genotype CC was associated with response to IFN (*P* = 0.025). HCV: Hepatitis C virus.

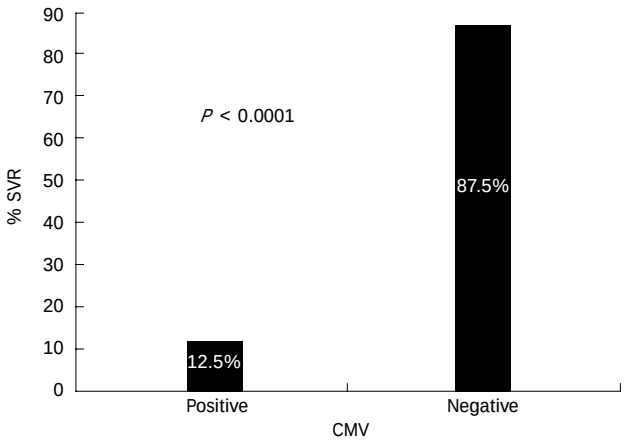


Figure 3 Association of cytomegalovirus reactivation with sustained viral response rates in C/C patients. The data shown clearly demonstrate that cytomegalovirus (CMV) reactivation has dramatically reduced the sustained viral response (SVR) rates in C/C genotypes as represented by a 12.5% SVR rate in CMV-positive patients compared with 87.5% in CMV-negative patients (*P* < 0.0001).

Rate of SVR in patients with the rs12979860 C/C allele

Among 166 CHC patients treated with combined IFN and ribavirin therapy, 79 patients did not achieve a SVR while 87 patients achieved a SVR. The effect of different IL28B genotypes and alleles on the HCV response to IFN treatment are shown in Table 1. The genotype CC was associated with response to IFN ($P = 0.025$). Of the 24 CHC patients bearing the C/C allele, 16 (67%) achieved a SVR, while the other two genotypes C/T and TT were associated with lower SVR rates: 50% and 48%, respectively (Figure 2).

Association of CMV reactivation with SVR rates in C/C patients

The PCR results of the CMV *gB* gene showed that 66/166 (40%) of chronic HCV patients have detectable CMV DNA. Since we have previously shown that coinfection with CMV seriously diminished the response to IFN therapy, it was tempting to examine whether this co-infection is associated with lower SVR rates in C/C holders. The data shown in Figure 3 clearly demonstrate that positive CMV DNA dramatically reduced the SVR rates in C/C genotypes as represented by a 12.5% SVR rate in CMV-positive patients compared with 87.5% in CMV-negative patients ($P < 0.0001$).

Sorting of C/T genotype according to association with CMV infection

Since the C/T allele represents the majority of CHC patients (117/166) (71%), the use of IL28B SNP genotyping may be of little value as a predictor of response prior to starting the treatment regimen. Therefore, there is a great need to include another factor that was previously known to affect SVR rates. Table 2 shows the results of CMV testing in CT genotype patients. Specifically, CMV DNA was detected in 71% of NR patients and in 29% of SVR patients. In Figure 4, the SVR rates among C/T carriers was reduced to < 50% of its value in cases where patients had positive CMV DNA On the other hand, the NR rate doubled in cases of positive CMV DNA. These data indicate that a supplemental assay for CMV viremia adds to the prognostic value of IL28B genotyping.

DISCUSSION

Several predictive factors have been reported recently for SVR rates for combination therapy in HCV infection. Viral factors, other than viral loads and genotypes were reported, including substitutions at core amino acids 70 and 91[30,31], IFN sensitivity determining region variations[32] and IFN and ribavirin response determining region variations[33]. SNPs within a number of host genes were found to be associated with different treatment outcomes to therapy. These include IFN-α pathway genes[34] and IFN-stimulated genes such as OAS[18]

Table 2 Influence of cytomegalovirus infection on response rate to interferon combined therapy in chronic hepatitis C virus infected patients with IL28B CT genotype *n* (%)

HCV response to treatment (*n* = 117)	IL 28B genotype (CT) +ve CMV patients (*n* = 45)	IL 28B genotype (CT) -ve CMV patients (*n* = 72)
NR (*n* = 59)	32 (71)	27 (38)
SVR (*n* = 58)	13 (29)	45 (62)

Reactivation of cytomegalovirus (CMV) was assessed by amplification of viral genome in all patients. Association of IL28B genotype CT and CMV emia with response to interferon based therapy is illustrated in this table. SVR: Sustained viral response; NR: Non-responder.

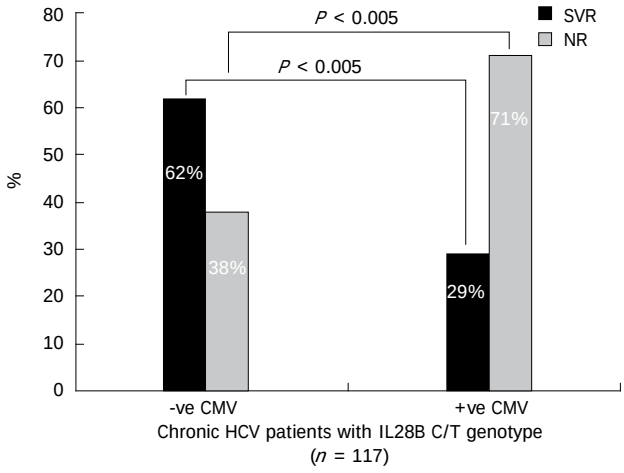


Figure 4 Sorting of C/T genotype according to association with cytomegalovirus infection. The sustained viral response (SVR) rates among C/T carriers, is reduced to < 50% of its value in cases where patients had cytomegalovirus (CMV) reactivation. On the other hand, the non-response rate is increased 2-fold in cases of CMV reactivation. HCV: Hepatitis C virus.

and MxA[19]. The widely studied IL28B SNP allele was reported to correlate with a favorable therapeutic response and was also associated with spontaneous clearance of HCV[6]. Thomas *et al*[6] reported that the allele related to HCV clearance, namely rs 12979860 C/C, was the major allele in the majority of Asian and European countries. Conversely, patients of African ancestry had lower C/C allele frequency and SVR rates. The current data indicate that the Egyptian population infected mainly with genotype 4 has an intermediate response between that of the populations of African and European ancestries, i.e., the C/C allele frequency in normal subjects was 48% *vs* > 50% in Europeans and ~35% in Africans while SVR rates in the currently studied CHC patients were 67% *vs* ~80% in Europeans and ~50% in Africans. One of the most interesting aspects of the current study is the dramatic decline in C/C frequency in CHC patients (from 48% in controls to 14% in genotype 4 infections), thus allowing speculation that protective IL28B variations provide a more substantial advantage in acute HCV genotype 4. In a similar study on a German cohort, the rs12979860 C/C genotype was found in 49% of uninfected subjects and declined in chronic infection to 42.7% of HCV genotype 2 and 3 patients and 33.9% of HCV genotype 1 patients[35]. Taking together the data from the German cohort and the data of the current study, we may speculate that HCV genotype 4 has the highest advantage of viral clearance during acute infection followed by genotype 1 and lastly genotypes 2 and 3.

Strong evidence from the current data on the significant protective effect of the IL28 B variant in acute HCV 4 is the high percentage of the C/C genotype among spontaneously cleared individuals, i.e., positive HCV Abs and negative HCV RNA compared with controls and CHC patients (86% *vs* 48 and 14%, respectively). The lack of a protective role of the C/C genotype in 14% of CHC patients suggests that, in CHC patients with the C/C genotype, other factors are involved in development of chronic infection. It is tempting to hypothesize that IL28B C/C in those 24 CHC patients was not sufficient to provide substantial tendency towards viral clearance and consequently explains the relatively lower figure for the C/C association with SVR rate in the present cohort compared with Asian and European studies. In a recent study from our laboratory co-infection with active CMV viremia induced severe failure to achieve acceptable rates of SVR[20]. Infection with CMV has evolved multiple mechanisms for disrupting the IFN-stimulated JAK/STAT signal transduction. It appears to inhibit IFN-α responsiveness by decreasing JAK1 protein, which is an essential component of IFN-α signaling[36]. It was reported that CMV blocks IFN-stimulated gene factor 3 (ISGF3)-dependent (MHC class I, 2',5'-OAS, and MxA) and ISGF3-independent gene expression in infected cells. Moreover, the essential component of ISGF3, p48, is significantly decreased by CMV. Therefore decreased JAK1 and p48 protein would inhibit IFN-α stimulated signal transduction, transcription factor activation, and gene expression, thus it is likely to globally block IFN-stimulated responses in CMV-infected patients[37]. The dual analysis of both roles of IL28B SNP and CMV co-infection clearly showed that C/C carriers not infected with CMV have a 7-fold higher rate of SVR than those C/C patients co-infected with CMV. One of the major problems in decision-making on an individual patient basis is the overwhelming preponderance of the IL28B C/T genotype among the current CHC cohort as well as other cohorts[38,39]. The present results indicate that the IL28B SNP is not the only factor that influences the IFN-induced antiviral activities of host cells against viral infection. The present results showed a 2-fold increase in SVR rate in CMV-negative over CMV-positive C/T carriers and clearly highlight the significance of additional testing for CMV viremia besides IL28 genotyping to set a model for accurate prediction of response to combined therapy in C/T carriers.

In conclusion the current results allow us to speculate that both genetic (i.e., spontaneous) and therapeutic (IFN-based therapy) arms are complementary in the battle against HCV where poor spontaneous clearance is

associated with higher IFN-mediated response as in genotypes 2 and 3. On the other hand, the difficult to treat genotypes 1 and 4 have clearly better chances for spontaneous resolution. Furthermore, CMV DNA testing may be of value at the moment to make better prognostication on response to IFN particularly in heterozygous carriers. Needless to say the search for supplemental predictive host factors will never stop at least in the near future.

COMMENTS

Background

The current recommended treatment for chronic hepatitis C virus (HCV) infection is the combination of pegylated α-interferon (IFN) and ribavirin given for 48 wk. Both viral and host factors may contribute to the phenomenon that some patients with chronic hepatitis C respond well to IFN therapy but others do not. The host genetic factors mediate the vigor of the immune response of the host through the control of inter-individual differences in the production of intracellular antiviral proteins or some cytokines. Different studies revealed that single nucleotide polymorphisms (SNPs) within or adjacent to IL28B (19q13) that codes for interferon-λ3, predict spontaneous resolution of HCV and likely SVR to IFN based treatment in chronic HCV patients infected with genotypes other than type 4. Since more than 93% of patients in Egypt are infected with HCV genotype 4, the authors investigated whether IL28B SNPs may be of predictive value for the outcome of IFN-based therapy in the difficult to treat HCV genotype 4 patients. Also the authors tested whether the status of positive cytomegalovirus (CMV) DNA detection added to the predictive value of IL28B and to further categorization of C/T allele carriers.

Research frontiers

HCV is a global health problem that infects more than 170 million people around the world and more than 15% of the Egyptian population. The response rate to combined IFN therapy does not exceed 50% of patients infected with HCV genotype 4, the most prevalent subtype in Egypt. Timing and rules for this therapy without major complications are not very well defined, so that infected patients not only suffer from severe adverse effects but there is also a contribution to evolution of more resistant strains of the virus. A research focus is how to determine and understand which HCV patients are likely to develop persistent infection, progressive liver disease or do not respond significantly to IFN therapy. These criteria are of utmost importance in disease control programs and treatment strategies. Therefore, there is an urgent need for biological models containing a variety of prognostic parameters to predict the response to IFN treatment in HCV-infected patients and to help make better decisions on treatment strategy.

Innovations and breakthroughs

The use of IL28B SNP analysis in predicting the response to combined IFN therapy is informative only in cases of CC or TT genotypes. Since the heterozygous genotype CT represents more than 70% of chronic HCV patients, there is a need for supplemental markers for making a decision on whether the HCV patient is a potential responder to combined IFN therapy or not. The authors' data clearly improve the decision-making by detecting CMV reactivation in the CT genotype group. CMV reactivation has been shown to dramatically interfere with the response to combined IFN treatment. The data presented in this manuscript are indeed important for hepatologists regarding the anticipated response to standard combined therapy for HCV genotype 4 patients.

Applications

The data of this manuscript help hepatologists make better decisions on response rate to combined interferon therapy for HCV genotype 4 patients.

Peer review

In this manuscript, the authors aimed to investigate whether the status of positive cytomegalovirus (CMV) DNA detection increases the predictive value of IL28B and to further categorize C/T allele carriers. The authors have found that CMV DNA testing might be valuable at the moment to make better decisions on response to IFN particularly in IL28B C/T carriers. Overall, this topic is very interesting.

REFERENCES

1 **Ferenci P**. Current Treatment for Chronic Hepatitis C. *Curr Treat Options Gastroenterol* 2004; **7**: 491-499 [PMID: 15527715 DOI: 10.1007/s11938-004-0008-2]

2 **Hadziyannis SJ**, Sette H, Morgan TR, Balan V, Diago M, Marcellin P, Ramadori G, Bodenheimer H, Bernstein D, Rizzetto M, Zeuzem S, Pockros PJ, Lin A, Ackrill AM. Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004; **140**: 346-355 [PMID: 14996676]

3 **Lagging M**, Wejstål R, Uhnoo I, Gerdén B, Fischler B, Friman S, Josephson F, Karlström O, Sangfelt P, Schvarz R, Weiland O. Treatment of hepatitis C virus infection: updated Swedish Consensus recommendations. *Scand J Infect Dis* 2009; **41**: 389-402 [PMID: 20001276 DOI: 10.1080/00365540902998271]

4 **Knapp S**, Yee LJ, Frodsham AJ, Hennig BJ, Hellier S, Zhang L, Wright M, Chiaramonte M, Graves M, Thomas HC, Hill AV, Thursz MR. Polymorphisms in interferon-induced genes and the outcome of hepatitis C virus infection: roles of MxA, OAS-1 and PKR. *Genes Immun* 2003; **4**: 411-419 [PMID: 12944978 DOI: 10.1038/sj.gene.6363984]

5 **Rauch A**, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T, Bochud M, Battegay M, Bernasconi E, Borovicka J, Colombo S, Cerny A, Dufour JF, Furrer H, Günthard HF, Heim M, Hirschel B, Malinverni R, Moradpour D, Müllhaupt B, Witteck A, Beckmann JS, Berg T, Bergmann S, Negro F, Telenti A, Bochud PY. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. *Gastroenterology* 2010; **138**: 1338-145, 1338-145, [PMID: 20060832]

6 **Thomas DL**, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, Kidd J, Kidd K, Khakoo SI, Alexander G, Goedert JJ, Kirk GD, Donfield SM, Rosen HR, Tobler LH, Busch MP, McHutchison JG, Goldstein DB, Carrington M. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 2009; **461**: 798-801 [PMID: 19759533 DOI: 10.1038/nature08463]

7 **Ge D**, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; **461**: 399-401 [PMID: 19684573 DOI: 10.1038/nature08309]

8 **Suppiah V**, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, Bassendine M, Spengler U, Dore GJ, Powell E, Riordan S, Sheridan D, Smedile A, Fragomeli V, Müller T, Bahlo M, Stewart GJ, Booth DR, George J. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 2009; **41**: 1100-1104 [PMID: 19749758 DOI: 10.1038/ng.447]

9 **Tanaka Y**, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, Nakagawa M, Korenaga M, Hino K, Hige S, Ito Y, Mita E, Tanaka E, Mochida S, Murawaki Y, Honda M, Sakai A, Hiasa Y, Nishiguchi S, Koike A, Sakaida I, Imamura M, Ito K, Yano K, Masaki N, Sugauchi F, Izumi N, Tokunaga K, Mizokami M. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. *Nat Genet* 2009; **41**: 1105-1109 [PMID: 19749757 DOI: 10.1038/ng.449]

10 **Zeuzem S**, Berg T, Moeller B, Hinrichsen H, Mauss S, Wedemeyer H, Sarrazin C, Hueppe D, Zehnter E, Manns MP. Expert opinion on the treatment of patients with chronic hepatitis C. *J Viral Hepat* 2009; **16**: 75-90 [PMID: 18761607]

11 **Berg T**, Sarrazin C, Herrmann E, Hinrichsen H, Gerlach T, Zachoval R, Wiedenmann B, Hopf U, Zeuzem S. Prediction

of treatment outcome in patients with chronic hepatitis C: significance of baseline parameters and viral dynamics during therapy. *Hepatology* 2003; **37**: 600-609 [PMID: 12601358 DOI: 10.1053/jhep.2003.50106]

12 **Thompson AJ**, Muir AJ, Sulkowski MS, Ge D, Fellay J, Shianna KV, Urban T, Afdhal NH, Jacobson IM, Esteban R, Poordad F, Lawitz EJ, McCone J, Shiffman ML, Galler GW, Lee WM, Reindollar R, King JW, Kwo PY, Ghalib RH, Freilich B, Nyberg LM, Zeuzem S, Poynard T, Vock DM, Pieper KS, Patel K, Tillmann HL, Noviello S, Koury K, Pedicone LD, Brass CA, Albrecht JK, Goldstein DB, McHutchison JG. Interleukin-28B polymorphism improves viral kinetics and is the strongest pretreatment predictor of sustained virologic response in genotype 1 hepatitis C virus. *Gastroenterology* 2010; **139**: 120-9.e18 [PMID: 20399780 DOI: 10.1053/j.gastro.2010.04.013]

13 **Bitetto D**, Fattovich G, Fabris C, Ceriani E, Falleti E, Fornasiere E, Pasino M, Ieluzzi D, Cussigh A, Cmet S, Pirisi M, Toniutto P. Complementary role of vitamin D deficiency and the interleukin-28B rs12979860 C/T polymorphism in predicting antiviral response in chronic hepatitis C. *Hepatology* 2011; **53**: 1118-1126 [PMID: 21480318 DOI: 10.1002/hep.24201]

14 **Lange CM**, Bojunga J, Ramos-Lopez E, von Wagner M, Hassler A, Vermehren J, Herrmann E, Badenhoop K, Zeuzem S, Sarrazin C. Vitamin D deficiency and a CYP27B1-1260 promoter polymorphism are associated with chronic hepatitis C and poor response to interferon-alfa based therapy. *J Hepatol* 2011; **54**: 887-893 [PMID: 21145801 DOI: 10.1016/j.jhep.2010.08.036]

15 **Lange CM**, von Wagner M, Bojunga J, Berg T, Farnik H, Hassler A, Sarrazin C, Herrmann E, Zeuzem S. Serum lipids in European chronic HCV genotype 1 patients during and after treatment with pegylated interferon-α-2a and ribavirin. *Eur J Gastroenterol Hepatol* 2010; **22**: 1303-1307 [PMID: 20729742 DOI: 10.1097/MEG.0b013e32833de92c]

16 **Petta S**, Cammà C, Scazzone C, Tripodo C, Di Marco V, Bono A, Cabibi D, Licata G, Porcasi R, Marchesini G, Craxí A. Low vitamin D serum level is related to severe fibrosis and low responsiveness to interferon-based therapy in genotype 1 chronic hepatitis C. *Hepatology* 2010; **51**: 1158-1167 [PMID: 20162613 DOI: 10.1002/hep.23489]

17 **Lagging M**, Askarieh G, Negro F, Bibert S, Söderholm J, Westin J, Lindh M, Romero A, Missale G, Ferrari C, Neumann AU, Pawlotsky JM, Haagmans BL, Zeuzem S, Bochud PY, Hellstrand K. Response prediction in chronic hepatitis C by assessment of IP-10 and IL28B-related single nucleotide polymorphisms. *PLoS One* 2011; **6**: e17232 [PMID: 21390311 DOI: 10.1371/journal.pone.0017232]

18 **El Awady MK**, Anany MA, Esmat G, Zayed N, Tabll AA, Helmy A, El Zayady AR, Abdalla MS, Sharada HM, El Raziky M, El Akel W, Abdalla S, Bader El Din NG. Single nucleotide polymorphism at exon 7 splice acceptor site of OAS1 gene determines response of hepatitis C virus patients to interferon therapy. *J Gastroenterol Hepatol* 2011; **26**: 843-850 [PMID: 21182542 DOI: 10.1111/j.1440-1746.2010.06605.x]

19 **Hijikata M**, Ohta Y, Mishiro S. Identification of a single nucleotide polymorphism in the MxA gene promoter (G/T at nt -88) correlated with the response of hepatitis C patients to interferon. *Intervirology* 2000; **43**: 124-127 [PMID: 10971132 DOI: 10.1159/000025035]

20 **Bader el-Din NG**, Abd el-Meguid M, Tabll AA, Anany MA, Esmat G, Zayed N, Helmy A, el-Zayady AR, Barakat A, el-Awady MK. Human cytomegalovirus infection inhibits response of chronic hepatitis-C-virus-infected patients to interferon-based therapy. *J Gastroenterol Hepatol* 2011; **26**: 55-62 [PMID: 21175794 DOI: 10.1111/j.1440-1746.2010.06319.x]

21 **Askar M**, Avery R, Corey R, Lopez R, Thomas D, Pidwell D, Eghtesad B, Miller C, Fung J, Zein NN. Lack of killer immunoglobulin-like receptor 2DS2 (KIR2DS2) and KIR2DL2 is associated with poor responses to therapy of recurrent hepatitis C virus in liver transplant recipients. *Liver Transpl* 2009; **15**: 1557-1563 [PMID: 19877200 DOI: 10.1002/lt.21878]

22 **Carneiro VL**, Lemaire DC, Bendicho MT, Souza SL, Cavalcante LN, Angelo AL, Freire SM, Mendes CM, Santana N, Lyra LG, Lyra AC. Natural killer cell receptor and HLA-C gene polymorphisms among patients with hepatitis C: a comparison between sustained virological responders and non-responders. *Liver Int* 2010; **30**: 567-573 [PMID: 20456039 DOI: 10.1111/j.1478-3231.2010.02212.x]

23 **Khakoo SI**, Thio CL, Martin MP, Brooks CR, Gao X, Astemborski J, Cheng J, Goedert JJ, Vlahov D, Hilgartner M, Cox S, Little AM, Alexander GJ, Cramp ME, O'Brien SJ, Rosenberg WM, Thomas DL, Carrington M. HLA and NK cell inhibitory receptor genes in resolving hepatitis C virus infection. *Science* 2004; **305**: 872-874 [PMID: 15297676 DOI: 10.1126/science.1097670]

24 **Fabris C**, Falleti E, Cussigh A, Bitetto D, Fontanini E, Bignulin S, Cmet S, Fornasiere E, Fumolo E, Fangazio S, Cerutti A, Minisini R, Pirisi M, Toniutto P. IL-28B rs12979860 C/T allele distribution in patients with liver cirrhosis: role in the course of chronic viral hepatitis and the development of HCC. *J Hepatol* 2011; **54**: 716-722 [PMID: 21146242 DOI: 10.1016/j.jhep.2010.07.019]

25 **Nattermann J**, Vogel M, Nischalke HD, Danta M, Mauss S, Stellbrink HJ, Baumgarten A, Mayr C, Bruno R, Tural C, Klausen G, Clotet B, Naumann U, Lutz T, Rausch M, Schewe K, Bienek B, Haerter G, Sauerbruch T, Rockstroh JK, Spengler U. Genetic variation in IL28B and treatment-induced clearance of hepatitis C virus in HIV-positive patients with acute and chronic hepatitis C. *J Infect Dis* 2011; **203**: 595-601 [PMID: 21257738 DOI: 10.1093/infdis/jiq098]

26 **Rallón NI**, Naggie S, Benito JM, Medrano J, Restrepo C, Goldstein D, Shianna KV, Vispo E, Thompson A, McHutchison J, Soriano V. Association of a single nucleotide polymorphism near the interleukin-28B gene with response to hepatitis C therapy in HIV/hepatitis C virus-coinfected patients. *AIDS* 2010; **24**: F23-F29 [PMID: 20389235 DOI: 10.1097/QAD.0b013e3283391d6d]

27 **Bedossa P**, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996; **24**: 289-293 [PMID: 8690394 DOI: 10.1002/hep.510240201]

28 **Jones RN**, Neale ML, Beattie B, Westmoreland D, Fox JD. Development and application of a PCR-based method including an internal control for diagnosis of congenital cytomegalovirus infection. *J Clin Microbiol* 2000; **38**: 1-6 [PMID: 10618053]

29 **Tabll A**, Shoman S, Ghanem H, Nabil M, El Din NG, El Awady MK. Assessment of human cytomegalovirus co-infection in Egyptian chronic HCV patients. *Virol J* 2011; **8**: 343 [PMID: 21740595 DOI: 10.1186/1743-422X-8-343]

30 **Akuta N**, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Kumada H. Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. *J Hepatol* 2007; **46**: 403-410 [PMID: 17126448 DOI: 10.1016/j.jhep.2006.09.019]

31 **Akuta N**, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, Kobayashi M, Saitoh S, Watahiki S, Sato J, Kobayashi M, Arase Y, Ikeda K, Kumada H. Predictive factors of virological non-response to interferon-ribavirin combination therapy for patients infected with hepatitis C virus of genotype 1b and high viral load. *J Med Virol* 2006; **78**: 83-90 [PMID: 16299715 DOI: 10.1002/jmv.20507]

32 **Enomoto N**, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, Ogura Y, Izumi N, Marumo F, Sato C. Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. *N Engl J Med* 1996; **334**: 77-81 [PMID: 8531962 DOI: 10.1056/NEJM199601113340203]

33 **El-Shamy A**, Nagano-Fujii M, Sasase N, Imoto S, Kim SR, Hotta H. Sequence variation in hepatitis C virus nonstructural protein 5A predicts clinical outcome of pegylated interferon/ribavirin combination therapy. *Hepatology* 2008; **48**: 38-47 [PMID: 18537193 DOI: 10.1002/hep.22339]

34 **Welzel TM**, Morgan TR, Bonkovsky HL, Naishadham D, Pfeiffer RM, Wright EC, Hutchinson AA, Crenshaw AT, Bashirova A, Carrington M, Dotrang M, Sterling RK, Lindsay KL, Fontana RJ, Lee WM, Di Bisceglie AM, Ghany MG, Gretch DR, Chanock SJ, Chung RT, O'Brien TR. Variants in interferon-alpha pathway genes and response to pegylated interferon-Alpha2a plus ribavirin for treatment of chronic hepatitis C virus infection in the hepatitis C antiviral long-term treatment against cirrhosis trial. *Hepatology* 2009; **49**: 1847-1858 [PMID: 19434718 DOI: 10.1002/hep.22877]

35 **Sarrazin C**, Susser S, Doehring A, Lange CM, Müller T, Schlecker C, Herrmann E, Lötsch J, Berg T. Importance of IL28B gene polymorphisms in hepatitis C virus genotype 2 and 3 infected patients. *J Hepatol* 2011; **54**: 415-421 [PMID: 21112657 DOI: 10.1016/j.jhep.2010.07.041]

36 **Miller DM**, Cebulla CM, Sedmak DD. Human cytomegalovirus inhibition of major histocompatibility complex transcription and interferon signal transduction. *Curr Top Microbiol Immunol* 2002; **269**: 153-170 [PMID: 12224507 DOI: 10.1007/978-3-642-59421-2_10]

37 **Miller DM**, Zhang Y, Rahill BM, Waldman WJ, Sedmak DD. Human cytomegalovirus inhibits IFN-alpha-stimulated antiviral and immunoregulatory responses by blocking multiple levels of IFN-alpha signal transduction. *J Immunol* 1999; **162**: 6107-6113 [PMID: 10229853]

38 **Scott J**, Holte S, Urban T, Burgess C, Coppel E, Wang C, Corey L, McHutchison J, Goldstein D. IL28B genotype effects during early treatment with peginterferon and ribavirin in difficult-to-treat hepatitis C virus infection. *J Infect Dis* 2011; **204**: 419-425 [PMID: 21742841 DOI: 10.1093/infdis/jir264]

39 **Lutz P**, Wasmuth JC, Nischalke HD, Vidovic N, Grünhage F, Lammert F, Oldenburg J, Rockstroh JK, Sauerbruch T, Spengler U. Progression of liver fibrosis in HIV/HCV genotype 1 co-infected patients is related to the T allele of the rs12979860 polymorphism of the IL28B gene. *Eur J Med Res* 2011; **16**: 335-341 [PMID: 21813376 DOI: 10.1186/2047-783X-16-8-335]

P- Reviewer Hung CH **S- Editor** Lv S **L- Editor** Cant MR
E- Editor Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i2.299

World J Gastroenterol 2013 January 14; 19(2): 299-303
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Lubiprostone induced ischemic colitis

Muhammed Sherid, Humberto Sifuentes, Salih Samo, Parakkal Deepak, Subbaramiah Sridhar

Muhammed Sherid, Salih Samo, Department of Internal Medicine, Division of Gastroenterology, Saint Francis Hospital, Evanston, IL 60202, United States
Humberto Sifuentes, Subbaramiah Sridhar, Section of Gastroenterology and Hepatology, Medical College of Georgia, Georgia Health Sciences University, Augusta, GA 30912, United States
Parakkal Deepak, Department of Internal Medicine, Division of Gastroenterology, NorthShore University HealthSystem, Highland Park, IL 60035, United States
Author contributions: Sherid M contributed to Study design, case presenter, literature review, data collection, data analysis, initial manuscript writing, manuscript review, approval of final version; Sifuentes H, Samo S, Deepak P, Sridhar S contributed to study design, literature review, data collection, data analysis, initial manuscript writing, manuscript review, approval of final version.
Correspondence to: Subbaramiah Sridhar, MBBS, MPH, FRCP (Edin), FRCP (Glasg), FRSS (Lond), FRCPC (Med and Gastro), FASGE, FACP, FACG, FASLM and S (United States), FAAG, MAAG, Professor of Medicine, Section of Gastroenterology and Hepatology, Medical College of Georgia, Georgia Health Sciences University, Augusta, GA 30912,
United States. ssridhar@georgiahealth.edu
Telephone: +1-706-7212238 Fax: +1-706-7210331
Received: August 27, 2012 Revised: October 30, 2012
Accepted: November 6, 2012
Published online: January 14, 2013

Abstract

Ischemic colitis accounts for 6%-18% of the causes of acute lower gastrointestinal bleeding. It is often multifactorial and more commonly encountered in the elderly. Several medications have been implicated in the development of colonic ischemia. We report a case of a 54-year old woman who presented with a two-hour history of nausea, vomiting, abdominal pain, and bloody stool. The patient had recently used lubiprostone with close temporal relationship between the increase in the dose and her symptoms of rectal bleeding. The radiologic, colonoscopic and histopathologic findings were all consistent with ischemic colitis. Her condition improved without any serious complications after the cessation of lubiprostone. This is the first reported case of ischemic colitis with a clear relationship with lubiprostone (Naranjo score of 10). Clinical vigilance for ischemic colitis is recommended for patients receiving lubiprostone who are presenting with abdominal pain and rectal bleeding.

© 2013 Baishideng. All rights reserved.

Key words: Lubiprostone; Ischemic colitis; Gastrointestinal bleeding; Rectal bleeding; Colonoscopy

Sherid M, Sifuentes H, Samo S, Deepak P, Sridhar S. Lubiprostone induced ischemic colitis. *World J Gastroenterol* 2013; 19(2): 299-303 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i2/299.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i2.299

INTRODUCTION

Ischemic colitis in patients with nonocclusive vascular disease is thought to result from a transient low flow state through an inadequate vascular system. Advanced age, aortic surgery, diabetes mellitus, hypertension, and peripheral vascular disease have been also suggested to be predisposing factors for ischemic colitis[1]. It occurs more often in the splenic flexure and rectosigmoid junction which are known as the watershed areas. These areas are the distributions of the superior mesenteric artery and the inferior mesenteric artery for the splenic flexure and between the inferior mesenteric artery and the internal iliac artery for the rectosigmoid junction[1].

The pathophysiology of developing ischemic colitis includes transient hypoperfusion that involve segments of the colon. Affected patients are often elderly or have a cardiovascular condition (congestive heart failure or an arrhythmia) which puts them at risk to develop a transient reduction in bowel perfusion. Less common causes of ischemic colitis include hypercoagulable states, vasculitis, embolus, colonic obstruction (strictures, colon cancer, diverticulitis)[1,2]. Other causes include medications and substances such as amphetamines, alosetron, catechol-

amines (epinephrine, norepinephrine), cocaine, digitalis, nonsteriodal anti-inflammatory drugs, pseudoephedrine and triptans, *etc*[3].

Lubiprostone was initially approved in 2006 by the US Food and Drug Administration for the treatment of idiopathic chronic constipation at a dose of 24 mcg twice daily[4-6]. In 2008, it was approved for women with constipation predominant irritable bowel syndrome at dose of 8 mcg twice daily[4,5,7]. Lubiprostone is a prostaglandin E1 derivative which acts by activating chloride channel type-2 (CLC-2) in the apical membrane of the intestinal epithelial cells. Consequently, it produces chloride rich fluid into the intestinal lumen with a passive sodium and water shift which increases the liquidity of the stool and promotes the gastrointestinal tract motility which in turn accelerates the bowel transit[8]. Lubiprostone acts locally on the bowel epithelium and is minimally absorbed. It is well known to cause nausea, diarrhea, abdominal distention, and abdominal pain. Here we describe the first case report of ischemic colitis associated with lubiprostone usage.

CASE REPORT

A 54-year old female presented with a two-hour history of nausea, non-bloody, non-bilious vomiting associated with crampy generalized abdominal pain located to the epigastrium and left upper quadrant. She did not have any bowel movements 3 d prior to her presentation. In the emergency department, she had one bowel movement with hard stool followed by 6 watery, bloody stools. She did not have a fever or urinary symptoms. She did not have a history of recent antibiotic use, travel outside the United States, recent diet changes, or history of symptoms suggestive of inflammatory bowel disease. She suffers from diabetes mellitus, hypertension, hypercholesterolemia, hypothyroidism and chronic constipation. Her current medications included metformin (taking for 8 years), lisinopril (taking for 3 years), atorvastatin (taking for 4 years) and replacement thyroxin (taking for 4 years). She did not smoke or use illicit drugs.

On admission, her vital signs were essentially normal with a blood pressure of 134/70 mmHg without orthostatic changes, a heart rate of 61 bpm, temperature of 97.7 F°, respiratory rate of 16/min, oxygen saturation of 97% on room air, height of 162 cm, weight of 60.8 kg, and body mass index of 23.2. She did not have any episodes of hypotension either in the emergency department or during her hospitalization. Physical examination revealed a soft abdomen with mild epigastric and left upper quadrant tenderness. There was no distension, guarding, rebound tenderness, or organomegaly. The bowel sounds were normal. Rectal exam was unremarkable except for positive occult blood. The remainder of physical exam revealed no findings.

The laboratory studies were unremarkable including complete blood count, comprehensive metabolic panel, amylase, lipase, urinalysis, and urine toxicology. The stool studies were negative for ova, parasites, clostridium difficile, and pathogenic bacteria. The hemoglobin A1c was 6.5%. A plain abdominal X-ray was unremarkable. A computed tomography (CT) scan of the abdomen and pelvis revealed thickening of the colonic wall involving the transverse and the proximal portions of the descending colon near the splenic flexure (Figure 1A). A colonoscopic examination showed mucosal changes consistent with ischemic colitis (mucosal edema and hyperemia with areas of hemorrhage, erosions, and frank ulcerations) affecting the descending colon, between 30 to 40 cm from the anal verge (Figure 1B-D). The histopathology examination showed ischemic loss of crypts with acute hemorrhage in lamnia propria associated with acute fibrinopurulent exudate at colonic epithelial surface, consistent with ischemic colitis (Figure 1E, F).

A diagnosis of ischemic colitis was made based on the above findings. To clarify the etiology, a detailed history was taken including the use of over the counter and herbal medications. She stated that she had been taking lubiprostone for a period of 2 mo prior to her admission after she failed to respond to conventional laxatives for chronic constipation. The patient was initially started on 24 mcg of lubiprostone, but several hours after taking the drug, she developed nausea, vomiting and abdominal pain with bloody diarrhea which lasted for two days. She did not undergo any investigations, but her dose of lubiprostone was changed to 8 mcg daily as needed after she called her primary care physician. Twenty-four hours prior to her current admission she had taken a total of 24 mcg of lubiprostone. Her symptoms developed two hours after the last dose. This temporal relationship between the recurrence of her symptoms and the rechallenging of the drug confirms the diagnosis of ischemic colitis in the light of absence of other causes of ischemic colitis. This case scored a 10 on the Naranjo Nomogram for adverse drug reactions which places lubiprostone as a definitive cause of ischemic colitis in this case (definitive $\geqslant$ 9). The patient was treated with discontinuation of lubiprostone, intravenous fluids, bowel rest and intravenous antibiotics. Her hospitalization course was uneventful and she was discharged home without any complications 4 d later.

DISCUSSION

Ischemic colitis accounts for 6%-18% of the causes of acute lower gastrointestinal bleeding[2]. The causes of ischemic colitis vary from systemic hypotension, aortoiliac surgery, atherosclerosis, thromboembolic events, vasculitis, and medications[9].

The medications that have been implicated in the development of ischemic colitis have different mechanisms including decreasing blood flow *via* systemic hypotension with angiotensin-converting enzyme inhibitors, vasospasm with pseudoephedrine, thromboembolic events with oral contraceptives, vasculitis secondary to gold salts, and increasing intracolonic pressures with alos-

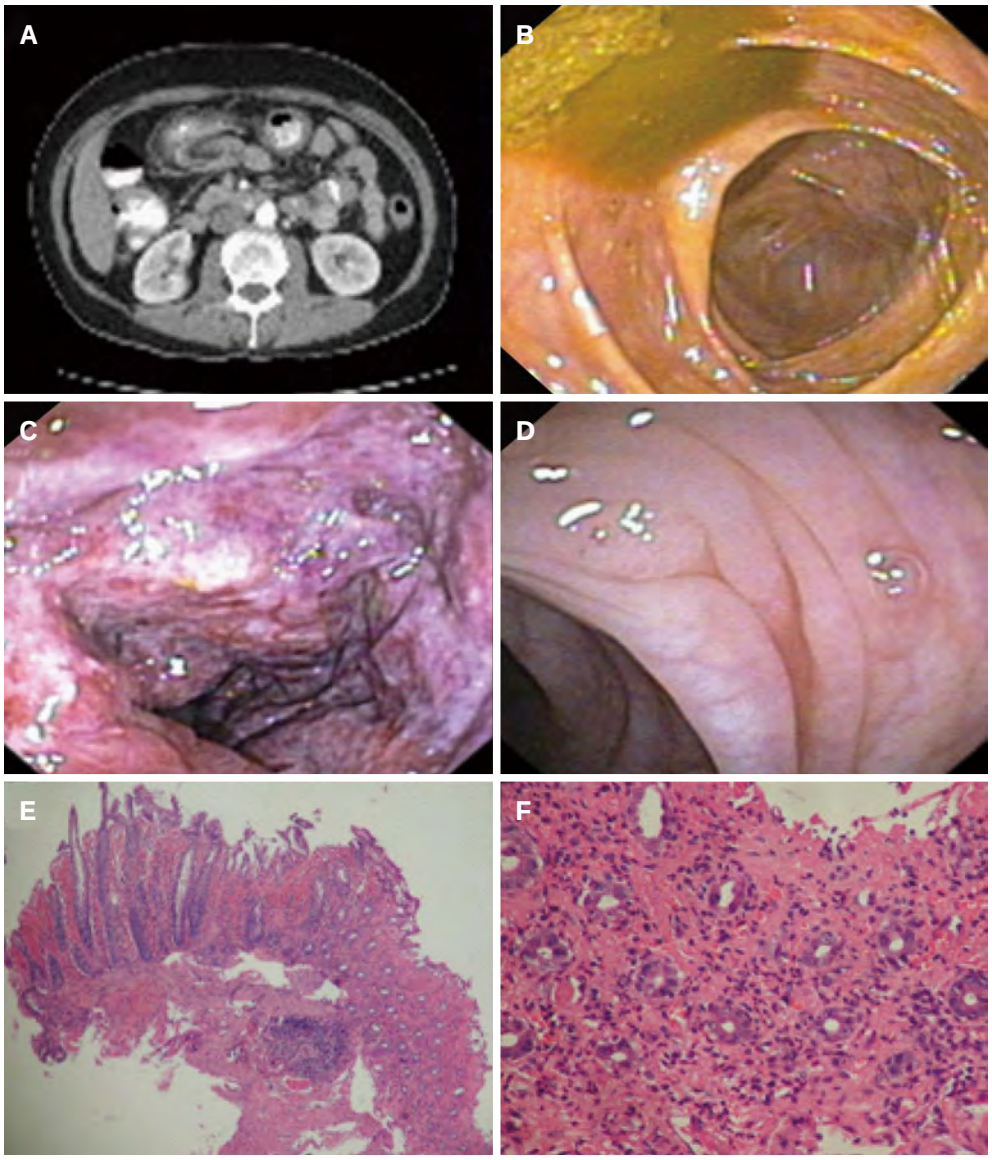


Figure 1 The laboratory studies results. A: Computed tomography scan shows thickening of the colonic wall involving the splenic flexure of the colon; B: Normal mucosa in the right colon (appendiceal orifice); C: Ischemic colitis features in the splenic flexure of the colon; D: Normal mucosa in sigmoid with a small polyp; E, F: Histopathology shows ischemic loss of crypts and acute hemorrhage in lamnia propria (acute hemorrhagic necrosis replacing glands) associated with acute fibrinopurulent exudate at colonic epithelial surface, consistent with ischemic colitis.

etron[3]. The mechanism of some drugs reported to cause ischemic colitis has not yet determined. Table 1 shows a list of medications and their mechanisms for causing ischemic colitis[3].

Many case reports have linked ischemic colitis and other unusual associations such as herbal remedies (ma huang/ephedra), weight loss medications such as phentermine, following screening colonoscopies, certain chemotherapy agents such as bevacizumab and irinotecan, hepatitis C therapy with peginterferon and ribavirin, scuba diving, flying, snake bites, acute carbonic monoxide poisoning, using electrical muscle stimulation devices on the abdominal wall, and following long distance running[3].

Lubiprostone has been shown to improve symptoms in patients with chronic idiopathic constipation. In a 48 wk prospective, multicenter, open-labeled trial performed by Lembo *et al*[10] patients with chronic idiopathic constipation were taking lubiprostone 24 mcg twice daily as needed and the most common side effects were nausea (19.8%), diarrhea (9.7%), abdominal distension (6.9%), headache (6.9%), and abdominal pain (5.2%).

Lubiprostone has also been approved for the treatment of constipation predominant irritable bowel syndrome (IBS-C). In a randomized, placebo controlled trial, the overall rate of response based on a patient ranked assessment tool was significantly higher in the lubiprostone group using 8 mcg twice daily compared to placebo. The side effects were similar in both groups[11].

In a phase 2, placebo controlled trial by Johanson *et al*[12] lubiprostone was superior to placebo in IBS-C regarding the severity of constipation, stool consistency,

Table 1 Medications associated with ischemic colitis

Agent	Mechanism
Amphetamines	Vasoconstriction
Alosetron	
Catecholamines (epinephrine, norepinephrine)	
Cocaine	
Cyclosporine	
Digitalis	
Dopamine	
Ergot derivatives	
Nonsteriodal anti-inflammatory drugs	
Pseudoephedrine	
Triptans (Naratriptan, Rizatriptan, Sumatriptan)	
Vasopressin and vasopressin analogues	
Glycerin enema	Local vasospasm effect
Phosphosoda solution	
Angiotensin-converting enzyme inhibitors	Systemic hypotension
Antipsychotic (chlorpromazine)	
Beta blockers	
Barbiturates	
Diuretics	
Interleukin-2	
Tricyclic antidepressants	
Amphetamines	Vasculitis
Gold compounds	
Estrogens	Thrombotic lesion induction
Progestational agents	
Alosetron	Increased intracolonic pressure
Danazol	
Glycerin enema	
Carboplatin	Undetermined
Flutamide	
Glutaraldehyde	
Hyperosmotic saline laxatives	
Interferon-alpha	
Mycophenolate mofetil	
Paclitaxol	
Simvastatin	
Tegaserod	

With permission to reuse from Elsevier[3].

frequency, straining, bloating, and abdominal pain in 1, 2, and 3 mo follow ups. Both diarrhea and nausea occurred more often in the lubiprostone group, especially with 24 mcg twice daily compared to 8 mcg twice daily.

Lubiprostone is a bicyclic fatty acid, derived from prostaglandin E1. It is a member of a class of drugs called prostones which, unlike prostaglandins, are not believed to cause direct smooth muscle contraction. It is metabolized in the upper part of the gastrointestinal tract by the microsomal carbonyl reductase system. Lubiprostone acts locally in gastrointestinal epithelium and its active metabolite, M3, absorbs systemically. The half-life of M3 is 0.9-1.4 h. M3 is used for the pharmacokinetic parameters of lubiprostone because the drug itself has limited systemic absorption and low bioavailability[4,8].

Lubiprostone mainly acts on CLC-2 activators in the intestinal epithelium which is one of the nine types of chloride channels in the body which also includes the cystic fibrosis transmembrane conductance regulator (CFTR) (Figure 2)[13].

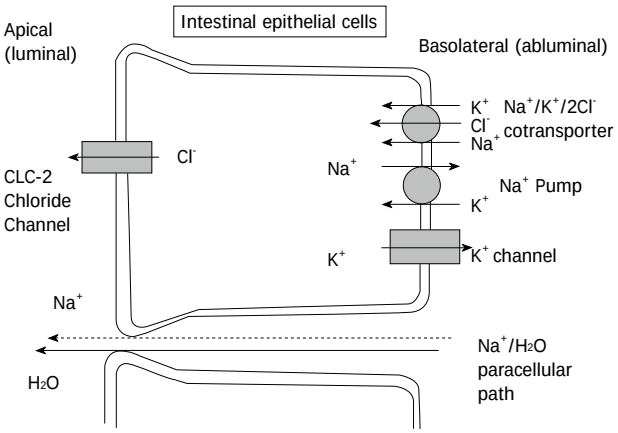


Figure 2 Mechanism of action of lubiprostone across epithelial intestinal cells[8].

Activation of CLC-2 in the apical membrane of intestinal epithelial cells causes an active secretion of chloride ions from cells into the intestinal lumen followed by a passive secretion of sodium ions and water which results in increased isotonic fluid in the lumen which promotes small bowel and colonic transit by stimulating stretch receptors and smooth muscles in the intestine (Figure 2)[8].

There is now controversy whether lubiprostone activates CLC-2 in be basolateral or apical aspect of intestinal epithelium, whether it activates mainly CFTR or CLC-2, or whether it directly stimulates smooth muscles *via* prostaglandin receptors[8]. Bao *et al*[14] showed in an experimental study that lubiprostone activates CLC-2 at low concentrations but activates CFTR at higher concentrations. It has also been shown that lubiprostone weakly activates prostaglandin receptors in different levels of the GI tract which could be responsible for some side effects such as nausea[15].

In our patient, despite having other risk factors for ischemic colitis such as chronic constipation which has been shown to increase the risk of ischemic colitis by 2.7 folds in some studies[16], the close temporal relationship between lubiprostone and occurrence of the symptoms of ischemic colitis makes the drug the most likely culprit of this event. When applying the Naranjo Nomogram in our patient, a score of 10 was obtained indicating a definite likelihood of lubiprostone as the cause of ischemic colitis in this case (Table 2).

To the best of our knowledge, this is the first report of lubiprostone induced ischemic colitis. We report that a direct causal relation by exclusion of other causes, the disappearance of symptoms after cessation of lubiprostone, recurrence of symptoms with self rechallenge, and a Naranjo Nomogram score of 10 all go in favor of the drug-induced ischemic colitis. The actual mechanism of lubiprostone causing ischemic colitis is not known, however, fast fluid shift into the intestinal lumen causing local hypoperfusion, rapid increase of the intracolonic pressure due to rapid fluid secretion into the bowel, or direct vasoconstriction caused by lubiprostone in higher doses

Table 2 Naranjo adverse drug reaction nomogram in our patient

	Yes	No	Our patient
Are there previous conclusive reports on this reaction?	1	0	0
Did the adverse event appear after the suspected drug was administered?	2	-1	2
Did the adverse reaction improve when the drug was discontinued or a specific antagonist was administered?	1	0	1
Did the adverse reaction reappear when the drug was readministered?	2	-1	2
Are there alternative causes (other than the drug) that could have, on their own, caused the reaction?	-1	2	2
Did the reaction appear when a placebo was given?	-1	1	1
Was the drug detected in the blood (or other fluids) in concentration known to be toxic?	1	0	0
Was the reaction more severe when the dose was increased or less severe when dose was decreased?	1	0	0
Did the patient have a similar reaction to the same or similar drugs in any previous exposure?	1	0	1
Was the adverse event confirmed by any objective evidence?	1	0	1
			10

Definite: score ⩾ 9; Probable: 5-8; Possible: 1-4; Doubtful: ⩽ 0.

by stimulating prostaglandin receptors are the proposed pathophysiological mechanisms. In conclusion, lubiprostone may cause ischemic colitis, especially in some susceptible persons and greater awareness of this side effect is warranted with the increasing use of lubiprostone.

REFERENCES

1 **Theodoropoulou A**, Koutroubakis IE. Ischemic colitis: clinical practice in diagnosis and treatment. *World J Gastroenterol* 2008; **14**: 7302-7308 [PMID: 19109863 DOI: 10.3748/wjg.14.7302]

2 **Strate LL**. Lower GI bleeding: epidemiology and diagnosis. *Gastroenterol Clin North Am* 2005; **34**: 643-664 [PMID: 16303575 DOI: 10.1016/j.gtc.2005.08.007]

3 **Sherid M**, Ehrenpreis ED. Types of colitis based on histology. *Dis Mon* 2011; **57**: 457-489 [PMID: 21944389 DOI: 10.1016/j.disamonth.2011.05.004]

4 AMITIZA® (lubiprostone) [package insert]. Bethesda, MD: Sucampo Pharmaceuticals, 2009

5 **Schiller LR**, Camilleri M. Lubiprostone: viewpoints. *Drugs* 2006; **66**: 880-881 [PMID: 16706563 DOI: 10.2165/00003495-200666060-00016]

6 **Ford AC**, Suares NC. Effect of laxatives and pharmacological therapies in chronic idiopathic constipation: systematic review and meta-analysis. *Gut* 2011; **60**: 209-218 [PMID: 21205879 DOI: 10.1136/gut.2010.227132]

7 **Lang L**. The Food and Drug Administration approves lubiprostone for irritable bowel syndrome with constipation. *Gastroenterology* 2008; **135**: 7 [PMID: 18541153 DOI: 10.1053/j.gastro.2008.06.004]

8 **Lunsford TN**, Harris LA. Lubiprostone: evaluation of the newest medication for the treatment of adult women with constipation-predominant irritable bowel syndrome. *Int J Womens Health* 2010; **2**: 361-374 [PMID: 21151683 DOI: 10.2147/IJWH.S4537]

9 **Stamatakos M**, Douzinas E, Stefanaki C, Petropoulou C, Arampatzi H, Safioleas C, Giannopoulos G, Chatziconstantinou C, Xiromeritis C, Safioleas M. Ischemic colitis: surging waves of update. *Tohoku J Exp Med* 2009; **218**: 83-92 [PMID: 19478463 DOI: 10.1620/tjem.218.83]

10 **Lembo AJ**, Johanson JF, Parkman HP, Rao SS, Miner PB, Ueno R. Long-term safety and effectiveness of lubiprostone, a chloride channel (ClC-2) activator, in patients with chronic idiopathic constipation. *Dig Dis Sci* 2011; **56**: 2639-2645 [PMID: 21769655 DOI: 10.1007/s10620-011-1801-0]

11 **Drossman DA**, Chey WD, Johanson JF, Fass R, Scott C, Panas R, Ueno R. Clinical trial: lubiprostone in patients with constipation-associated irritable bowel syndrome--results of two randomized, placebo-controlled studies. *Aliment Pharmacol Ther* 2009; **29**: 329-341 [PMID: 19006537 DOI: 10.1111/j.1365-2036.2008.03881.x]

12 **Johanson JF**, Drossman DA, Panas R, Wahle A, Ueno R. Clinical trial: phase 2 study of lubiprostone for irritable bowel syndrome with constipation. *Aliment Pharmacol Ther* 2008; **27**: 685-696 [PMID: 18248656 DOI: 10.1111/j.1365-2036.2008.03629.x]

13 **Lipecka J**, Bali M, Thomas A, Fanen P, Edelman A, Fritsch J. Distribution of ClC-2 chloride channel in rat and human epithelial tissues. *Am J Physiol Cell Physiol* 2002; **282**: C805-C816 [PMID: 11880269 DOI: 10.1152/ajpcell.00291.2001]

14 **Bao HF**, Liu L, Self J, Duke BJ, Ueno R, Eaton DC. A synthetic prostone activates apical chloride channels in A6 epithelial cells. *Am J Physiol Gastrointest Liver Physiol* 2008; **295**: G234-G251 [PMID: 18511742 DOI: 10.1152/ajpgi.00366.2007]

15 **Bassil AK**, Borman RA, Jarvie EM, McArthur-Wilson RJ, Thangiah R, Sung EZ, Lee K, Sanger GJ. Activation of prostaglandin EP receptors by lubiprostone in rat and human stomach and colon. *Br J Pharmacol* 2008; **154**: 126-135 [PMID: 18332851 DOI: 10.1038/bjp.2008.84]

16 **Suh DC**, Kahler KH, Choi IS, Shin H, Kralstein J, Shetzline M. Patients with irritable bowel syndrome or constipation have an increased risk for ischaemic colitis. *Aliment Pharmacol Ther* 2007; **25**: 681-692 [PMID: 17311601 DOI: 10.1111/j.1365-2036.2007.03250.x]

P- Reviewers King TM, Chung WC **S- Editor** Song XX
L- Editor A **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i2.304

World J Gastroenterol 2013 January 14; 19(2): 304-306
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Lesson from an intriguing case of cryoglobulinemia

Michele Barone, Raffaele Licinio, Annabianca Amoruso, Maria Teresa Viggiani, Angela Maria Vittoria Larocca, Alfredo Di Leo

Michele Barone, Raffaele Licinio, Annabianca Amoruso, Maria Teresa Viggiani, Alfredo Di Leo, Department of Emergency and Organ Transplantation, University of Bari, 70100 Bari, Italy
Angela Maria Vittoria Larocca, Department of Biomedical Science and Human Oncology, University of Bari, 70100 Bari, Italy
Author contributions: Barone M, Licinio R, Amoruso A, Viggiani MT and Larocca AMV contributed equally to this work; Di Leo A approved the final version.
Correspondence to: Alfredo Di Leo, Professor, Department of Emergency and Organ Transplantation, University of Bari, Policlinico G. Cesare Sq 11, 70124 Bari, Italy. alfredo.dileo@uniba.it
Telephone: +39-80-5592577 Fax: +39-80-5593251
Received: May 5, 2012 Revised: July 30, 2012
Accepted: August 14, 2012
Published online: January 14, 2013

Abstract

Cryoglobulinemia is a pathological condition usually associated with hepatitis C virus (HCV) chronic liver disease and less commonly with autoimmune or lymphoproliferative disorders. The possible association of cryoglobulinemia with hepatitis B virus (HBV) infection is not widely accepted. In our patient, serum negativity for HCV markers initially led us to consider two other causes of cryoglobulinemia. Myelodysplastic disorders were excluded on the basis of hematological studies, while serum markers for active HBV infection were positive. Surprisingly, the detection of HCV RNA in the cryocrit, even in the absence of anti-HCV antibodies, suggested a pathogenetic role of HCV in this case of cryoglobulinemia. Negative "first level" tests for HCV in the serum do not completely exclude HCV involvement in the pathogenesis of cryoglobulinemia. Analysis of the cryoprecipitate is always essential for diagnosis.

© 2013 Baishideng. All rights reserved.

Key words: Cryocrit; Hepatitis B virus; Hepatitis C virus; Myelodysplastic disorders; Cryoglobulinemia

Barone M, Licinio R, Amoruso A, Viggiani MT, Larocca AMV, Di Leo A. Lesson from an intriguing case of cryoglobulinemia. *World J Gastroenterol* 2013; 19(2): 304-306 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i2/304.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i2.304

INTRODUCTION

Cryoglobulinemia is a pathological condition usually associated with hepatitis C virus (HCV) chronic liver disease[1,2]. Cases of cryoglobulinemia associated with autoimmune or lymphoproliferative diseases are less common[2], while the association with hepatitis B virus (HBV) infection is not widely accepted, the HBV virus being mostly associated with other immunocomplex-related disorders[3-5].

Cryoglobulins are proteins that can precipitate at low temperatures (< 4 ℃). The final product of this precipitate, termed the cryocrit, can be characterized on the basis of its composition: polyclonal immunoglobulins, monoclonal immunoglobulins or the presence of rheumatoid activity (RA). Three types of cryoglobulinemia have been identified, namely: type 1, including monoclonal cryoglobulins (IgM, IgG and IgA) without any RA; type 2, that includes a monoclonal component (usually IgM with RA) and a polyclonal component (usually IgM/IgG); type 3, that includes several polyclonal components and a component with RA (usually IgG/IgM)[6].

Normally, cryoglobulinemia type 1 is associated with lymphoproliferative/myelodysplastic diseases such as multiple myeloma, Waldenstrom macroglobulinemia, chronic lymphatic leukemia, non-Hodgkin lymphoma, *etc.* Cryoglobulinemia type 2 is associated not only with lymphoproliferative diseases and plasma cellular dyscrasias but also with infectious and autoimmune diseases (rheumatoid arthritis, Sjogren's syndrome, *etc.*). Finally, cryoglobulinemia type 3 is frequently associated with autoimmune or infectious diseases. The mechanism responsible

for cryoglobulin formation during lymphoproliferative or autoimmune diseases is known, but the etiopathogenesis of the forms defined as "essential cryoglobulinemias", which occur as isolated events, has still to be clarified. Among the essential forms, the most common variant is cryoglobulinemia type 2[2]. The amount of cryoglobulins, expressed as a percentage of the serum volume, can vary significantly among patients and may change over time. It is therefore useful to evaluate the cryocrit, also during follow-up, in order to assess prognosis and treatment. Cryoglobulinemia characterization by protein electrophoresis and immunofixation is equally important to define the type[2]. In 1966, Meltzer *et al*[7] described a clinical triad characterized by palpable purpura, arthralgia and asthenia associated with nephropathy and neuropathy. It is widely accepted that presentation of the complete triad is rare in clinical practice. In fact, most patients are asymptomatic and purpura, often fleeting, is the only clinical manifestation. The frequent association of HCV infection with the great majority of cryoglobulins, initially defined as essential, suggested the involvement of this virus in the pathogenesis of mixed cryoglobulinemia. Actually, mixed cryoglobulinemia (type 2 or 3) is found in 50% of patients with chronic HCV infection[1,4]. Therefore, in patients with mixed cryoglobulinemia, the possibility of HCV infection should always be considered, and serum tests should be performed for the detection of anti-HCV antibodies and HCV RNA.

CASE REPORT

Our patient was in apparent good health until the age of 73 when he underwent percutaneous angioplasty for acute non-Q myocardial infarction, after which he was prescribed anticoagulant and antihypertensive therapy. At the age of 77, he had an outpatient medical visit because of the first manifestation of purpura of the lower limbs, arthralgia and a sense of postural instability, raising the suspicion of a cryoglobulinemic syndrome. At this time, the cryocrit was positive (35%), and the patient underwent several serological and molecular investigations that excluded HCV (negative anti-HCV and HCV RNA) and HIV infection and demonstrated chronic HBV infection (surface antigen of the hepatitis B virus (HBsAg) 11 700 IU/mL, anti-HBsAg negative, hepatitis B core antibody positive, anti-hepatitis Be antibody positive, HBV-DNA 2 410 000 IU/mL, anti-HDV negative). Immediately after, he was admitted to our unit because of the onset of a hypertensive crisis that was not controlled by the administration of calcium antagonists, beta-blockers and loop diuretics. Renal function monitoring demonstrated the following values: creatinine 1.85 mg/dL, creatinine clearance 20 mL/min and 24-h proteinuria 1.3 g/24 h. Echotomographic examination showed ultrasonographic signs of cirrhosis, and hepatic elastometry yielded a stiffness value of 47.2 kPa. Other laboratory tests demonstrated aspartate aminotransferase × 6.7 upper limit of normal (ULN), alanine aminotransferase × 4.9 ULN and gamma-glutamyl transpeptidase × 5.2 ULN, and total bilirubin 1.8 mg/dL. On the basis of these data, the patient was started on antiviral treatment (0.5 mg entecavir every 72 h) and prednisone, 50 mg/d, to be tapered to 10 mg/d. Although the hypertensive crisis could presumably be ascribed to a cryoglobulin-induced nephropathy attributable to HBV, considering that the serum β2-microglobulin levels were markedly increased (7.2 mg/L, < 2 ULN) and gamma-globulin showed a monoclonal peak, we also considered the possibility of cryoglobulinemia associated with a myeloproliferative disorder. The characteristics of the cryoprecipitate were therefore evaluated in order to define the type of cryoglobulinemia. We found rheumatoid factor positivity, lower C3 and C4 levels, 24% cryocrit, while serum immunofixation showed a K monoclonal IgM component, and cryocrit immunofixation a K monoclonal IgM component with polyclonal IgG. On the basis of these elements we made a diagnosis of mixed cryoglobulinemia type 2, that did not exclude a myeloproliferative disorder, so a peripheral blood smear and bone marrow biopsy were performed, and these excluded a myeloproliferative disease. During the period of hospitalization we observed an improvement in renal function (creatinine decreased to 1.4 mg/dL, 24-h creatinine clearance increased to 35 mL/min, and 24-h proteinuria decreased to 0.9 g) and a striking reduction of the cryocrit (6.1%). Surprisingly, cryoprecipitate characterization demonstrated the presence of HCV RNA (807 copies/mL, by m2000 Real Time System, Abbott, IL, United States, Figure 1) and for this reason, we rechecked the serum anti-HCV antibodies and HCV RNA, and the absence of both was confirmed (HCV RNA undetectable using 50 IU/mL as cut-off).

DISCUSSION

In the present case, the detection of HCV components in the cryocrit cast some doubt on the pathogenesis of the cryoglobulinemia that we initially attributed to HBV infection.

It has been reported that in chronic HCV patients with mixed cryoglobulinemia, HCV RNA may be undetectable in the plasma (due to the entrapment of HCV RNA in the cryoprecipitate) and the diagnosis of HCV infection is based on the presence of anti-HCV antibodies[8]. However, in our patient anti-HCV antibodies were undetectable in the serum. This could be explained by interference between the two viruses that may even lead to inhibition of HCV replication in patients with chronic hepatitis C with a superimposed HBV infection[9,10].

This experience suggests that negative tests for HCV detection in the serum (anti-HCV antibodies and HCV RNA) do not completely exclude the involvement of HCV in the pathogenesis of cryoglobulinemia, and show that analysis of the cryoprecipitate is always essential, since detection of HCV RNA in the cryoprecipitate is the most sensitive method. Once HCV infection has been excluded, investigations must take into account other causes

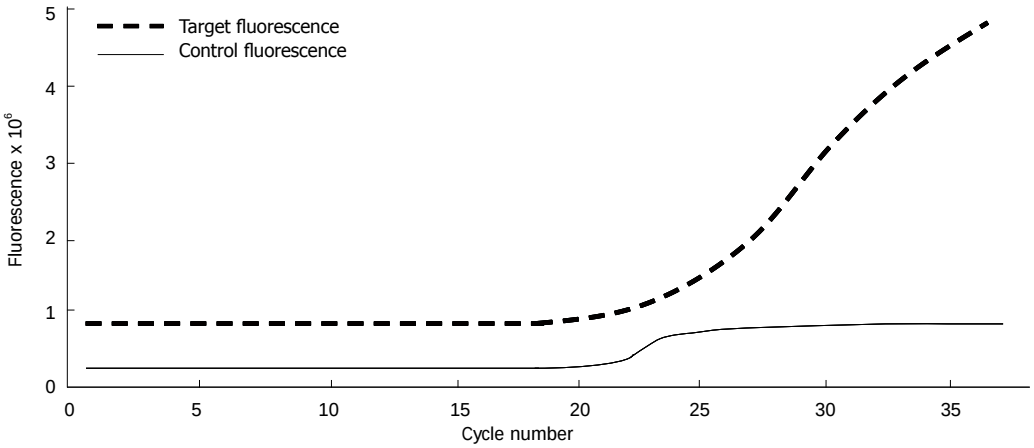


Figure 1 Real time polymerase chain reaction amplification plot for hepatitis C virus RNA. The presence of viral RNA started to become evident after 19.4 cycles and the amount of RNA was 807 copies/mL.

of cryoglobulinemia, such as autoimmune and myelo- or lymphoproliferative disorders and HBV infection.

REFERENCES

1 **Ferri C**, La Civita L, Longombardo G, Greco F, Bombardieri S. Hepatitis C virus and mixed cryoglobulinaemia. *Eur J Clin Invest* 1993; **23**: 399-405 [PMID: 8397090 DOI: 10.1111/j.1365-2362.1993.tb00782.x]

2 **Trendelenburg M**, Schifferli JA. Cryoglobulins are not essential. *Ann Rheum Dis* 1998; **57**: 3-5 [PMID: 9536813 DOI: 10.1136/ard.57.1.3]

3 **Johnson RJ**, Couser WG. Hepatitis B infection and renal disease: clinical, immunopathogenetic and therapeutic considerations. *Kidney Int* 1990; **37**: 663-676 [PMID: 1968522 DOI: 10.1038/ki.1990.32]

4 **Liang TJ**. Hepatitis B: the virus and disease. *Hepatology* 2009; **49**: S13-S21 [PMID: 19399811 DOI: 10.1002/hep.22881]

5 **Cakir N**, Pamuk ON, Umit H, Midilli K. Successful treatment with adefovir of one patient whose cryoglobulinemic vasculitis relapsed under lamivudine therapy and who was diagnosed to have HBV virologic breakthrough with YMDD mutations. *Intern Med* 2006; **45**: 1213-1215 [PMID: 17139120 DOI: 10.2169/internalmedicine.45.1816]

6 **Brouet JC**, Clauvel JP, Danon F, Klein M, Seligmann M. Biologic and clinical significance of cryoglobulins. A report of 86 cases. *Am J Med* 1974; **57**: 775-788 [PMID: 4216269 DOI: 10.1016/0002-9343(74)90852-3]

7 **Meltzer M**, Franklin EC, Elias K, McCluskey RT, Cooper N. Cryoglobulinemia--a clinical and laboratory study. II. Cryoglobulins with rheumatoid factor activity. *Am J Med* 1966; **40**: 837-856 [PMID: 4956871]

8 **Bichard P**, Ounanian A, Girard M, Baccard C, Rolachon A, Renversez JC, Cordonnier D, Seigneurin JM, Debru JL, Zarski JP. High prevalence of hepatitis C virus RNA in the supernatant and the cryoprecipitate of patients with essential and secondary type II mixed cryoglobulinemia. *J Hepatol* 1994; **21**: 58-63 [PMID: 7525697]

9 **Alberti A**, Pontisso P, Chemello L, Fattovich G, Benvegnù L, Belussi F, De Mitri MS. The interaction between hepatitis B virus and hepatitis C virus in acute and chronic liver disease. *J Hepatol* 1995; **22**: 38-41 [PMID: 7602074]

10 **Sagnelli E**, Coppola N, Messina V, Di Caprio D, Marrocco C, Marotta A, Onofrio M, Scolastico C, Filippini P. HBV superinfection in hepatitis C virus chronic carriers, viral interaction, and clinical course. *Hepatology* 2002; **36**: 1285-1291 [PMID: 12395342]

P- Reviewers Santos DCM, Hung CH **S- Editor** Gou SX
L- Editor Cant MR **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i2.307

World J Gastroenterol 2013 January 14; 19(2): 307-310
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Recurrent cervical esophageal stenosis after colon conduit failure: Use of myocutaneous flap

Young Jo Sa, Young Du Kim, Chi Kyung Kim, Jong Kyung Park, Seok Whan Moon

Young Jo Sa, Young Du Kim, Chi Kyung Kim, Seok Whan Moon, Department of Thoracic and Cardiovascular Surgery, St. Paul Hospital, The Catholic University of Korea, Seoul 130-709, South Korea
Jong Kyung Park, Department of General Surgery, St. Paul Hospital, The Catholic University of Korea, Seoul 130-709, South Korea
Author contributions: Sa YJ and Moon SW were responsible for research design and wrote the paper; Sa YJ, Kim YD, Park JK, and Moon SW performed surgical research; Sa YJ, Kim CK, and Moon SW analyzed and interpreted the data; Sa YJ and Moon SW designed the paper, drafted and revised the article, and obtained final approval.
Correspondence to: Seok Whan Moon, MD, PhD, Department of Thoracic and Cardiovascular Surgery, St. Paul Hospital, The Catholic University of Korea, Seoul 130-709, South Korea. swmoon@catholic.ac.kr
Telephone: +82-2-9582447 Fax: +82-2-9582447
Received: August 9, 2012 Revised: October 3, 2012
Accepted: October 30, 2012
Published online: January 14, 2013

Abstract

A 53-year-old male developed cervical esophageal stenosis after esophageal bypass surgery using a right colon conduit. The esophageal bypass surgery was performed to treat multiple esophageal strictures resulting from corrosive ingestion three years prior to presentation. Although the patient underwent several endoscopic stricture dilatations after surgery, he continued to suffer from recurrent esophageal stenosis. We planned cervical patch esophagoplasty with a pedicled skin flap of sternocleidomastoid (SCM) muscle. Postoperative recovery was successful, and the patient could eat a solid meal without difficulty and has been well for 18 mo. SCM flap esophagoplasty is an easier and safer method of managing complicated and recurrent cervical esophageal strictures than other operations.

© 2013 Baishideng. All rights reserved.

Key words: Cervical esophageal stenosis; Corrosive stricture; Esophagus; Esophageal conduit; Esophagoplasty; Myocutaneous flap; Surgery

Sa YJ, Kim YD, Kim CK, Park JK, Moon SW. Recurrent cervical esophageal stenosis after colon conduit failure: Use of myocutaneous flap. *World J Gastroenterol* 2013; 19(2): 307-310 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i2/307.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i2.307

INTRODUCTION

A variety of total esophageal reconstruction procedures have been described; however, there is no universally-accepted method[1]. Cervical reconstruction for esophageal stenosis of any etiology is complicated and can be more challenging in cases of recurrent cervical stenosis with previous colon conduit failure. Recently, we successfully treated upper cervical stenosis caused by corrosive strictures using colon conduits. However, these operations have been followed by recurrent stenosis. Many different approaches have been utilized in the treatment of cervical stenosis. These include local fasciocutaneous flaps, pedicled myocutaneous flaps, pedicled visceral flaps, free flaps, and free fasciocutaneous flaps[2]. We present a successful patch-plasty with myocutaneous flap of sternocleidomastoid (SCM) muscle for treatment of recurrent cervical esophageal stenosis.

CASE REPORT

A 53-year-old male developed recurrent cervical esophageal stenosis 1.5 years after undergoing esophageal bypass surgery using a right colon conduit. He had received corrosive esophageal strictures three years earlier after a suicide attempt by potassium hydroxide ingestion. His presentation was complicated, with multi-level stenotic segments down the entire esophagus. He underwent

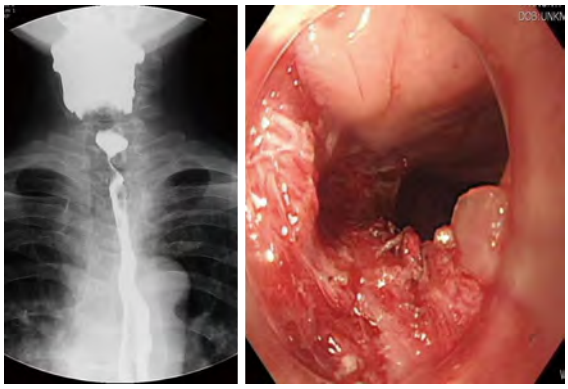


Figure 1 Esophagogram and endoscopic findings before patch esophagoplasty. The native esophagus was visualized with the esophagogram, but the colon conduit from bypass surgery was not visible. After insertion of an Endoscopic Varix Ligation tube, a stenotic lesion of the esophago-colonic anastomosis site was opened. Focal luminal stenosis by fibrosis was evident. Endoscopic dilatation was repeated using electrocauterization.

repeated balloon dilatation, but one cervical stricture progressed to near-complete obstruction just below the entrance of the esophagus as seen on endoscopy and barium esophagogram. The severity of stenosis below the proximal esophagus could not be evaluated. He had severe weight loss of 15 kg and required total parenteral nutrition, afterwards gaining back about 2.5 kg (43 kg and 173 cm, body mass index 14.37). Instead of feeding gastrostomy insertion, esophageal bypass surgery using a segment of the right colon was performed one year later. At surgery, the ileum was anastomosed with the cervical esophagus in a side-to-end manner, and its blood supply was preserved. Postoperative esophagogram showed that barium passed through the native esophagus and newly constructed colon conduit. The patient began to eat 7 d after surgery and returned home 14 d after surgery with a weight gain of 3 kg. On follow-up, he gained weight by additional 3 kg, but suffered from painful swallowing of solid food three mo after discharge. Follow-up endoscopy revealed cicatricial stenosis in the native esophagus proximal to the interposed colon, though the site of gastro-colon anastomosis was patent. Barium esophagogram showed mild stenosis at the level of the thoracic inlet and severe stenosis just above the anastomosis of the esophagus. The patient underwent repeated 7 times esophageal dilatation using Savary-Guillard or balloon dilators, and an episode of electrocauterization ablation to improve swallowing before the 2nd operation. Despite endoscopic dilatation using electrocauterization, he again developed pain on swallowing. Finally, he was admitted for surgical correction. We reviewed the esophagogram and endoscopic findings (Figure 1). The stenotic segment was 3 cm long and located in the proximal esophagus near the site of the previous esophago-colonic anastomosis. Fortunately, the lesion was easily opened with a dilator or balloon. This finding suggested that the stenotic segment was not caused by mechanical stenosis resulting from circumferential fibrosis, but was caused by twisted axis of esophageal anastomosis site possibly due to cicatricial obstruction resulting from colon conduit failure. We planned to perform a patch enlargement operation with a myocutaneous flap of SCM muscle. Under endotracheal anesthesia, the patient was placed supine with neck extension and slight rotation for better exposure. A skin incision in the neck was made along the previous incision while preserving an elliptical skin island over the SCM muscle (Figure 2). Improved exposure of the stenotic cervical esophagus was obtained after SCM division with a skin island near the clavicle. There was a fibrotic band near the proximal esophagus instead of near the colon. The proximal esophagus and hypopharynx were exposed, and a small esophagotomy at the level of the cricoid cartilage was initially made and extended upward to the hypopharynx with a longitudinal incision of about 5 cm (Figure 2). The attached skin island of the SCM myocutaneous flap was rotated medially over the esophagopharyngeal opening and sutured in interrupted fashion with 3-0 black silk. The SCM muscle attached to the skin flap was then tacked into place around the esophagus and pharynx. A drain was placed, and the neck wound was closed after a skin tension-releasing procedure (Figure 2). Seven days later, an esophagogram showed good passage without tracheal aspiration (Figure 3). The patient could eat a solid meal without pain or resistance and returned home in ten days. He then returned to work and has been well with a gradual weight gain about 10 kg for 18 mo after the esophagoplasty.

DISCUSSION

Graft necrosis is the most dangerous complication of colon graft interposition with a reported mortality approaching 90% or more[3]. Most instances of colonic graft ischemia are secondary to arterial insufficiency[3]. Ischemic complications are expressed as frank colon necrosis, anastomotic leakage, and colonic stricture[3]. Colonic segment necrosis and anastomotic leakage usually occur in the first postoperative week[4], but in our case, localized strictures of the cervical region developed 1.5 years after the first bypass operation. The slow course is likely explained by slowly progressive ischemia from graft torsion, resulting in a non-necrotic colonic cicatricial stricture rather than colonic necrosis. This may be the cause of esophageal obstruction in our case. This cicatricial change in the esophageal anastomosis site might twist the esophageal axis, and cause painful swallowing. Also this lesion was easily dilated with esophageal ballooning.

Although dilatation is the primary method of treating esophageal strictures[5], alimentary tract reconstruction is necessary for correction of severe and extensive strictures. Invasive surgeries like stomach pull-up, colon bypass, and free jejunal flap are not always possible when the prior operation was similarly invasive[6].

In our case, the right colon had already been used during the previous bypass operation, and the graft stricture of the neo-esophagus resulted from ischemia. Thus, patch dilative strictureplasty with viable tissue was

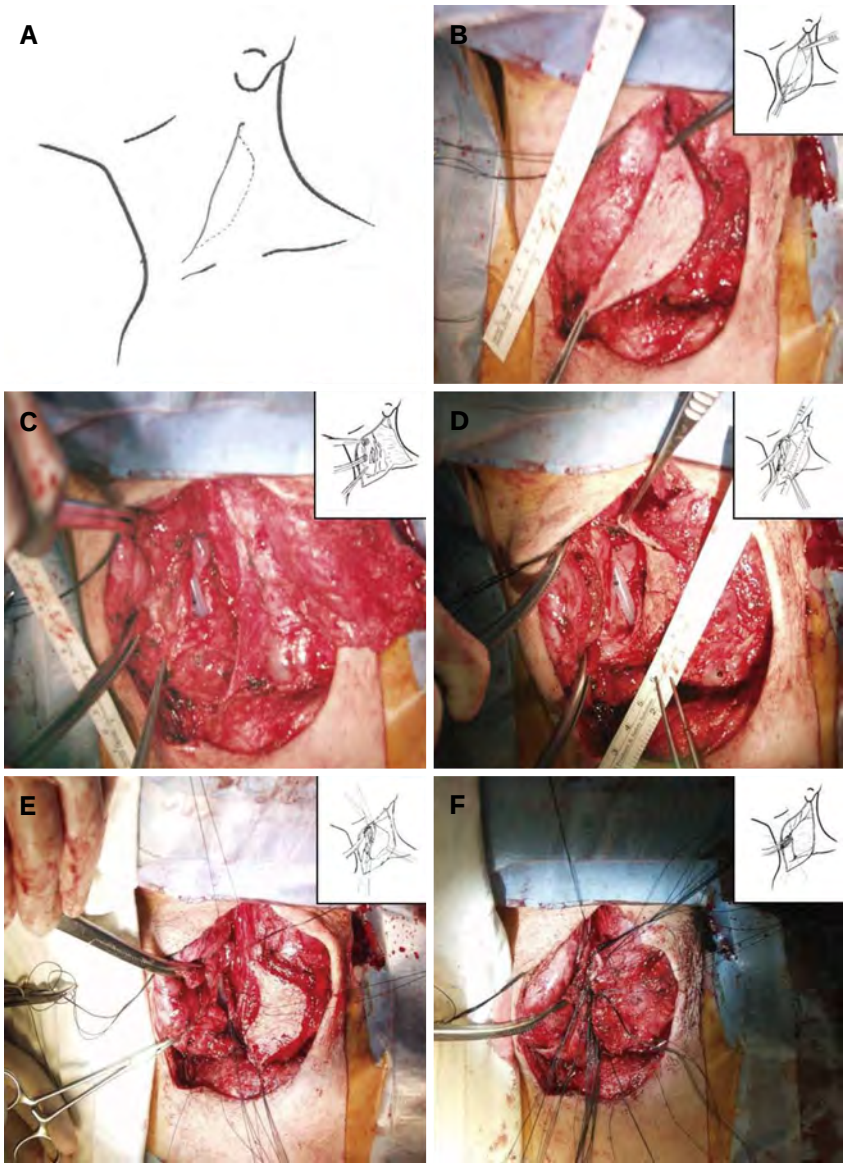


Figure 2 Photograph of the musculocutaneous sternocleidomastoid flap and esophagotomy during the operation. A: A skin incision was designed for an elliptical skin island based on the sternocleidomastoid muscle; B: A skin island (6 cm × 4 cm) on the sternocleidomastoid (SCM) muscle was prepared; C, D: Esophagotomy at the level of the cricoid cartilage was extended upward to pharynx and downward; E: The SCM muscle was severed near the clavicle and rotated. The skin flap was fixated to esophagus with interrupted silk sutures; F: The esophagoplasty site was covered with SCM muscle *via* multiple fixation sutures.

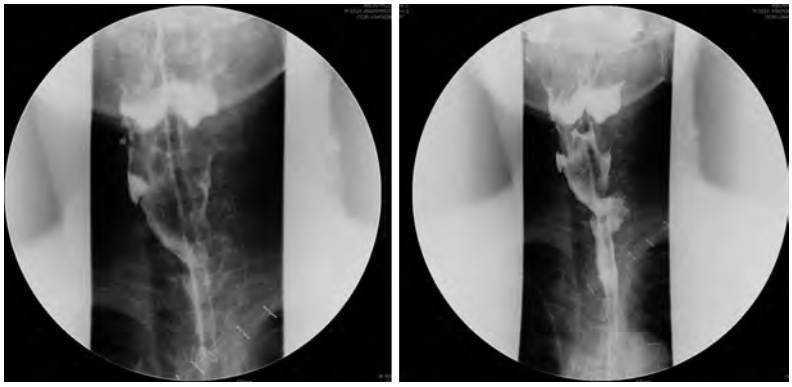


Figure 3 Esophagogram after sternocleidomastoid myocutaneous patch esphagoplasty. The stenotic lesion at the esophago-colonic anastomosis site was widened successfully and the dye passed through the colon conduit without resistance.

thought to be an ideal option for relieving the short, benign, recurrent cervical esophageal stricture.

The choice of tissue to be used as a patch to widen the esophagus varies. Among flaps, a myocutaneous flap has several advantages: its skin provides an adequate patch for the esophageal defect and the well-vascularized muscle forms a seal around the ischemic anastomosis site, obliterates dead space, and conveys antibiotics[7]. The SCM muscle is a logical choice for myocutaneous flap in this case because its anatomic proximity lessens trauma[7]. Because SCM patch esophagoplasty avoids complete transection of the esophagus, coordinated peristalsis is also preserved and vagotomy is avoided[7]. The SCM muscle has a segmental blood supply from branches of three

main vessels: the occipital artery superiorly, the superior thyroid artery in the middle segment, and a branch of the thyrocervical trunk inferiorly. The superior pedicle is more commonly used because it provides greater rotation and allows the SCM to reach the entire length of the ipsilateral cervical esophagus[2]. The SCM myocutaneous flap does, however, have limitations. Safety is a major concern when the SCM flap is used in oncologic patients[8]. In cases of radiation therapy, the SCM myocutaneous flap may be used only if the overlying skin is soft and supple[2]. Furthermore, use of a SCM pedicled skin flap is limited to the cervical esophagus. In conclusion esophagoplasty with SCM myocutaneous flap is thought to be an efficient treatment of recurrent cervical esophageal strictures.

REFERENCES

1 **Park JK**, Sim SB, Lee SH, Jeon HM, Kwack MS. Pharyngo-enteral anastomosis for esophageal reconstruction in diffuse corrosive esophageal stricture. *Ann Thorac Surg* 2001; **72**: 1141-1143 [PMID: 11603426 DOI: 10.1016/S0003-4975(01)02961-7]

2 **Noland SS**, Ingraham JM, Lee GK. The sternocleidomastoid myocutaneous "patch esophagoplasty" for cervical esophageal stricture. *Microsurgery* 2011; **31**: 318-322 [PMID: 21500276 DOI: 10.1002/micr.20880]

3 **Wormuth JK**, Heitmiller RF. Esophageal conduit necrosis. *Thorac Surg Clin* 2006; **16**: 11-22 [PMID: 16696279 DOI: 10.1016/j.thorsurg.2006.01.003]

4 **Jain V**, Rodrigues GS, Gupta K. Ischaemic necrosis of subcutaneous colonic neoesophagus: an unusual complication of presternal hypertrophic scar. *Singapore Med J* 2006; **47**: 235-236 [PMID: 16518560]

5 **Ananthakrishnan N**, Parthasarathy G, Maroju NK, Kate V. Sternocleidomastoid muscle myocutaneous flap for corrosive pharyngoesophageal strictures. *World J Surg* 2007; **31**: 1592-1596 [PMID: 17551780 DOI: 10.1007/s00268-007-9120-5]

6 **Lin YD**, Jiang YG, Wang RW, Gong TQ, Zhou JH. Platysma myocutaneous flap for patch stricturoplasty in relieving short and benign cervical esophageal stricture. *Ann Thorac Surg* 2006; **81**: 1090-1094 [PMID: 16488729 DOI: 10.1016/j.athoracsur.2005.09.005]

7 **Cunningham DK**, Stern SJ, Burnett HF. Sternocleidomastoid myocutaneous esophagoplasty for benign cervical stricture. *Ann Thorac Surg* 2005; **79**: 1406-1407 [PMID: 15797094 DOI: 10.1016/j.athoracsur.2003.10.035]

8 **Lin CH**, Lin CH, Wu CW, Liao CT. Sternocleidomastoid muscle flap: an option to seal off the esophageal leakage after free jejunal flap transfer--a case report. *Chang Gung Med J* 2009; **32**: 224-229 [PMID: 19403014]

P- Reviewer Roman S **S- Editor** Jiang L
L- Editor A **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i2.311

World J Gastroenterol 2013 January 14; 19(2): 311-315
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

A case of ascending colon variceal bleeding treated with venous coil embolization

Bong Suk Ko, Woo Tae Kim, Su Sun Chang, Eun Hye Kim, Seung Woo Lee, Won Seok Park, Yeon Soo Kim, Soon Woo Nam, Dong Soo Lee, Ji Chang Kim, Sang Bum Kang

Bong Suk Ko, Woo Tae Kim, Su Sun Chang, Eun Hye Kim, Seung Woo Lee, Won Seok Park, Yeon Soo Kim, Soon Woo Nam, Dong Soo Lee, Sang Bum Kang, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Daejeon St. Mary's Hospital, Daejeon 301-723, South Korea
Ji Chang Kim, Department of Radiology, College of Medicine, The Catholic University of Korea, Daejeon St. Mary's Hospital, Daejeon 301-723, South Korea
Author contributions: Ko BS wrote the paper; Kim WT, Chang SS, Kim EH, Lee SW, Park WS, Kim YS, Nam SW, and Lee DS collected the materials; Kim JC contributed radiologic interventions; and Kang SB designed the study.
Correspondence to: Sang Bum Kang, MD, Department of Internal Medicine, College of Medicine, The Catholic University of Korea, Daejeon St. Mary's Hospital, 64 Daeheungro, Jung-gu, Daejeon 301-723, South Korea. ksb1999@hanmail.net
Telephone: +82-42-2209823 Fax: +82-42-2219038
Received: August 14, 2012 Revised: November 8, 2012
Accepted: November 14, 2012
Published online: January 14, 2013

Abstract

A 38-year-old female with a history of alcoholic liver cirrhosis visited our hospital with a massive hematochezia. An esophagogastroduodenoscopy did not demonstrate any bleeding source, and a colonoscopy showed a massive hemorrhage in the ascending colon but without an obvious focus. The source of the bleeding could not be found with a mesenteric artery angiography. We performed an enhanced abdominal computed tomography, which revealed a distal ascending colonic varix, and assumed that the varix was the source of the bleeding. We performed a venous coil embolization and histoacryl injection to obliterate the colon varix. The intervention appeared to be successful because the vital signs and hemoglobin laboratory data remained stable and because the hematochezia was no longer observed. We report here on a rare case of colonic variceal bleeding that was treated with venous coil embolization.

© 2013 Baishideng. All rights reserved.

Key words: Colon ascending; Varicose veins; Liver cirrhosis; Therapeutic embolization

Ko BS, Kim WT, Chang SS, Kim EH, Lee SW, Park WS, Kim YS, Nam SW, Lee DS, Kim JC, Kang SB. A case of ascending colon variceal bleeding treated with venous coil embolization. *World J Gastroenterol* 2013; 19(2): 311-315 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i2/311.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i2.311

INTRODUCTION

Colonic variceal bleeding is a very rare cause of lower gastrointestinal bleeding. Fewer than 100 cases of colonic variceal bleeding have been reported in English literature and few case reports exist in South Korea. However, most cases in South Korea were caused by different varix locations, etiologies and treatments. A single case in South Korea with ascending colonic variceal bleeding caused by alcoholic liver cirrhosis was treated with a hemicolectomy[1]. We report the first case of an ascending colon variceal bleed caused by alcoholic liver cirrhosis that was treated with venous coil embolization in South Korea.

CASE REPORT

A 38-year-old female was admitted to our hospital with massive hematochezia and hemodynamic instability. She was diagnosed with alcoholic liver cirrhosis (Child-Pugh classification C) 6 years ago and had multiple hospitalizations with complications of esophageal variceal bleeding and hepatic encephalopathy. Upon physical examination, her initial blood pressure was 80/50 mmHg, her heart rate was 110 bpm, her respiration was 28 bpm and her

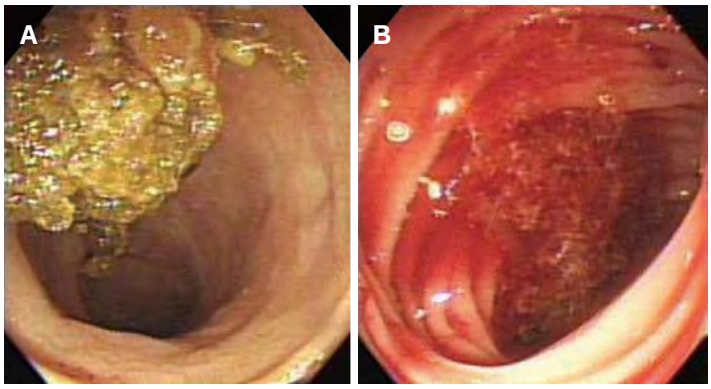


Figure 1 Initial colonoscopy. A: The terminal ileum showing yellowish-colored stool with no evidence of upper gastrointestinal bleeding; B: The ascending colon showing fresh blood without an observable source.

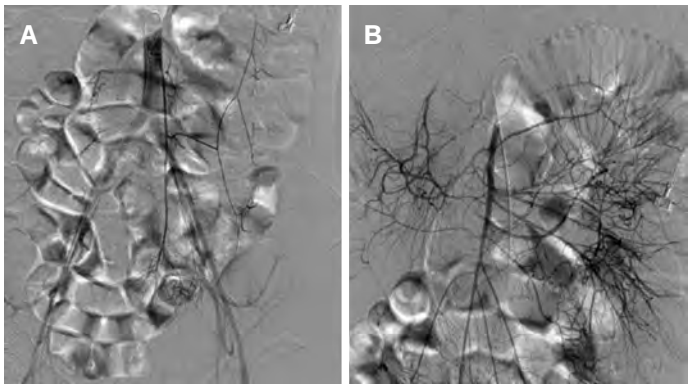


Figure 2 Mesenteric artery angiography. A: The inferior mesenteric artery angiography showing no source of bleeding; B: The superior mesenteric artery angiography showing no source of bleeding.

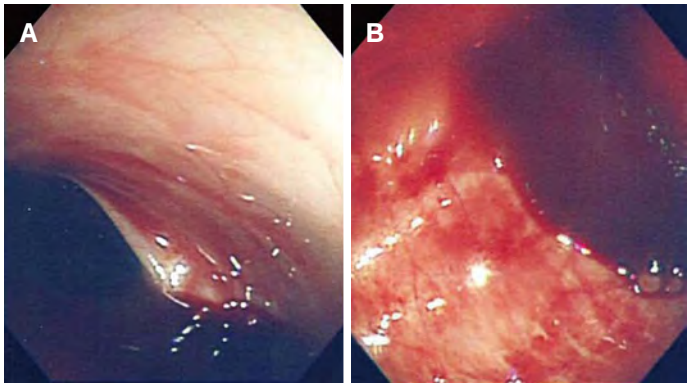


Figure 3 The second colonoscopy. A: Massive hemorrhage within the proximal ascending colon without a definite bleeding focus; B: Fresh blood within the distal ascending colon without a definite bleeding focus.

body temperature was 37 ℃. No evidence of bleeding was detected with nasogastric tube irrigation. Abnormal laboratory data upon admission included the following: hemoglobin 7.0 g/dL, hematocrit 22.3%, platelets 59 000/mm^3, serum creatinine 2.31 mg/dL, international normalized ratio 1.98, and albumin 2.1 g/dL.

An esophagogastroduodenoscopy demonstrated esophageal varices grade Ⅲ but did not show signs of recent bleeding. The colonoscopy showed fresh blood from the ascending colon to the rectum, but there was no evidence of bleeding in the terminal ileum, which demonstrated that the origin of the bleeding could be assumed to be in the ascending colon (Figure 1). Diagnostic angiographies of the superior and inferior mesenteric arteries were performed, but the source of the bleeding could not be found (Figure 2). Even with 2 units of packed RBCs, the hemoglobin level dropped to 5.6 g/dL the next day, and the vital signs remained unstable.

A second colonoscopy was still unable to visualize the bleeding focus and only detected a massive hemorrhage in the ascending colon (Figure 3). We performed an enhanced abdominal computed tomography (CT), which revealed the formation of a varix surrounding and protruding inside the distal ascending colon by the portacaval shunt (Figure 4). The varix was thought to be the source of the gastrointestinal bleeding. We planned an operation, but a right hemicolectomy could not be performed due to poor vital signs and abnormal coagulopathy. Initially, we tried to perform a Balloon-Occluded Retrograde Transvenous Obliteration (BRTO) but failed due to the selection of the portacaval shunt. Because the patient had unstable vital signs that required immediate intervention, we performed a coil embolization and his-

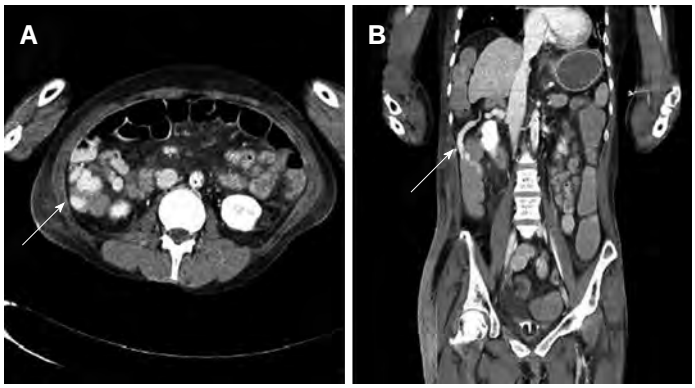


Figure 4 Abdominal computed tomography. A: Portal phase axial view with white arrow showing the varix surrounding and protruding inside the ascending colon formed by portacaval shunt; B: Coronal view with white arrow showing the colon varix.

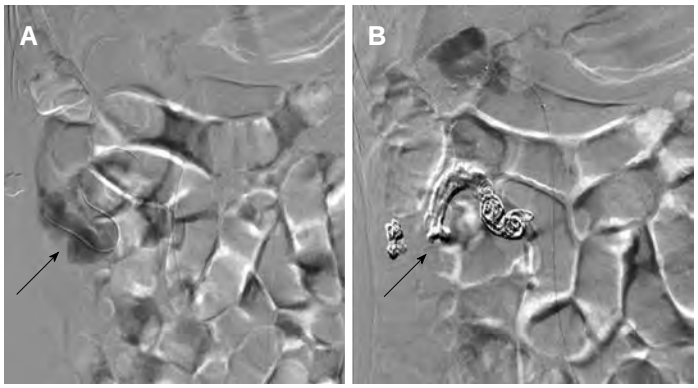


Figure 5 Venous coil embolization. A: Venography showing the varix within the ascending colon (black arrow); B: Coil embolization (black arrow) and histoacryl injection was performed to obliterate the varix.

toacryl injection to obliterate the varix (Figure 5). After the coil embolization and 4 units of packed red blood cells, the patient's hemoglobin level rose to 10.0 g/dL, where it stabilized, and the vital signs (blood pressure and heart rate) recovered. There were no further signs of bleeding, such as hematochezia. However, the patient developed complications of pneumonia and renal failure, which led to multiorgan failure. Even with ventilator care and continuous renal replacement therapy, the patient expired 2 wk after the intervention.

DISCUSSION

Colon varices are a very rare cause of lower gastrointestinal bleeding, with a reported incidence of 0.07%[2]. Colon varices can be associated with several conditions, such as portal hypertension, portal venous obstruction, postsurgical changes and idiopathic factors, but the vast majority of colonic varices are related to portal hypertension caused by cirrhosis or portal vein obstruction. Embryologically derived anastomoses between the portal and systemic systems exist in the esophagus, terminal ileum, colon, retroperitoneum, and anterior abdominal wall[3]. The most common sites of colonic varices are the rectum and cecum[4]. Colon varices with portal hypertension are hypothesized to arise in patients in whom the colonic portosystemic venous collateral network is highly developed[5]. The rate of colonic variceal bleeding that causes lower gastrointestinal bleeding with liver cirrhosis is approximately 1%-8%[6,7]. Thus, if a patient has a history of liver cirrhosis and has massive gastrointestinal bleeding, the possibility of colon variceal bleeding should also be considered. Other than portal hypertension, mesenteric venous or splenic vein obstruction, which may be due to thrombosis, tumor invasion, extrinsic compression, acute or chronic pancreatitis, mesenteric adhesions, or congenital anatomic variations, may cause a colon varix. With no etiology, colon varix is classified as idiopathic or primary[5].

A colonoscopy is the principal method for the diagnosis of colon varices, but with massive bleeding, the diagnostic rate of a colonoscopy is 69% (range: 48%-90%) because the varices may be obscured by blood[8]. Thus, in case of massive hematochezia, magnetic resonance imaging, mesenteric angiography and abdominal CT are alternative diagnostic tools[9,10].

Management of colonic variceal bleeding has not been standardized. In a report describing a case of massive lower gastrointestinal bleeding with some hemodynamic instability caused by colonic varix, an operation was the optimal choice[11]. However, most cases with colonic variceal bleeding are associated with cirrhotic liver, which makes many cases inoperable due to coagulopathy. Thus, interventional therapies are tested depending on the underlying etiology and the distribution of the varices. These therapies include transhepatic intravenous portosystemic shunt (TIPS), BRTO, endoscopic variceal ligation, somatostatin infusion, argon plasma coagulation, histoacryl injection, and coil embolization[12-15]. Four other cases of colon variceal bleeding have been

described in South Korea. Theses cases had different varix locations, etiologies and treatments compared to our case. The first case was a 64-year-old female with liver cirrhosis who had descending colon variceal bleeding that was treated with a left hemicolectomy[16]. The second case was a 24-year-old male who had idiopathic rectal variceal bleeding that was treated with low anterior resection[2]. The third case was a 43-year-old male with ascending colon variceal bleeding due to alcoholic liver cirrhosis. This case was very similar to our case but was treated with a right hemicolectomy[1]. The last case was a 33-year-old woman with a history of systemic lupus erythematosus who developed antiphospholipid syndrome and had ascending colon variceal bleeding that was treated with octreotide, a beta-blocker and warfarin[17]. In our case, the patient was inoperable because of the poor vital signs and coagulopathy caused by the abnormal liver function. We initially tried to perform BRTO to obliterate the portacaval shunt, but the angiographic selection of the portacaval shunt failed. Thus, venous coil embolization along with a histoacryl injection was performed on the ascending colon varix. This technique seemed to be successful because the vital signs (blood pressure and heart rate) along with hemoglobin level remained stable, and no further evidence of gastrointestinal bleeding was observed after the intervention. This result is despite the fact that our patient expired due to complications of multiorgan failure because the patient already had terminal liver cirrhosis and had developed unstable vital signs before arriving in the emergency room. Together, these conditions made the patient susceptible to secondary infection and renal failure. Complications of the varix coil embolization procedure could also be considered after the intervention, such as necrosis, vessel perforation, non-targeting embolization and migration of the coil. However, although rate of complications has not been reported, this rate is thought to be very low because the procedure proceeds through a venous approach, and migration of the coil could be corrected during the procedure, and the patient did not complain of post-interventional symptoms such as abdominal pain, fever, or dyspnea. Thus, acute or delayed complications of the venous coil embolization were eliminated. A similar case in Switzerland included a 43-year-old male patient with ascending colon variceal bleeding due to alcoholic liver cirrhosis. The patient had TIPS initially to reduce the portosystemic pressure gradient, but the bleeding did not stop; the patient then underwent histoacryl injection and venous coil embolization. The patient recovered without further incidents[18]. As noted above, there is no standardized management of colon variceal bleeding, but venous coil embolization could be an effective treatment option for patients with massive hematochezia by the colon varix. Even though our patient could not survive due to multiple organ failure and secondary infection, we believe that the successful venous coil embolization had stopped the bleeding, which prevented a more aggressive progression. Our case is thought to be the first case in South Korea that used venous coil embolization to treat ascending colon variceal bleeding caused by alcoholic liver cirrhosis.

In conclusion, the treatment of colon variceal bleeding has not been well established. However, this case has shown that treatment with venous coil embolization on an ascending colon variceal bleeding caused by alcoholic liver cirrhosis is effective. We report here on the first case of ascending colon variceal bleeding caused by alcoholic liver cirrhosis that was treated with venous coil embolization in South Korea.

REFERENCES

1 **Kim HU**, Her KH, Kim SH, Kim BS, Kang YJ, Lee J, Kim KS. A case of variceal bleeding of the ascending colon associated with alcoholic liver cirrhosis. *Korean J Med* 2008; **75**: 215-220

2 **Han JH**, Jeon WJ, Chae HB, Park SM, Youn SJ, Kim SH, Bae IH, Lee SJ. A case of idiopathic colonic varices: a rare cause of hematochezia misconceived as tumor. *World J Gastroenterol* 2006; **12**: 2629-2632 [PMID: 16688816]

3 **Edwards EA**. Functional anatomy of the porta-systemic communications. *AMA Arch Intern Med* 1951; **88**: 137-154 [PMID: 14856444]

4 **Sato T**, Akaike J, Toyota J, Karino Y, Ohmura T. Clinicopathological features and treatment of ectopic varices with portal hypertension. *Int J Hepatol* 2011; **2011**: 960720 [PMID: 21994879 DOI: 10.4061/2011/960720]

5 **Francois F**, Tadros C, Diehl D. Pan-colonic varices and idiopathic portal hypertension. *J Gastrointestin Liver Dis* 2007; **16**: 325-328 [PMID: 17925930]

6 **Ganguly S**, Sarin SK, Bhatia V, Lahoti D. The prevalence and spectrum of colonic lesions in patients with cirrhotic and noncirrhotic portal hypertension. *Hepatology* 1995; **21**: 1226-1231 [PMID: 7737627]

7 **Hosking SW**, Smart HL, Johnson AG, Triger DR. Anorectal varices, haemorrhoids, and portal hypertension. *Lancet* 1989; **1**: 349-352 [PMID: 2563507]

8 **Abraham-Igwe C**, Patel R. Idiopathic colonic varices: a case report. *Endoscopy* 2002; **34**: 680 [PMID: 12173098 DOI: 10.1055/s-2002-33235]

9 **Chevallier P**, Motamedi JP, Demuth N, Caroli-Bosc FX, Oddo F, Padovani B. Ascending colonic variceal bleeding: utility of phase-contrast MR portography in diagnosis and follow-up after treatment with TIPS and variceal embolization. *Eur Radiol* 2000; **10**: 1280-1283 [PMID: 10939490]

10 **Choi JW**, Lee CH, Kim KA, Park CM, Kim JY. Ectopic varices in colonic stoma: MDCT findings. *Korean J Radiol* 2006; **7**: 297-299 [PMID: 17143035]

11 **Lopes LM**, Ramada JM, Certo MG, Pereira PR, Soares JM, Ribeiro M, Areias J, Pinho C. Massive lower gastrointestinal bleeding from idiopathic ileocolonic varix: report of a case. *Dis Colon Rectum* 2006; **49**: 524-526 [PMID: 16395635 DOI: 10.1007/s10350-005-0279-2]

12 **Allgaier HP**, Ochs A, Haag K, Hauenstein KH, Tittor W, Rössle M, Blum HE. [Recurrent bleeding from colonic varices in portal hypertension. The successful prevention of recurrence by the implantation of a transjugular intrahepatic stent-shunt (TIPS)]. *Dtsch Med Wochenschr* 1995; **120**: 1773-1776 [PMID: 8549262 DOI: 10.1055/s-2008-1055541]

13 **Anan A**, Irie M, Watanabe H, Sohda T, Iwata K, Suzuki N, Yoshikane M, Nakane H, Hashiba T, Yokoyama M, Hi-

gashihara H, Okazaki M, Sakisaka S. Colonic varices treated by balloon-occluded retrograde transvenous obliteration in a cirrhotic patient with encephalopathy: a case report. *Gastrointest Endosc* 2006; **63**: 880-884 [PMID: 16650568 DOI: 10.1016/j.gie.2005.11.038]

14 **Misra SP**, Dwivedi M. Ligation of a bleeding colonic varix using an upper gastrointestinal endoscope. *Endoscopy* 2006; **38**: 657 [PMID: 16673302 DOI: 10.1055/s-2006-925189]

15 **Chen WC**, Hou MC, Lin HC, Chang FY, Lee SD. An endoscopic injection with N-butyl-2-cyanoacrylate used for colonic variceal bleeding: a case report and review of the literature. *Am J Gastroenterol* 2000; **95**: 540-542 [PMID: 10685765 DOI: 10.1111/j.1572-0241.2000.01782.x]

16 **Song C**, Lee Y, Cho H, Kim D, Choi W, Park H. Massive gastrointestinal hemorrhage from the colonic varices. *J Korean Surg Soc* 1993; **44**: 923-928

17 **Sohn W**, Lee HL, Lee KN. Variceal hemorrhage of ascending colon. *Clin Gastroenterol Hepatol* 2012; **10**: A24 [PMID: 21893130 DOI: 10.1016/j.cgh.2011.08.024]

18 **Wiegand N**, Pfiffner R, Bauerfeind P. Ascending colonic variceal bleeding. *Gastrointest Endosc* 2006; **63**: 1073-104; discussion 1074 [PMID: 16733135 DOI: 10.1016/j.gie.2005.11.021]

P- Reviewer De Nardi P **S- Editor** Gou SX
L- Editor A **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i2.316

World J Gastroenterol 2013 January 14; 19(2): 316-318
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Atypical presentation of pseudomembranous colitis localized in adenomatous polyps

Cristian Hernández-Rocha, Jonathan Barra-Carrasco, Ana María Guzmán, Daniel Paredes-Sabja, Gabriel Lezcano, Pablo Zoroquiaín, Manuel Álvarez-Lobos

Cristian Hernández-Rocha, Manuel Álvarez-Lobos, Department of Gastroenterology, Faculty of Medicine, Pontificia Universidad Católica de Chile, Marcoleta 367, Santiago 6510260, Chile
Jonathan Barra-Carrasco, Daniel Paredes-Sabja, Laboratory of Mechanisms of Bacterial Pathogenesis, Department of Biological Sciences, Faculty of Biological Sciences, Universidad Andrés Bello, Santiago 6618000, Chile
Ana María Guzmán, Department of Clinical Laboratory, Faculty of Medicine, Pontificia Universidad Católica de Chile, Marcoleta 367, Santiago 6510260, Chile
Gabriel Lezcano, Pablo Zoroquiaín, Department of Anatomophatology, Faculty of Medicine, Pontificia Universidad Católica de Chile, Marcoleta 367, Santiago 6510260, Chile
Daniel Paredes-Sabja, Department of Biomedical Sciences, College of Veterinary Medicine, Oregon State University, Corvallis, OR 97333, United States
Author contributions: Hernández-Rocha C, Barra-Carrasco J, Guzman AM, Paredes-Sabja D and Álvarez-Lobos M wrote the paper; Lezcano G and Zoroquiaín P reviewed the histopathological slides.
Correspondence to: Dr. Manuel Álvarez-Lobos, Department of Gastroenterology, Faculty of Medicine, Pontificia Universidad Católica de Chile, Marcoleta 367, Santiago, Chile. manalvarezl@gmail.com
Telephone: +56-2-3543820 Fax: +56-2-6397780
Received: July 17, 2012 Revised: September 30, 2012
Accepted: October 16, 2012
Published online: January 14, 2013

Abstract

The most frequent cause of pseudomembranous colitis is *Clostridium difficile* (*C. difficile*) infection. This type of colitis is characterized by an endoscopic pattern of numerous small, yellowish or whitish plaques diffusely distributed, which typically compromises the rectum extending to proximal colon. Occasionally, the pseudomembranes compromise only the transverse or right colon, but their exclusive localization over polyps has not been reported. In this case report we have described a patient with symptoms compatible with *C. difficile* infection and positive for *C. difficile* toxigenic culture. Colonoscopy examination showed two small polyps with a whitish surface, and histopathological analysis confirmed them to be pseudomembranes over tubular adenomas. The rest of the colonic mucosa was normal and no other cause was demonstrated. We suggest that this particular distribution might be due to a higher affinity for dysplastic cells such as adenomatous polyps of colon by *C. difficile* and/or its toxins.

© 2013 Baishideng. All rights reserved.

Key words: *Clostridium difficile*; Pseudomembranous colitis; Adenomatous polyps; Antibiotic-associated colitis; *Clostridium difficile* infections

Hernández-Rocha C, Barra-Carrasco J, Guzmán AM, Paredes-Sabja D, Lezcano G, Zoroquiaín P, Álvarez-Lobos M. Atypical presentation of pseudomembranous colitis localized in adenomatous polyps. *World J Gastroenterol* 2013; 19(2): 316-318 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i2/316.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i2.316

INTRODUCTION

The clinical spectrum of *Clostridium difficile* (*C. difficile*) infections may vary from mild diarrhea to life-threatening pseudomembranous colitis (PMC), which may localize from the proximal colon to the rectum[1]. Localized forms of PMC have been reported to compromise the transverse and right colon[2], but to the best of our knowledge, we have not found cases with exclusive localization of PMC over polyps. Below, we report a case with the pseudomembranes only covering the surface of adenomatous polyps.

CASE REPORT

An 86-year-old female receiving anticoagulant therapy due

to an aortic valve prosthesis with auricular fibrillation secondary to aortic stenosis was admitted with an incarcerated umbilical hernia. A hernioplasty was performed without complication, and she was treated with sodium piperacillin-tazobactam (4.5 g *tid*) intravenously for 7 d and discharged 10 d after surgery. Twenty-five days later, she presented hematochezia with hemodynamic compromise. She was admitted to the coronary unit, and an upper endoscopy showed a hiatal hernia with Cameron's ulcers; she was treated with omeprazole. The next day a colonoscopy showed a clean colonic mucosa, absence of blood, and two sessile 6-mm polyps in the transverse colon and in the right colon. The upper surface of both polyps was covered by an adherent and whitish layer and the surrounding mucosa was normal (Figure 1). The polyps were resected with cold biopsy forceps and sent for histopathological study. Strikingly, the day after colonoscopy, the patient developed fever with no apparent cause in the physical exam, while laboratory results showed a hematocrit of 29%, white blood cell count of 8700 cell/μL without band forms and C reactive protein was 10.2 mg/dL. The chest X-ray was normal and the blood and urine cultures were negative. She was empirically treated with sodium piperacillin-tazobactam, but three days later she presented frequent loose stools and diffuse abdominal pain. An enzyme immune assay (EIA) for *C. difficile* toxin was positive. The antibiotic was stopped and she was treated with oral metronidazole with an excellent response. Presence of *C. difficile* was further confirmed by real time-polymerase chain reaction (PCR) and toxigenic culture. PCR-ribotyping indicated that the clinical isolate that caused *C. difficile* infection was not an epidemic strain (non-ribotype 027 strain). A few days later, the histopathological study of the polyps showed two tubular adenomas with a low-grade epithelial dysplasia and pseudomembranes on their surface with a typical volcanic appearance (Figure 2).

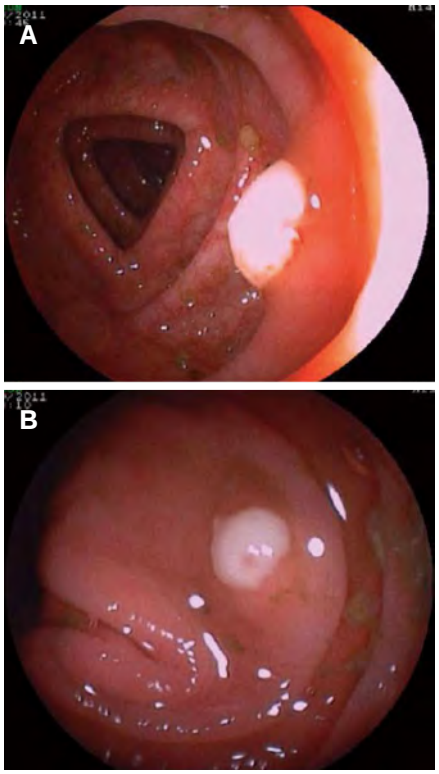


Figure 1 Colonoscopy images showing two polyps, 4 and 6 mm in size, in the right colon with adherent whitish surface. A: Polyp of 6 mm; B: Polyp of 4 mm. The mucosa round the polyps and in the rest of colon is normal.

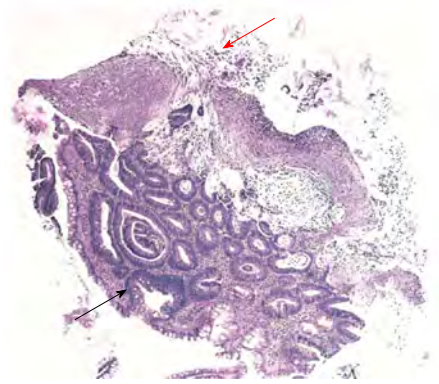


Figure 2 Low power view of a mucosal fragment. Histopathology shows: at the bottom, tubular structures lined by columnar epithelial cells with pseudostratified nuclei, consistent with low grade tubular adenoma (black arrow); at the top, ballooned crypts and intercrypt necrosis, an exudate featuring the classical "volcano" lesion (red arrow) surrounded by a laminated pseudomembrane composed of neutrophils, mucin and fibrin (hematoxylin and eosin, x 100).

DISCUSSION

It is our understanding that this is the first documented case in which the pseudomembranes are exclusively localized over adenomatous polyps with the rest of the colonic mucosa being intact. The term PMC is nonspecific and describes an acute mucosal injury characterized by an endoscopic pattern of numerous, discrete and small (2-5 mm), raised, round and yellowish plaques (pseudomembranes) distributed over an erythematous but unulcerated mucosa[1]. The rectum is frequently involved, but in a few cases the sigmoid and/or right colon is compromised[2]. The histological pattern is characterized by a neutrophil-rich edema fluid in the lamina propria that bursts through tiny breeches to the surface epithelium, like a volcanic eruption, to form a characteristic punctate inflammatory pseudomembrane[1,3]. The PMC is due, in nearly all cases, to *C. difficile*. Other infrequent causes are ischemic or infectious colitis[4]. Piperacillin-tazobactam has been associated at lower rates with *C. difficile* infection than other antibiotics[5]; however, in this case report, the clinical course and the study with EIA, PCR and toxigenic culture confirmed an association with *C. difficile* and the response to treatment with oral metronidazole was concordant.

The specific type of colonic cells where *C. difficile* infections starts is unclear, but it is known that at least enterotoxin A, one of its main virulence factors, has remarkable cytotoxicity for cells derived from human or animal cancer, more than for normal colonic epithelial

cells. One possible explanation, but by no means proven, is that the surface of neoplastic cells possesses a greater density of specific receptors for toxin A and/or toxin B as compared to less sensitive cell lines[6]. Alternatively, it is well known that members of the *Clostridium* genus have a tropism for tumors mainly due to the hypoxic environment as a consequence of poor vascular irrigation[7,8]. Thus, it seems plausible that colonic neoplastic cells might favor *C. difficile* colonization and PMC formation.

In conclusion, we propose that dysplastic changes of the colonic epithelium occurring as adenomatous polyps might represent a site of greater affinity for *C. difficile* toxin and/or tropism of *C. difficile* cells with the consequent formation of pseudomembranes, which were observed by an early colonoscopy in this patient.

REFERENCES

1 **Carpenter HA**, Talley NJ. The importance of clinicopathological correlation in the diagnosis of inflammatory conditions of the colon: histological patterns with clinical implications. *Am J Gastroenterol* 2000; **95**: 878-896 [PMID: 10763932 DOI: S0002-9270(00)00716-4]

2 **Tedesco FJ**, Corless JK, Brownstein RE. Rectal sparing in antibiotic-associated pseudomembranous colitis: a prospective study. *Gastroenterology* 1982; **83**: 1259-1260 [PMID: 7129030 DOI: S0016508582002509]

3 **Price AB**, Davies DR. Pseudomembranous colitis. *J Clin Pathol* 1977; **30**: 1-12 [PMID: 838865 DOI: 10.1136/jcp.30.1.1]

4 **Bartlett JG**. Clinical practice. Antibiotic-associated diarrhea. *N Engl J Med* 2002; **346**: 334-339 [PMID: 11821511 DOI: 10.1056/NEJMcp011603]

5 **Mendez MN**, Gibbs L, Jacobs RA, McCulloch CE, Winston L, Guglielmo BJ. Impact of a piperacillin-tazobactam shortage on antimicrobial prescribing and the rate of vancomycin-resistant enterococci and Clostridium difficile infections. *Pharmacotherapy* 2006; **26**: 61-67 [PMID: 16509027]

6 **Kushnaryov VM**, Redlich PN, Sedmak JJ, Lyerly DM, Wilkins TD, Grossberg SE. Cytotoxicity of Clostridium difficile toxin A for human colonic and pancreatic carcinoma cell lines. *Cancer Res* 1992; **52**: 5096-5099 [PMID: 1516066]

7 **Wei MQ**, Ren R, Good D, Anné J. Clostridial spores as live 'Trojan horse' vectors for cancer gene therapy: comparison with viral delivery systems. *Genet Vaccines Ther* 2008; **6**: 8 [PMID: 18279524 DOI: 10.1186/1479-0556-6-8]

8 **Minton NP**. Clostridia in cancer therapy. *Nat Rev Microbiol* 2003; **1**: 237-242 [PMID: 15035028 DOI: 10.1038/nrmicro777]

P- Reviewer Chiarioni G **S- Editor** Gou SX **L- Editor** Logan S **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i2.319

World J Gastroenterol 2013 January 14; 19(2): 319-320
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

Anesthetic management of the SRS™ endoscopic stapling system for gastro-esophageal reflux disease

Ufuk Topuz, Tarik Umutoglu, Mefkur Bakan, Erdogan Ozturk

Ufuk Topuz, Tarik Umutoglu, Mefkur Bakan, Erdogan Ozturk, Department of Anesthesiology and Reanimation, Bezmialem Vakif University Faculty of Medicine, 34093 Istanbul, Turkey

Author contributions: Topuz U and Umutoglu T wrote this letter; Bakan M and Ozturk E revised the letter.

Correspondence to: Mefkur Bakan, MD, Assistant Professor, Bezmialem Vakif University, Vatan cad, Fatih, 34093 Istanbul, Turkey. mefkur@yahoo.com

Telephone: +90-536-2602699 Fax: +90-212-6217580

Received: September 28, 2012 Revised: December 5, 2012
Accepted: December 15, 2012
Published online: January 14, 2013

Abstract

The SRS™ Endoscopic Stapling System (Medigus, Tel Aviv, Israel) is a new tool capable of creating a totally endoscopic fundoplication, combined with an endoscope, endoscopic ultrasound and a surgical stapler. SRS™ endoscopic stapling for gastro-esophageal reflux disease is a minimally invasive, outpatient procedure, which requires general anesthesia with positive-pressure ventilation. Keeping the patient on positive end-expiratory pressure (PEEP) may minimize the pressure gradient between the esophagus and the mediastinum, as well as help to prevent air from leaking around the screws and causing pneumomediastinum. In addition, in patients with hiatal hernia, higher PEEP levels may be required to increase intra-thoracic pressure and to force the stomach to slide into the abdomen for ease of endoscopy. We advise smoother emergence from anesthesia, taking precautions for retching, postoperative nausea and vomiting (PONV), while coughing and gagging during extubation and PONV may affect the success of the procedure. Total intravenous anesthesia with propofol and remifentanil seems to be a good choice for these reasons.

© 2013 Baishideng. All rights reserved.

Key words: Gastro-esophageal reflux disease; Endoscopy; Anesthesia; SRS™ endoscopic stapling system; Positive end-expiratory pressure

Topuz U, Umutoglu T, Bakan M, Ozturk E. Anesthetic management of the SRS™ endoscopic stapling system for gastro-esophageal reflux disease. *World J Gastroenterol* 2013; 19(2): 319-320 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i2/319.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i2.319

TO THE EDITOR

Endoscopic ultrasound was developed as a useful diagnostic tool and is being used in the treatment of various gastrointestinal diseases[1,2]. The SRS™ Endoscopic Stapling System (Medigus, Tel Aviv, Israel) is a new tool capable of creating a totally endoscopic fundoplication, combined with an endoscope, endoscopic ultrasound, and a surgical stapler[3]. This modified endoscope fires 3 rows of staples plicating the fundus onto the esophagus. While the anesthesia management of the SRS™ endoscopic fundoplication procedure has not been described, we would like to share our initial experiences with it on 12 patients.

After obtaining written informed consent, patients diagnosed with gastro-esophageal reflux disease, without or with ≤ 3 cm hiatal hernia, were scheduled for this procedure. Following at least 8 h fasting time, patients were premedicated with midazolam, metoclopramide, and a proton pump inhibitor. Total intravenous anesthesia (TIVA) with propofol and remifentanil was used for both induction and maintenance of general anesthesia. After muscle relaxation was achieved with rocuronium, patients were endotracheally intubated. Patients were mechanically ventilated with oxygen/air mixture. During the procedure, the lumen of the stomach was inflated with air for better visualization. To prevent possible air leakage,

positive end-expiratory pressure (PEEP) 4-6 mmHg was applied. If a hiatal hernia was discovered during preliminary gastroscopy, the patient should be tilted 15 degrees head up and the PEEP level must be increased to 7-10 mmHg in order to depress the diaphragm and reduce the gastro-esophageal junction, so that it can be repositioned into the abdomen. Mean interventional time was 75 min; range was between 47-102 min according to the expertise of the operator. Paracetamol 1 g *iv* was administered for postoperative analgesia and ondansetron 4 mg *iv* for postoperative nausea and vomiting (PONV). Recovery was uneventful in all patients.

In conclusion, SRS™ endoscopic fundoplication for gastro-esophageal reflux disease is a minimally invasive, outpatient procedure, which requires general anesthesia with positive-pressure ventilation. Keeping the patient on PEEP may minimize the pressure gradient between the esophagus and the mediastinum and may help to prevent air from leaking around the screws and causing pneumomediastinum. In patients with hiatal hernia, higher PEEP levels may be required to increase intra-thoracic pressure and force the stomach to slide into the abdomen for ease of endoscopy. It must be kept in mind that higher PEEP levels may be associated with arterial hypotension. We advise smoother emergence from anesthesia, taking precautions for retching, PONV, while coughing and gagging during extubation and PONV may affect the success of the procedure. TIVA with propofol and remifentanil seems to be a good choice for these reasons.

REFERENCES

1 **Nishimura M**, Togawa O, Matsukawa M, Shono T, Ochiai Y, Nakao M, Ishikawa K, Arai S, Kita H. Possibilities of interventional endoscopic ultrasound. *World J Gastrointest Endosc* 2012; **4**: 301-305 [PMID: 22816010 DOI: 10.4253/wjge.v4.i7.301]

2 **Chavalitdhamrong D**, Draganov PV. Endoscopic ultrasound-guided biliary drainage. *World J Gastroenterol* 2012; **18**: 491-497 [PMID: 22363114 DOI: 10.3748/wjg.v18.i6.491]

3 **Jafri SM**, Arora G, Triadafilopoulos G. What is left of the endoscopic antireflux devices? *Curr Opin Gastroenterol* 2009; **25**: 352-357 [PMID: 19342950 DOI: 10.1097/MOG.0b013e32832ad8b4]

P- Reviewer Dahaba A **S- Editor** Wen LL **L- Editor** A **E- Editor** Zhang DN



ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

World J Gastroenterol 2013 January 21; 19(3): 321-430



Baishideng Publishing Group
www.wjgnet.com

World Journal of Gastroenterology

Contents

Weekly Volume 19 Number 3 January 21, 2013

EDITORIAL

321 Clinicopathological characteristics in the differential diagnosis of hepatoid adenocarcinoma: A literature review
Su JS, Chen YT, Wang RC, Wu CY, Lee SW, Lee TY

FIELD OF VISION

328 Alpha-fetoprotein: A controversial prognostic biomarker for small hepatocellular carcinoma
Asrih M, Lenglet S, Mach F, Montecucco F

REVIEW

331 Clinical significance of heterotopic gastric mucosal patch of the proximal esophagus
Chong VH

ORIGINAL ARTICLE

339 Diagnostic value of AFP-L3 and PIVKA-Ⅱ in hepatocellular carcinoma according to total-AFP
Choi JY, Jung SW, Kim HY, Kim M, Kim Y, Kim DG, Oh EJ

BRIEF ARTICLE

347 Epidemiology of perforated peptic ulcer: Age- and gender-adjusted analysis of incidence and mortality
Thorsen K, Søreide JA, Kvaløy JT, Glomsaker T, Søreide K

355 Biliary fistula after treatment for hydatid disease of the liver: When to intervene
Zeybek N, Dede H, Balci D, Coskun AK, Ozerhan IH, Peker S, Peker Y

362 Right recumbent position on gastric emptying of water evidenced by ^{13}C breath testing
Sanaka M, Urita Y, Yamamoto T, Shirai T, Kimura S, Aoyagi H, Kuyama Y

366 Role of surgical resection for multiple hepatocellular carcinomas
Choi SH, Choi GH, Kim SU, Park JY, Joo DJ, Ju MK, Kim MS, Choi JS, Han KH, Kim SI

375 Comparison of concomitant and subsequent cholangiocarcinomas associated with hepatolithiasis: Clinical implications
Lin CC, Lin PY, Chen YL

381 Acupuncture transcutaneous electrical nerve stimulation reduces discomfort associated with barostat-induced rectal distension: A randomized-controlled study
Leung WW, Jones AYM, Ng SSM, Wong CYN, Lee JFY

389 Preoperative carcinoembryonic antibody is predictive of distant metastasis in pathologically T1 colorectal cancer after radical surgery
Lou Z, Meng RG, Zhang W, Yu ED, Fu CG

394 Single-incision *vs* three-port laparoscopic cholecystectomy: Prospective randomized study
Pan MX, Jiang ZS, Cheng Y, Xu XP, Zhang Z, Qin JS, He GL, Xu TC, Zhou CJ, Liu HY, Gao Y

399 *Helicobacter pylori* tumor necrosis factor-α inducing protein promotes cytokine expression *via* nuclear factor-κB
Tang CL, Hao B, Zhang GX, Shi RH, Cheng WF

404 Magnifying chromoendoscopy combined with immunohistochemical staining for early diagnosis of gastric cancer
Meng XM, Zhou Y, Dang T, Tian XY, Kong J

CASE REPORT

411 Gastric ischemia after epinephrine injection in a patient with liver cirrhosis
Kim SY, Han SH, Kim KH, Kim SO, Han SY, Lee SW, Baek YH

415 Primary aortoduodenal fistula: A case report
Alzobydi AH, Guraya SS

418 Emergency caudate lobectomy for ruptured hepatocellular carcinoma with multiple primary cancers
Sun LH, Han HQ, Wang PZ, Tian WJ

422 Primary histiocytic sarcoma of the stomach: A case report with imaging findings
Shen XZ, Liu F, Ni RJ, Wang BY

LETTERS TO THE EDITOR

426 Inflammatory bowel disease: A proposal to facilitate the achievement of an unequivocal diagnosis
Canavese G, Bassotti G, Astegiano M, Castellano I, Cassoni P, Sapino A, Villanacci V

429 Association of inducible nitric oxide synthetase genotype and *Helicobacter pylori* infection gastric cancer risk may be due to faulty primer design
Abadi ATB, Kusters JG

World Journal of Gastroenterology
Volume 19 Number 3 January 21, 2013

Contents

APPENDIX	I-VI	Instructions to authors
ABOUT COVER		Editorial Board Member of *World Journal of Gastroenterology*, Giuseppe Sica, MD, PhD, Associate Professor, Department of Surgery, University Hospital Tor Vergata, Viale Oxford 81, 00133 Rome, Italy

AIMS AND SCOPE

World Journal of Gastroenterology (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. *WJG* was established on October 1, 1995. It is published weekly on the 7th, 14th, 21st, and 28th each month. The *WJG* Editorial Board consists of 1352 experts in gastroenterology and hepatology from 64 countries.

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

INDEXING/ABSTRACTING

World Journal of Gastroenterology is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, Digital Object Identifier, and Directory of Open Access Journals. ISI, Journal Citation Reports®, Gastroenterology and Hepatology, 2011 Impact Factor: 2.471 (32/74); Total Cites: 16951 (7/74); Current Articles: 677 (1/74); and Eigenfactor® Score: 0.06035 (5/74).

FLYLEAF	I-IX	Editorial Board

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Shuai Ma*
Responsible Electronic Editor: *Jun-Yao Li*
Proofing Editor-in-Chief: *Lian-Sheng Ma*
Responsible Science Editor: *Su-Xin Gou*
Proofing Editorial Office Director: *Jin-Lei Wang*

NAME OF JOURNAL
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

EDITOR-IN-CHIEF
Ferruccio Bonino, MD, PhD, Professor of Gastroenterology, Director of Liver and Digestive Disease Division, Department of Internal Medicine, University of Pisa, Director of General Medicine 2 Unit University Hospital of Pisa, Via Roma 67, 56124 Pisa, Italy

Myung-Hwan Kim, MD, PhD, Professor, Head, Department of Gastroenterology, Director, Center for Biliary Diseases, University of Ulsan College of Medicine, Asan Medical Center, 388-1 Pungnap-2dong, Songpa-gu, Seoul 138-736, South Korea

Kjell Öberg, MD, PhD, Professor, Department of Endocrine Oncology, Uppsala University Hospital, SE-751 85 Uppsala, Sweden

Matt D Rutter, MBBS, MD, FRCP, Consultant Gastroenterologist, Senior Lecturer, Director, Tees Bowel Cancer Screening Centre, University Hospital of North Tees, Durham University, Stockton-on-Tees, Cleveland TS19 8PE, United Kingdom

Andrzej S Tarnawski, MD, PhD, DSc (Med), Professor of Medicine, Chief Gastroenterology, VA Long Beach Health Care System, University of California, Irvine, CA, 5901 E. Seventh Str., Long Beach, CA 90822, United States

EDITORIAL OFFICE
Jian-Xia Cheng, Director
Jin-Lei Wang, Vice Director
World Journal of Gastroenterology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Co., Limited
Room 1701, 17/F, Henan Building,
No.90 Jaffe Road, Wanchai, Hong Kong, China
Fax: +852-31158812
Telephone: +852-58042046
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

PUBLICATION DATE
January 21, 2013

COPYRIGHT
© 2013 Baishideng. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT
All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i3.321

World J Gastroenterol 2013 January 21; 19(3): 321-327
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

EDITORIAL

Clinicopathological characteristics in the differential diagnosis of hepatoid adenocarcinoma: A literature review

Jiann-Sheng Su, Yu-Tso Chen, Ren-Ching Wang, Chun-Ying Wu, Shou-Wu Lee, Teng-Yu Lee

Jiann-Sheng Su, Division of Gastroenterology and Hepatology, Kuang Tien General Hospital, Taichung 433, Taiwan
Yu-Tso Chen, Chun-Ying Wu, Shou-Wu Lee, Teng-Yu Lee, Division of Gastroenterology and Hepatology, Taichung Veterans General Hospital, Taichung 407, Taiwan
Ren-Ching Wang, Department of Pathology and Laboratory Medicine, Taichung Veterans General Hospital, Taichung 407, Taiwan
Chun-Ying Wu, Teng-Yu Lee, Graduate Institute of Clinical Medical Science, China Medical University, Taichung 404, Taiwan
Shou-Wu Lee, Teng-Yu Lee, Department of Medicine, Chung Shan Medical University, Taichung 402, Taiwan
Author contributions: Su JS, Chen YT and Lee TY designed this study; Su JS and Chen YT collected data; Wu CY and Lee SW provided executive support for this work; Su JS, Chen YT, Wang RC and Lee TY wrote the manuscript.
Correspondence to: Dr. Teng-Yu Lee, Division of Gastroenterology and Hepatology, Taichung Veterans General Hospital, 160, Section 3, Taichung Harbor Road, Taichung 407, Taiwan. tylee@vghtc.gov.tw
Telephone: +886-4-23592525 Fax: +886-4-23741331
Received: May 7, 2012 Revised: July 16, 2012
Accepted: August 14, 2012
Published online: January 21, 2013

Abstract

Hepatoid adenocarcinoma (HAC) is a rare but important special type of extrahepatic adenocarcinoma with clinicopathological presentation mimicking hepatocellular carcinoma (HCC), and prompt and correct diagnosis can be a challenge, especially in endemic areas with a high incidence of HCC. To date, HAC has only been reported in case series or single case reports, so we aimed to review the clinicopathological characteristics of HAC to obtain a more complete picture of this rare form of extrahepatic adenocarcinoma. All the articles about HAC published from 2001 to 2011 were reviewed, and clinicopathological findings were extracted for analysis. A late middle-aged male with high serum α-fetoprotein and atypical image finding of HCC should raise the suspicion of HAC, and characteristic pathological immunohistochemical stains can help with the differential diagnosis. Novel immunohistochemical markers may be useful to clearly differentiate HAC from HCC. Once metastatic HAC is diagnosed, the primary tumor origin should be identified for adequate treatment. The majority of HAC originates from the stomach, so pan-endoscopy should be arranged first.

© 2013 Baishideng. All rights reserved.

Key words: Hepatocellular carcinoma; Alpha-fetoprotein-producing tumor; Alpha fetoprotein; Gastric adenocarcinoma; Pathology

Su JS, Chen YT, Wang RC, Wu CY, Lee SW, Lee TY. Clinicopathological characteristics in the differential diagnosis of hepatoid adenocarcinoma: A literature review. *World J Gastroenterol* 2013; 19(3): 321-327 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i3/321.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i3.321

INTRODUCTION

Hepatoid adenocarcinoma (HAC) is a rare but important special type of extrahepatic adenocarcinoma with clinicopathological presentation mimicking hepatocellular carcinoma (HCC). HAC was first reported as an α fetoprotein (AFP)-producing tumor in 1970[1], and Ishikura *et al*[2,3], in a report of seven AFP-producing gastric adenocarcinomas (GAC), proposed the term hepatoid adenocarcinoma due to the high AFP levels, ranging from 4730 to 700 000 ng/mL in serum. A serum AFP level higher than 200 ng/mL has been one of the screening criteria for HCC in international guidelines, and HCC may be diagnosed when liver tumors with high serum AFP are found among patients with liver cirrhosis[4,5]. Because of high serum AFP levels among patients with HAC, identification of tumor origin may be difficult, especially when liver tumors aris-

ing from HAC metastasis are initially found. Distinguishing between HAC and HCC is important, especially in areas with a high incidence of chronic hepatitis B, chronic hepatitis C and HCC.

HAC can originate from different organs such as the stomach[6], gallbladder[7], colon[8], lung[9] and urinary bladder[10], and the stomach is the most common origin of tumors in the literature. Finding the primary tumor origin of HAC is crucial, and prompt and adequate treatment may improve outcomes of patients. However, HCC with various extrahepatic metastases such as to the stomach has been reported[11], and determining the tumor origin may become a challenge for physicians, especially when clinicopathological findings of liver tumors are not typical for HCC. Pathological findings in immunohistochemical (IHC) stains may help in the differential diagnosis of HAC, and some specific IHC stains such as cytokeratin (CK)19, AFP, Hep-Par 1 and palate, lung, and nasal epithelium carcinoma-associated protein (PLUNC) have been used in the literature[12]. A systemic literature review regarding differential diagnosis of HAC by IHC stains is lacking, and the IHC presentations of HAC, HCC and common GAC (except HAC) are compared in this review.

The majority of HAC originates from the stomach, and the incidence of gastric HAC among GAC cases was reported to be 0.3%-1% in some recent articles[6,13,14]. The clinical features of gastric HAC are old age, aggressive clinical course, poor survival and frequent metastasis to the liver and lymph nodes, and data have suggested that HAC of the stomach has a poorer prognosis than common GAC[14]. Detection of tumors in the early stage, followed by surgical resection is still considered to be the only way to cure HAC. Because clinical presentations of HAC differ from those of common GAC, studies focusing on gastric HAC may be important in the future, and clinical characteristics may provide useful clues for further research. Moreover, a review of HAC originating from organs other than stomach is still lacking. To date, HAC has only been reported in case series or single case reports, so we aimed to review the clinicopathological characteristics of HAC to obtain a more complete picture of this rare form of extrahepatic adenocarcinoma.

LITERATURE REVIEW

All articles cited in the Medline/PubMed database from January 2001 to December 2011 were searched to identify relevant medical literature, and the search terms included "hepatoid adenocarcinoma", "AFP-producing tumor", "α fetoprotein-producing tumor" and "α fetoprotein-producing gastric cancer". In addition, a manual search of all relevant articles was conducted. All studies with full text were included. The search limits were: (1) type of article: all types; (2) languages: English; (3) species: humans; (4) sexes: both male and female; (5) subsets: all types and fields; (6) ages: all ages; and (7) search field tags: titles. Studies without pathological confirmation of HAC or without clinical data were excluded.

A total of 98 articles were initially identified from the literature search. Among the 98 articles, 32 were excluded because the diagnosis was not confirmed as HAC, and 13 were excluded because the full text was not available. In total, 217 patients with HAC were included in our review. (All 53 reviewed articles are listed in Supplementary References online).

CLINICAL CHARACTERISTICS

The clinical features of HAC cases are summarized in Table 1. Most HAC originated from the stomach (83.9%), and origins other than the stomach, including gallbladder (3.7%), uterus (3.2%), lung (2.3%) and urinary bladder (1.8%), were rare. Moreover, HAC originating from the esophagus and peritoneum was very rare (0.9%), and HACs of the rectum, transverse colon, testis, ovary, jejunum or ureter were only reported in single case report form (< 0.5%). The mean age of HAC patients was 63.0 ± 12.8 years, with a range of 21 to 100 years. Patients with HAC were usually male and the male-to-female ratio was 2.3:1. Most patients had elevated serum AFP (84.8%), with a range from less than 1.0 to 475 000 ng/mL.

When HAC was diagnosed, metastases from original organs were usually found. The most common sites of metastasis were lymph nodes (57.5%), followed by liver (46.3%) and lung (3.4%) metastasis. Liver and gastric tumors were often found simultaneously, and liver tumors could be detected before gastric tumors. As in common gastric cancer, surgical resection was the primary curative treatment in HAC. Most patients received surgical resection (80.2%), even when lymph node or distant metastases were found. In addition, about half of patients (52.1%) received adjuvant chemotherapy following surgical resection. In our literature review, survival data were not reported for all patients, but we were able to analyze data from 125 cases with various tumor origins. The median survival of all HAC patients was 12 mo, and 64 (51.2%) patients died within the first 12 mo.

PATHOLOGICAL CHARACTERISTICS

Hematoxylin and eosin stain

HAC usually showed morphologic similarity to HCC in histology, and polygonal tumor cells were found in hematoxylin and eosin (HE) stains. Polygonal tumor cells proliferating in both trabecular and intestinal-like structures could be found (Figure 1), but definite diagnosis of HAC was difficult only based on findings in histology. Further IHC stains were usually done for differential diagnosis.

Immunohistochemical staining

The major IHC presentations of HAC tumor tissues are depicted in Table 2. Positive AFP stains (Figure 2A) were found in the majority of HAC (91.6%), but positive Hep Par 1 stains were only found in some HAC patients (38.1%) (Figure 2B). In addition, positive carcinoembryonic antigen (CEA) stains were found in most HAC patients (78.7%). Among epithelial markers, all HAC tumors were

Table 1 Clinical characteristics of patients with hepatoid adenocarcinoma in the literature review *n* (%)

	Number	Male (total), *n*	Female (total), *n*	Ratio M:F	Age (range), yr	Elevated serum AFP	Lymph node metastasis	Liver metastasis	Lung metastasis	Other metastasis	Operation	Chemotherapy	Median survival (range), mo
Total	217 (100)	135 (194)	59 (194)	2.3:1	63 (21–100)	78 (84.8)	122 (57.5)	94 (46.3)	7 (3.4)	16 (7.9)	138 (80.2)	49 (52.1)	12 (1.2-66)
Stomach	182 (83.9)	121 (159)	38 (159)	3.2:1	63.3 (28–100)	56 (87.5)	115 (63.9)	84 (48.8)	5 (2.9)	9 (5.2)	107 (77.5)	34 (50)	13 (1.2-66)
Gallbladder	8 (3.7)	2	6	1:03	66.1 (55–76)	3 (75)	2 (28.6)	3 (42.9)	0 (0)	1 (14.3)	7 (100)	2 (28.6)	12 (5-20)
Uterus	7 (3.2)	–	7	–	69.4 (61–86)	7 (100)	2 (28.6)	2 (28.6)	1 (14.3)	1 (14.3)	7 (100)	5 (71.4)	22 (12-36)
Lung	5 (2.3)	4	1	4:01	54.8 (49–68)	2 (60)	2 (40)	1 (20)	NA	2 (40)	5 (100)	2 (50)	15 (2-45)
Urinary bladder	4 (1.8)	4	–	–	70 (61–85)	4 (100)	NA	NA	NA	NA	4 (100)	NA	19 (12-26)
Esophagus	2 (0.9)	1	1	1:01	60 (44–76)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	2 (100)	1 (50)	N/A
Peritoneum	2 (0.9)	1	1	1:01	46.5 (21–72)	1 (50)	1 (50)	1 (50)	0 (0)	0 (0)	1 (50)	2 (100)	6
Retroperitoneum	1 (< 0.5)	1	–	–	47	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)	N/A
Testis	1 (< 0.5)	1	–	–	36	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)	NA	28
Ovary	1 (< 0.5)	–	1	–	59	1 (100)	0 (0)	0 (0)	0 (0)	1 (100)	0 (0)	1 (100)	N/A
Jejunum	1 (< 0.5)	–	1	–	40	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	NA	N/A
Transverse colon	1 (< 0.5)	–	1	–	59	NA	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	N/A
Rectum	1 (< 0.5)	–	1	–	50	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)	NA	N/A
Ureter	1 (< 0.5)	–	1	–	80	NA	0 (0)	1 (100)	0 (0)	1 (100)	1 (100)	0 (0)	N/A

AFP: α-fetoprotein; NA: Not available.

Table 2 Immunohistochemical staining characteristics of patients with hepatoid adenocarcinoma in the literature review *n* (%)

	Number	AFP	Hep Par1	CEA	CK18	CK19	CK20	CK7	AE1/AE3	α1-AT	CD10	GPC3
Total	217 (100)	177 (91.6)	24 (38.1)	37 (78.7)	22 (100)	27 (100)	6 (28.6)	4 (15.4)	12 (92.3)	10 (91)	5 (62.5)	10 (100)
Stomach	182 (83.9)	156 (90.6)	12 (26.7)	27 (75.0)	17 (100)	16 (100)	5 (38.5)	0 (0)	10 (100)	7 (87.5)	0 (0)	10 (100)
Gallbladder	8 (3.7)	5 (83.3)	4 (66.7)	3 (75)	1 (100)	6 (100)	0 (0)	2 (33.3)	1 (100)	1 (100)	3 (100)	NA
Uterus	7 (3.2)	6 (85.7)	NA	1 (100)	NA	NA	NA	NA	NA	NA	1 (100)	NA
Lung	5 (2.3)	4 (100)	0 (0)	1 (100)	1 (100)	1 (100)	1 (50)	2 (0)	NA	NA	1 (100)	NA
Urinary bladder	4 (1.8)	4 (100)	4 (100)	4 (100)	NA	NA	NA	NA	NA	NA	NA	NA
Esophagus	2 (0.9)	2 (100)	1 (50)	NA	NA	NA	NA	NA	NA	2 (100)	NA	NA
Peritoneum	2 (0.9)	1 (50)	1 (100)	NA	1 (100)	NA	0 (0)	NA	1 (50)	NA	NA	NA
Retroperitoneum	1 (< 0.5)	NA	NA	NA	NA	NA	NA	NA	0 (0)	NA	NA	NA
Testis	1 (< 0.5)	1 (100)	0 (0)	NA	NA	NA	NA	NA	NA	NA	NA	NA
Ovary	1 (< 0.5)	1 (100)	NA	1 (100)	NA	NA	NA	NA	NA	NA	NA	NA
Jejunum	1 (< 0.5)	1 (100)	1 (100)	NA	1 (100)	1 (100)	0 (0)	0 (0)	NA	NA	NA	NA
Transverse colon	1 (< 0.5)	NA	NA	NA	NA	1 (100)	NA	NA	NA	NA	NA	NA
Rectum	1 (< 0.5)	1 (100)	1 (100)	NA	1 (100)	1 (100)	0 (0)	0 (0)	NA	NA	NA	NA
Ureter	1 (< 0.5)	1 (100)	NA	NA	NA	1 (100)	0 (0)	NA	NA	NA	0 (0)	NA

CEA: Carcinoembryonic antigen; CK: Cytokeratin; AE1/AE3: Anti-pancytokeratin AE1/AE3; CD10: Cluster of differentiation 10; α1-AT: α1-Antitrypsin; GPC-3: Glypican 3; AFP: α-fetoprotein; NA: Not available.

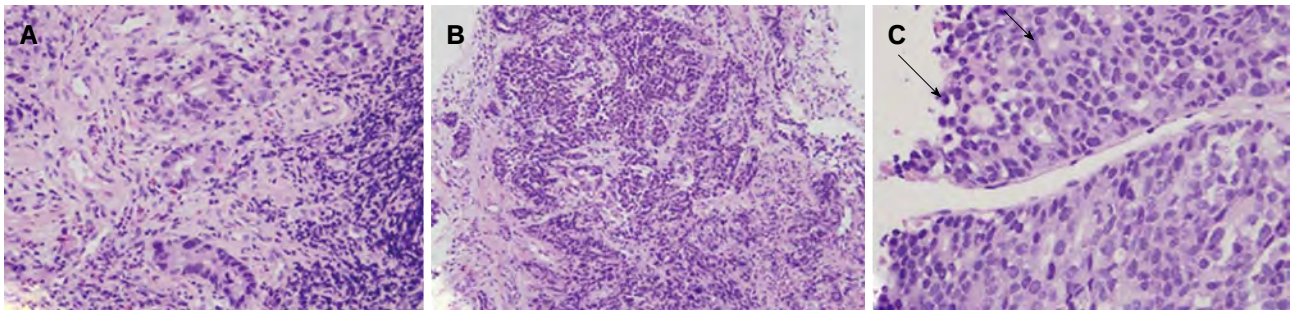


Figure 1 Presentations of hematoxylin and eosin stains: Gastric hepatoid adenocarcinoma. A: Polygonal cells with glandular pattern; B: Trabecular pattern; C: Metastatic liver tumor tissue from gastric hepatoid adenocarcinoma showed focal glandular pattern (arrows).

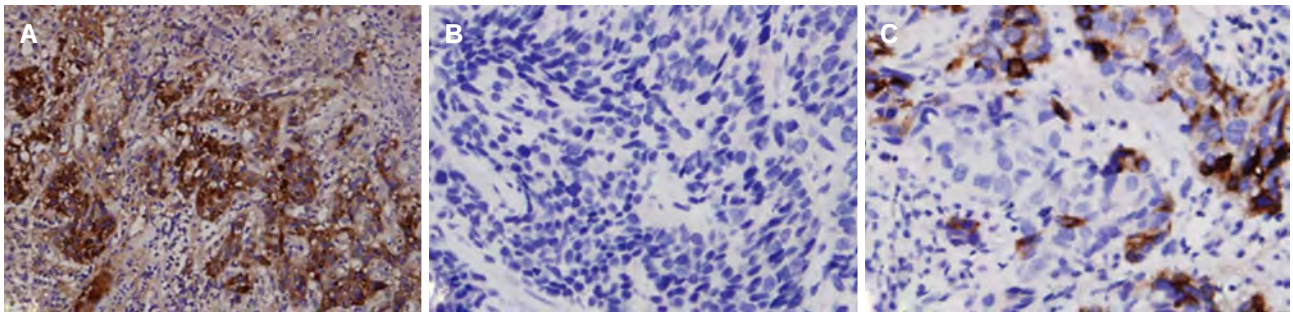


Figure 2 Presentations of immunohistochemical stains: Gastric hepatoid adenocarcinoma. A: Positive finding in α fetoprotein stain; B: Metastatic liver tumor tissue from gastric hepatoid adenocarcinoma showed negative for Hep Par 1 stain; C: Metastatic liver tumor tissue from gastric hepatoid adenocarcinoma showed positive for glypican 3 stain.

positive for both CK18 and CK19 stains (100%), and only a small proportion of HAC patients showed positive stains for CK20 (25%) and CK7 (15.4%). High proportions of HAC tumors were positive for pancytokeratin (AE1/AE3) stain (92.3%) and α1-antitrypsin (α1-AT) stain (91%), and 62.5% of HAC patients were positive for CD10 antigen. In addition, positive glypican 3 (GPC-3) staining (Figure 2C) was found in all HAC patients (100%).

DIFFERENTIAL DIAGNOSIS

Clinical presentation

Although the stomach is the most common origin of HAC, the clinical presentation of HAC may vary greatly depending on the anatomic location of the tumor. Among gastric HAC cases, clinical symptoms were usually non-specific, such as fatigue, weight loss, nausea, poor appetite, emesis, diarrhea, epigastric distress, epigastric mass, abdominal pain or abdominal distension[15-17]. It is difficult to make a differential diagnosis from the clinical symptoms alone.

HAC is usually found in late middle-aged males, and elevated serum AFP is commonly noted among patients with HAC. Due to similar clinicopathological features, HAC may closely resemble and even be indistinguishable from HCC. Serum AFP has long been used for the screening and diagnosis of HCC, and high AFP in HAC may confuse physicians[4,5]. HAC may be incorrectly diagnosed as HCC, especially in endemic areas with a high incidence of HCC. AFP may be elevated in various conditions including HAC, so AFP is not suggested for diagnosis of HCC nowadays[18]. Diagnosis of HCC should be based on dynamic image studies (such as computed tomography or magnetic resonance imaging) and/or liver tumor biopsy, and the typical dynamic image of HCC shows intense arterial uptake followed by "washout" of contrast in the venous and/or delayed phases[18]. Even in liver tumors with elevated AFP, secondary malignancies still should be considered especially when the presentations of imaging studies are atypical, and liver tumor biopsy is recommended for differential diagnosis. In addition, risk factors such as liver cirrhosis, chronic hepatitis B or chronic hepatitis C infection can usually be found among patients with HCC, but those risk factors may not be observed among patients with HAC. Once AFP-producing metastatic liver tumors are found, an intensive search for primary tumor origin should be initiated.

HAC is a very aggressive neoplasm with metastasis in a high proportion of patients at the time of diagnosis. Lymph nodes and the liver are the most common sites of metastasis. Once metastatic HAC is suspected, panendoscopy should be arranged because the stomach is the most common origin of HAC. HCC with metastasis to the stomach has been reported[11], and a clinical challenge of differentiating primary tumor origin arises when liver and gastric tumors are found simultaneously. Pathology markers, especially IHC stains, may provide clues to make a correct diagnosis. In addition, finding the tumor origin may be challenging when no tumor can be detected in the liver and/or stomach, so other organs

Table 3 Comparison of positive rates of commonly-used key immunohistochemical stains in hepatocellular carcinoma, hepatoid adenocarcinoma, and common gastric adenocarcinoma

IHC stains	HCC[1]	HAC[2]	Common GAC[3,4]
AFP	8.2%-37%	92.1%	0%-0.8%
GPC-3	84%	100%	3.4%
Hep Par 1	73%-93%	38.1%	4%-47%
CK19	8.2%-15%	100%	83.6%-89.7%

IHC: Immunohistochemical; HCC: Hepatocellular carcinoma; HAC: Hepatoid adenocarcinoma; GAC: Gastric adenocarcinoma. [1]HCC: AFP stain in 218 cases[16,29,30], GPC-3 stain in 56 cases[30], Hep Par 1 stain in 308 cases[16,29-32] and CK19 stain in 233 cases[16,29,33]; [2]HAC data source: Data based on our review in Table 2; [3]Common GAC data source: AFP stain in 122 cases[34,35], GPC-3 stain in 118 cases[35], Hep Par 1 stain in 108 cases[22,32], CK19 stain in 384 cases[33,36]; [4]Common GAC means GAC except HAC.

such as the gallbladder, urogenital tract, lung and intestine should be carefully checked.

As a result of the high rate of metastasis and a disappointing response to chemotherapy in HAC, poor patient survival can be predicted. Data have suggested that HAC has a poorer prognosis than more common types of tumors[14], and more than half of patients died within the first 12 mo in our literature review. There has been no systemic therapy proven to be effective for HAC until now, but target therapy such as sorafenib is recommended as a standard treatment in HCC with extrahepatic metastasis to prolong patient survival[18].

Pathological presentation

Because HAC bears a striking morphologic similarity to HCC in histology and IHC stains, HCC could be mistakenly diagnosed when liver tumors are the only initial finding, especially in regions with a high incidence of HCC. However, HAC and HCC differ in histologic presentation, and specific findings suggesting HAC may provide important clues. Kodama *et al*[19] described two histologic types of HAC: one was the medullary type, characterized by polygonal cells arranged in a solid nest or sheets, with scattered large pleomorphic or multinucleated giant cells, and the other was well-differentiated papillary or tubular type with clear cytoplasm. However, two types of HAC sometimes coexist in a single tumor. If polygonal tumor cells proliferating in both trabecular and intestinal-like structures are found in HE staining, HAC should be considered (Figure 1).

HAC usually secretes some special chemomediators, so it could be characterized by some IHC stains (Table 2). As an AFP-producing tumor, HAC is commonly > 90% stained for AFP regardless of organ origin (Figure 2A). Although AFP is a characteristic stain for identifying HAC, it can also cause confusion in differentiating HAC from HCC. AFP, a well-known tumor marker of HCC, is usually secreted by HCC and gonadal tumors, and AFP can be positive in tissues of these tumors[20,21]. Hep Par 1, a highly specific marker of HCC, may help in the differential diagnosis between HAC and HCC (Figure 2B), but some HAC tumors can still present with positive staining. In addition, although a low percentage of gastric HAC was stained positive for Hep Par 1 (Table 2), high percentages of non-gastric HAC such as HAC of the gallbladder and urinary bladder were stained positive. However, due to the small case numbers of non-gastric HAC, large cohort studies are needed to confirm this finding. Moreover, other tumors such as adrenal cortical carcinoma, yolk sac tumor, colonic adenocarcinoma, lung carcinoma, ovarian carcinoma and endocervical adenocarcinoma may be occasionally positive for Hep Par 1 stain[22].

The proportion of HAC with a positive CEA stain was high in both gastric (75%) and non-gastric HAC (75%-100%), but it can also be positive in HCC and other metastatic adenocarcinoma of the liver[23] arising from the gastrointestinal tract such as the colon[24,25], stomach[26] and pancreas[27]. Among epithelial markers, AE1/AE3, CK18 and CK19 are usually positive for HAC (92.3%, 100% and 100%, respectively), but the proportions of positive CK20 and CK7 stains are low (25% and 15.4%, respectively). CK7 and CK20 have diagnostic value in differentiating the tumors of unknown primary sites, and epithelial neoplasms with negative staining for CK7 and CK20 include adrenal cortical carcinoma, germ cell tumor, prostate carcinoma, renal cell carcinoma, and HCC[28]. In the differential diagnosis between HAC and HCC for AFP-producing liver tumors, HAC is strongly suggested if AE1/AE3, CK18 and C19 stains show strong positive findings.

Regarding other IHC stains for HAC such as α1-AT, α1-antichemotrypsin, CD10, CDX2, MUC1, PLUNC and GPC-3 have been reported to be promising in the differential diagnosis of HAC[12]. For example, Sentani *et al*[29] reported that positive PLUNC staining was observed in all six gastric HAC, and PLUNC staining was also detected in both liver metastases. Furthermore, PLUNC staining was not observed in 52 cases of HCC, and it was a novel marker that might well distinguish HAC from HCC. However, case numbers in the literature were small (Table 2), and further studies to confirm sensitivity and specificity will be crucial.

In Table 3, we summarize and compare positive rates of commonly-used key IHC stains among HCC, HAC, and common GAC (except HAC) from our literature review[16,29-36] of the most common clinical malignancies needing differential diagnosis. Although the AFP stain can be positive in both HCC and HAC, it is overwhelmingly positive in HAC (91.6%) in our review. In other words, tumors with a negative AFP stain are rare in HAC, and they usually do not indicate HAC. Moreover, AFP staining is almost always negative in common GAC (0%-0.8%) in the literature, and it may be a useful tool to confirm HAC-type GAC. Similar to the AFP stain, the GPC-3 stain can be used in the differential diagnosis because of its low positive rate in common GAC but high positive rate in HAC (Figure 2C). However, although GPC-3 and AFP stains can be positive in both HCC and

HAC, HCC with a negative AFP stain may be confirmed by GPC-3 staining. Hep Par 1 is another important stain with a high positive rate in HCC, but it can also be positive in some HAC (38.1%) in our review. The Hep Par 1 stain is usually negative in common GAC, and liver tumors with a negative Hep Par 1 stain suggest metastasis from other origins such as the stomach. Epithelial marker CK19 stain has an almost 100% positive rate in HAC, and it is also frequently positive in common GAC. However, the positive rate of CK19 in HCC is very low, and HCC is usually not suggested with a positive CK19 stain in liver tumors. In summary, HAC is a special type of extrahepatic adenocarcinoma with pathological presentation mimicking HCC, and HAC should be suspected once polygonal tumor cells proliferating in both trabecular and intestinal-like structures are found in HE staining. There is no single IHC stain that can completely differentiate HAC from HCC, and a panel of IHC stains, such as CK19, PLUNC, Hap-Par 1 and CEA, with detailed clinical history and endoscopic findings are essential for definitive diagnosis.

CONCLUSION

HAC is a rare but important special type of extrahepatic adenocarcinoma with clinicopathological presentation mimicking HCC, and prompt and correct diagnosis can be a challenge, especially in endemic areas with a high incidence of HCC. A late middle-aged male with high serum AFP and atypical image findings of HCC should raise the suspicion of HAC, and characteristic pathological IHC stains can help in differential diagnosis. Novel IHC markers may be useful to clearly differentiate HAC from HCC. Once metastatic HAC is diagnosed, a search for the primary tumor origin should be initiated for adequate treatment. The majority of HAC arise from the stomach, so panendoscopy should be arranged first.

REFERENCES

1 **Bourreille J**, Metayer P, Sauger F, Matray F, Fondimare A. [Existence of alpha feto protein during gastric-origin secondary cancer of the liver]. *Presse Med* 1970; **78**: 1277-1278 [PMID: 5426134]

2 **Ishikura H**, Fukasawa Y, Ogasawara K, Natori T, Tsukada Y, Aizawa M. An AFP-producing gastric carcinoma with features of hepatic differentiation. A case report. *Cancer* 1985; **56**: 840-848 [PMID: 2410093 DOI: 10.1002/1097-0142.]

3 **Ishikura H**, Kirimoto K, Shamoto M, Miyamoto Y, Yamagiwa H, Itoh T, Aizawa M. Hepatoid adenocarcinomas of the stomach. An analysis of seven cases. *Cancer* 1986; **58**: 119-126 [PMID: 2423220]

4 **Bruix J**, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051 DOI: 10.1002/hep.20933]

5 **Bruix J**, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodés J. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; **35**: 421-430 [PMID: 11592607 DOI: 10.1016/S0168-8278(01)00130-1]

6 **Kumashiro Y**, Yao T, Aishima S, Hirahashi M, Nishiyama K, Yamada T, Takayanagi R, Tsuneyoshi M. Hepatoid adenocarcinoma of the stomach: histogenesis and progression in association with intestinal phenotype. *Hum Pathol* 2007; **38**: 857-863 [PMID: 17320150 DOI: 10.1016/j.humpath.2006.10.020]

7 **Gakiopoulou H**, Givalos N, Liapis G, Agrogiannis G, Patsouris E, Delladetsima I. Hepatoid adenocarcinoma of the gallbladder. *Dig Dis Sci* 2007; **52**: 3358-3362 [PMID: 17510803 DOI: 10.1007/s10620-007-9807-3]

8 **Liu Q**, Bannan M, Melamed J, Witkin GB, Nonaka D. Two cases of hepatoid adenocarcinoma of the intestine in association with inflammatory bowel disease. *Histopathology* 2007; **51**: 123-125 [PMID: 17532770 DOI: 10.1111/j.1365-2559.2007.02715.x]

9 **Hayashi Y**, Takanashi Y, Ohsawa H, Ishii H, Nakatani Y. Hepatoid adenocarcinoma in the lung. *Lung Cancer* 2002; **38**: 211-214 [PMID: 12399135 DOI: 10.1016/S0169-5002(02)00214-3]

10 **Lopez-Beltran A**, Luque RJ, Quintero A, Requena MJ, Montironi R. Hepatoid adenocarcinoma of the urinary bladder. *Virchows Arch* 2003; **442**: 381-387 [PMID: 12715173]

11 **Sano T**, Izuishi K, Takebayashi R, Akamoto S, Kakinoki K, Okano K, Masaki T, Suzuki Y. Surgical approach for extrahepatic metastasis of HCC in the abdominal cavity. *Hepatogastroenterology* 2011; **58**: 2067-2070 [PMID: 22024077 DOI: 10.5754/hge11188]

12 **Metzgeroth G**, Ströbel P, Baumbusch T, Reiter A, Hastka J. Hepatoid adenocarcinoma - review of the literature illustrated by a rare case originating in the peritoneal cavity. *Onkologie* 2010; **33**: 263-269 [PMID: 20502062 DOI: 10.1159/000305717]

13 **Nagai E**, Ueyama T, Yao T, Tsuneyoshi M. Hepatoid adenocarcinoma of the stomach. A clinicopathologic and immunohistochemical analysis. *Cancer* 1993; **72**: 1827-1835 [PMID: 7689918 DOI: 10.1002/1097-0142]

14 **Liu X**, Cheng Y, Sheng W, Lu H, Xu X, Xu Y, Long Z, Zhu H, Wang Y. Analysis of clinicopathologic features and prognostic factors in hepatoid adenocarcinoma of the stomach. *Am J Surg Pathol* 2010; **34**: 1465-1471 [PMID: 20871221 DOI: 10.1097/PAS.0b013e3181f0a873]

15 **Inagawa S**, Shimazaki J, Hori M, Yoshimi F, Adachi S, Kawamoto T, Fukao K, Itabashi M. Hepatoid adenocarcinoma of the stomach. *Gastric Cancer* 2001; **4**: 43-52 [PMID: 11706627 DOI: 10.1007/s101200100016]

16 **Terracciano LM**, Glatz K, Mhawech P, Vasei M, Lehmann FS, Vecchione R, Tornillo L. Hepatoid adenocarcinoma with liver metastasis mimicking hepatocellular carcinoma: an immunohistochemical and molecular study of eight cases. *Am J Surg Pathol* 2003; **27**: 1302-1312 [PMID: 14508391 DOI: 10.1097/00000478-200310000-00002]

17 **Caruso RA**, Basile F, Fedele F, Zuccalà V, Crisafulli C, Fracassi MG, Quattrocchi E, Venuti A, Fabiano V. Gastric hepatoid adenocarcinoma with autophagy-related necrosis-like tumor cell death: report of a case. *Ultrastruct Pathol* 2006; **30**: 301-307 [PMID: 16971355 DOI: 10.1080/01913120600820575.]

18 **Bruix J**, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]

19 **Kodama T**, Kameya T, Hirota T, Shimosato Y, Ohkura H, Mukojima T, Kitaoka H. Production of alpha-fetoprotein, normal serum proteins, and human chorionic gonadotropin in stomach cancer: histologic and immunohistochemical analyses of 35 cases. *Cancer* 1981; **48**: 1647-1655 [PMID: 6169423]

20 **Li P**, Wang SS, Liu H, Li N, McNutt MA, Li G, Ding HG. Elevated serum alpha fetoprotein levels promote pathological progression of hepatocellular carcinoma. *World J Gastroenterol* 2011; **17**: 4563-4571 [PMID: 22147961 DOI: 10.3748/wjg.v17.i41.4563]

21 **Hammad A**, Jasnosz KM, Olson PR. Expression of alpha-fetoprotein by ovarian Sertoli-Leydig cell tumors. Case report and review of the literature. *Arch Pathol Lab Med* 1995; **119**: 1075-1079 [PMID: 7487411]

22 **Fan Z**, van de Rijn M, Montgomery K, Rouse RV. Hep par 1 antibody stain for the differential diagnosis of hepatocellular carcinoma: 676 tumors tested using tissue microarrays and conventional tissue sections. *Mod Pathol* 2003; **16**: 137-144 [PMID: 12591966 DOI: 10.1097/01.MP.0000052103.13730.20]

23 **Porcell AI**, De Young BR, Proca DM, Frankel WL. Immunohistochemical analysis of hepatocellular and adenocarcinoma in the liver: MOC31 compares favorably with other putative markers. *Mod Pathol* 2000; **13**: 773-778 [PMID: 10912937 DOI: 10.1038/modpathol.3880134]

24 **Naghibalhossaini F**, Ebadi P. Evidence for CEA release from human colon cancer cells by an endogenous GPI-PLD enzyme. *Cancer Lett* 2006; **234**: 158-167 [PMID: 15893415 DOI: 10.1016/j.canlet.2005.03.028]

25 **Bombski G**, Gasiorowska A, Orszulak-Michalak D, Neneman B, Kotynia J, Strzelczyk J, Janiak A, Malecka-Panas E. Elevated plasma gastrin, CEA, and CA 19-9 levels decrease after colorectal cancer resection. *Int J Colorectal Dis* 2003; **18**: 148-152 [PMID: 12548418]

26 **Pectasides D**, Mylonakis A, Kostopoulou M, Papadopoulou M, Triantafillis D, Varthalitis J, Dimitriades M, Athanassiou A. CEA, CA 19-9, and CA-50 in monitoring gastric carcinoma. *Am J Clin Oncol* 1997; **20**: 348-353 [PMID: 9256887 DOI: 10.1097/00000421-199708000-00005]

27 **Brand RE**, Nolen BM, Zeh HJ, Allen PJ, Eloubeidi MA, Goldberg M, Elton E, Arnoletti JP, Christein JD, Vickers SM, Langmead CJ, Landsittel DP, Whitcomb DC, Grizzle WE, Lokshin AE. Serum biomarker panels for the detection of pancreatic cancer. *Clin Cancer Res* 2011; **17**: 805-816 [PMID: 21325298 DOI: 10.1158/1078-0432.CCR-10-0248]

28 **Chu P**, Wu E, Weiss LM. Cytokeratin 7 and cytokeratin 20 expression in epithelial neoplasms: a survey of 435 cases. *Mod Pathol* 2000; **13**: 962-972 [PMID: 11007036 DOI: 10.1038/modpathol.3880175]

29 **Sentani K**, Oue N, Sakamoto N, Arihiro K, Aoyagi K, Sasaki H, Yasui W. Gene expression profiling with microarray and SAGE identifies PLUNC as a marker for hepatoid adenocarcinoma of the stomach. *Mod Pathol* 2008; **21**: 464-475 [PMID: 18204429 DOI: 10.1038/modpathol.3801050]

30 **Yamauchi N**, Watanabe A, Hishinuma M, Ohashi K, Midorikawa Y, Morishita Y, Niki T, Shibahara J, Mori M, Makuuchi M, Hippo Y, Kodama T, Iwanari H, Aburatani H, Fukayama M. The glypican 3 oncofetal protein is a promising diagnostic marker for hepatocellular carcinoma. *Mod Pathol* 2005; **18**: 1591-1598 [PMID: 15920546 DOI: 10.1038/modpathol.3800436]

31 **Kakar S**, Muir T, Murphy LM, Lloyd RV, Burgart LJ. Immunoreactivity of Hep Par 1 in hepatic and extrahepatic tumors and its correlation with albumin in situ hybridization in hepatocellular carcinoma. *Am J Clin Pathol* 2003; **119**: 361-366 [PMID: 12645337 DOI: 10.1309/8L872RPHEJRKF5JJ]

32 **Lugli A**, Tornillo L, Mirlacher M, Bundi M, Sauter G, Terracciano LM. Hepatocyte paraffin 1 expression in human normal and neoplastic tissues: tissue microarray analysis on 3,940 tissue samples. *Am J Clin Pathol* 2004; **122**: 721-727 [PMID: 15491968 DOI: 10.1309/KC09YTF2M4DLUYQ6]

33 **Lee MJ**, Lee HS, Kim WH, Choi Y, Yang M. Expression of mucins and cytokeratins in primary carcinomas of the digestive system. *Mod Pathol* 2003; **16**: 403-410 [PMID: 12748245 DOI: 10.1097/01.MP.0000067683.84284.66]

34 **Lau SK**, Prakash S, Geller SA, Alsabeh R. Comparative immunohistochemical profile of hepatocellular carcinoma, cholangiocarcinoma, and metastatic adenocarcinoma. *Hum Pathol* 2002; **33**: 1175-1181 [PMID: 12514785 DOI: 10.1053/hupa.2002.130104]

35 **Hishinuma M**, Ohashi KI, Yamauchi N, Kashima T, Uozaki H, Ota S, Kodama T, Aburatani H, Fukayama M. Hepatocellular oncofetal protein, glypican 3 is a sensitive marker for alpha-fetoprotein-producing gastric carcinoma. *Histopathology* 2006; **49**: 479-486 [PMID: 17064293 DOI: 10.1111/j.1365-2559.2006.02522.x]

36 **Kim MA**, Lee HS, Yang HK, Kim WH. Cytokeratin expression profile in gastric carcinomas. *Hum Pathol* 2004; **35**: 576-581 [PMID: 15138932 DOI: 10.1016/j.humpath.2003.12.007]

P- Reviewers Currò G, Zhang JZ, Kobayashi T **S- Editor** Shi ZF
L- Editor Cant MR **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i3.328

World J Gastroenterol 2013 January 21; 19(3): 328-330
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

FIELD OF VISION

Alpha-fetoprotein: A controversial prognostic biomarker for small hepatocellular carcinoma

Mohamed Asrih, Sébastien Lenglet, François Mach, Fabrizio Montecucco

Mohamed Asrih, Sébastien Lenglet, François Mach, Fabrizio Montecucco, Cardiology Division, Department of Medicine, Geneva University Hospital, Foundation for Medical Research, 1211 Geneva, Switzerland
Fabrizio Montecucco, Clinic of Internal Medicine 1, Department of Internal Medicine, University of Genoa, 16126 Genoa, Italy
Author contributions: Asrih M and Montecucco F wrote the paper; Lenglet S, Mach F and Montecucco F approved the final version of the manuscript.
Supported by The Swiss National Science Foundation, No. 32003B-134963/1, to Dr. Montecucco F; EU FP7 AtheroRemo, No. 201668; and Swiss National Science Foundation, No. 310030B-133127, to Dr. Mach F
Correspondence to: Dr. Fabrizio Montecucco, MD, PhD, Cardiology Division, Department of Medicine, Geneva University Hospital, Foundation for Medical Researches, 64 Avenue Roseraie, 1211 Geneva, Switzerland. fabrizio.montecucco@unige.ch
Telephone: +41-22-3827238 Fax: +41-22-3827245
Received: October 15, 2012 Revised: November 23, 2012
Accepted: December 20, 2012
Published online: January 21, 2013

Abstract

The assessment of the prognosis in patients with early hepatocellular carcinoma represents a hot-topic issue that requires further improvements and clarifications. The life expectancy of the patients has been shown to depend on several clinical and histological parameters (such as patient's general conditions, macroscopic tumor morphology and histopathology). Recently, the prognostic role of some biomarkers [i.e., alpha-fetoprotein (AFP)] has been also investigated with controversial findings mainly on the assessment of patient survival. The study by Giannini *et al* failed to show a prognostic value of AFP on survival of patients with well-compensated cirrhosis and small hepatocellular carcinoma. Since the study presents some limitations, a larger clinical trial is needed to clarify the potential prognostic role of serum AFP levels in these patients.

© 2013 Baishideng. All rights reserved.

Key words: Hepatocellular carcinoma; Alpha-fetoprotein; Prognosis; Cirrhosis; Biomarker

Asrih M, Lenglet S, Mach F, Montecucco F. Alpha-fetoprotein: A controversial prognostic biomarker for small hepatocellular carcinoma. *World J Gastroenterol* 2013; 19(3): 328-330 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i3/328.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i3.328

INVITED COMMENTARY ON HOT ARTICLES

The incidence of hepatocellular carcinoma (HCC) has rapidly increased in the last decade to reach the sixth most common cancers worldwide[1]. HCC most often develops in patients with chronic liver disease, usually cirrhosis. Several therapies for HCC have been developed and can be divided into four categories: surgical interventions (tumor resection and liver transplantation), percutaneous interventions (ethanol injection, radiofrequency thermal ablation), transarterial interventions (embolization, chemoperfusion, or chemoembolization) and drugs including gene and immune therapy. However, the diagnosis and treatment of a HCC at an early stage remain the most relevant strategies to improve prognosis[2]. Several pathophysiological biomarkers have been investigated to predict HCC recurrence after liver transplantation[3]. In particular, among different gene, RNA and protein targets, the circulating levels of alpha-fetoprotein (AFP) before the liver transplantation have been recently shown to be independent predictors of HCC recurrence[3]. Firstly described by Abelev *et al*[4], AFP is a large serum glycoprotein, belonging to the class of onco-development protein[5]. It is synthesized by embryonic liver, the fetal intestinal tract and the vitelline sac cells. Similar to albumin, AFP binds and transports a large variety of ligands, such as fatty

acids, retinoids, steroids, heavy metals, dyes, flavonoids, phytoestrogens, dioxin, and various others drugs[6,7]. Previous findings by Toso *et al*[8], showing that a combined score with total tumor volume (TTV) and AFP levels (> 400 ng/mL) was capable of predicting posttransplant survival, further supported the clinical prognostic use of this biomarker in transplanted patients. In this analysis of 6478 adult recipients of an isolated first liver transplant included in the Scientific Registry of Transplant Recipients database, the authors suggested that more traditional Milan criteria might be less efficient especially in patients with large TTV[8]. Despite these promising results, the clinical use of AFP has been also shown to present some important limitations in sensitivity and specificity. In fact, low AFP levels have been described in HCC patients, while high levels might be detected in hepatic cirrhosis without HCC[2]. For instance, Sangiovanni *et al*[9] have found that the level of AFP was increased in only 38% patients developing small HCC nodules. Indeed, AFP might be also useful in detecting other tumors (such as germ cell tumors of the ovary and testis and colorectal cancer)[10,11]. Thus, although currently recommended as a fundamental parameter for HCC screening in patients with cirrhosis[2,12], the prognostic capability of AFP in patients with HCC remains controversial and with important limitations.

The article by Giannini *et al*[13] performed a retrospective analysis from the Italian Liver Cancer group and showed that serum AFP levels did not predict survival in a prospective cohort of 205 patients with well-compensated cirrhosis and small (< 3 cm) hepatocellular carcinoma (Child-Pugh class A and Eastern Cooperative Group Performance Status 0) treated with curative intent. Therefore, accordingly to the Child-Pugh score, which is used to assess the prognostic of the liver disease, and the status that indicates the severity of the disease, the patients for this study exhibit a very early stage of HCC. Interestingly, AFP failed to predict survival also when patients were sub-divided into three groups and analyzed accordingly to their alpha-fetoprotein serum levels (normal: 0-20 ng/mL, n = 116; altered: 21-200 ng/mL, n = 71; diagnostic: > 200 ng/mL, n = 18) at the time of HCC diagnosis. Authors also indicate some potential limitations underlying the study results. AFP levels were within the normal range in a large portion of the cohort, thus confirming the previously discussed limitations in sensitivity of this marker in patients with early HCC and cirrhosis[14]. These low AFP levels also contributed to determine a very low statistical power (22%) to detect differences in survival rates between patient groups. In addition, the stratification from AFP levels revealed a statistically significant difference in male gender between groups. Since AFP levels have been shown to be potentially influenced by the patient gender (increased values in women)[15], this point might represent a critical concern affecting the relevance of the negative results. Finally, potential comorbidities (such as human immunodeficiency virus co-infection previously shown to potentially influence AFP levels) were also not reported[16]. These limitations have been correctly considered by authors, who concluded that the evaluation of more accurate biomarkers (other than AFP) might improve the assessment of prognosis in patients with early HCC. We believe that the study by Giannini *et al*[13] critically confirmed important concerns on strategies targeting a single prognostic biomarker in early HCC. In fact, given several existing confounders and its potential association with HCC nodule size, AFP levels might be more perceptible and relevant in patients with advanced HCC[17]. In particular, the changes from AFP basal levels might be also useful for monitoring the treatment efficacy[18]. In agreement with Giannini *et al*[13], we support a multifactorial approach to improve the prognostic prediction in patients with early HCC. In addition, we would also suggest that larger prospective studies with appropriate statistical power are needed to really evaluate the role of AFP and other biomarkers in these patients.

In conclusion, we believe that the study by Giannini *et al*[13], focused on a hot-topic issue in the hepatological field, importantly contributed to the current debate on the clinical use of serum biomarkers in disease prognosis. Considering the substantial study limitations, larger clinical trials are needed to better evaluate the prognostic role of AFP in early HCC. The matter is opened for future studies and discussion.

REFERENCES

1 **Llovet JM**, Peña CE, Lathia CD, Shan M, Meinhardt G, Bruix J. Plasma biomarkers as predictors of outcome in patients with advanced hepatocellular carcinoma. *Clin Cancer Res* 2012; **18**: 2290-2300 [PMID: 22374331 DOI: 10.1016/S0140-6736(11)61347-0]

2 **Bertino G**, Neri S, Bruno CM, Ardiri AM, Calvagno GS, Malaguarnera M, Toro A, Malaguarnera M, Clementi S, Bertino N, Di Carlo I. Diagnostic and prognostic value of alpha-fetoprotein, des-γ-carboxy prothrombin and squamous cell carcinoma antigen immunoglobulin M complexes in hepatocellular carcinoma. *Minerva Med* 2011; **102**: 363-371 [PMID: 22193346]

3 **Qin LX**, Tang ZY. The prognostic significance of clinical and pathological features in hepatocellular carcinoma. *World J Gastroenterol* 2002; **8**: 193-199 [PMID: 11925590]

4 **Abelev GI**, Perova SD, Khramkova NI, Postnikova ZA, Irlin IS. Production of embryonal alpha-globulin by transplantable mouse hepatomas. *Transplantation* 1963; **1**: 174-180 [PMID: 14010646 DOI: 10.1097/00007890-196301020-00004]

5 **Gillespie JR**, Uversky VN. Structure and function of alpha-fetoprotein: a biophysical overview. *Biochim Biophys Acta* 2000; **1480**: 41-56 [PMID: 11004554 DOI: 10.1016/S0167-4838(00)00104-7]

6 **Arsenov DV**, Golubeva MB, Kisel' MA, Konoplya NA, Lyubin GS, Kuz'mitskii BB, Strel'chenok OA. Modification of humoral immune response in C57Bl/6 mice with a complex of alpha-fetoprotein and retinoid acid derivatives. *Bull Exp Biol Med* 2001; **132**: 963-965 [PMID: 11782793]

7 **Milligan SR**, Khan O, Nash M. Competitive binding of xenobiotic oestrogens to rat alpha-fetoprotein and to sex steroid binding proteins in human and rainbow trout (Oncorhynchus mykiss) plasma. *Gen Comp Endocrinol* 1998; **112**: 89-95 [PMID: 9748407 DOI: 10.1006/gcen.1998.7146]

8 **Toso C**, Asthana S, Bigam DL, Shapiro AM, Kneteman NM. Reassessing selection criteria prior to liver transplantation

for hepatocellular carcinoma utilizing the Scientific Registry of Transplant Recipients database. *Hepatology* 2009; **49**: 832-838 [PMID: 19152426 DOI: 10.1002/hep.22693]

9 **Sangiovanni A**, Del Ninno E, Fasani P, De Fazio C, Ronchi G, Romeo R, Morabito A, De Franchis R, Colombo M. Increased survival of cirrhotic patients with a hepatocellular carcinoma detected during surveillance. *Gastroenterology* 2004; **126**: 1005-1014 [PMID: 15057740 DOI: 10.1053/j.gastro.2003.12.049]

10 **Haibin Z**, Yue J, Yaxian X. Primary yolk sac tumor of the omentum: a case report and literature review. *Eur J Gynaecol Oncol* 2010; **31**: 682-684 [PMID: 21319517]

11 **Nakagawa K**, Koike S, Matsumura H, Yokoi K, Kitamura H. Alpha-fetoprotein producing rectal cancer. *Gan To Kagaku Ryoho* 2012; **39**: 671-674 [PMID: 22504700]

12 **Collier J**, Sherman M. Screening for hepatocellular carcinoma. *Hepatology* 1998; **27**: 273-278 [PMID: 9425947 DOI: 10.1002/hep.510270140]

13 **Giannini EG**, Marenco S, Borgonovo G, Savarino V, Farinati F, Del Poggio P, Rapaccini GL, Anna Di Nolfo M, Benvegnù L, Zoli M, Borzio F, Caturelli E, Chiaramonte M, Trevisani F. Alpha-fetoprotein has no prognostic role in small hepatocellular carcinoma identified during surveillance in compensated cirrhosis. *Hepatology* 2012; **56**: 1371-1379 [PMID: 22535689 DOI: 10.1002/hep.25814]

14 **Sterling RK**, Wright EC, Morgan TR, Seeff LB, Hoefs JC, Di Bisceglie AM, Dienstag JL, Lok AS. Frequency of elevated hepatocellular carcinoma (HCC) biomarkers in patients with advanced hepatitis C. *Am J Gastroenterol* 2012; **107**: 64-74 [PMID: 21931376 DOI: 10.1038/ajg.2011.312]

15 **Li T**, Qin LX, Gong X, Zhou J, Sun HC, Qiu SJ, Ye QH, Wang L, Fan J. Hepatitis B virus surface antigen-negative and hepatitis C virus antibody-negative hepatocellular carcinoma: Clinical characteristics, outcome, and risk factors for early and late intrahepatic recurrence after resection. *Cancer* 2013; **119**: 126-135 [PMID: 22736338 DOI: 10.1002/cncr.27697]

16 **Carrat F**, Bedossa P, Lunel-Fabiani F, Morand P, Pialoux G, Piroth L, Salmon-Céron D, Bani-Sadr F, Perronne C, Cacoub P, Pol S. Serum alpha-fetoprotein predicts virologic response to hepatitis C treatment in HIV coinfected patients. *AIDS* 2008; **22**: 1513-1515 [PMID: 18614875 DOI: 10.1097/QAD.0b013e3282f7370d]

17 **Tandon P**, Garcia-Tsao G. Prognostic indicators in hepatocellular carcinoma: a systematic review of 72 studies. *Liver Int* 2009; **29**: 502-510 [PMID: 19141028 DOI: 10.1111/j.1478-3231.2008.01957.x]

18 **Bang MH**, Van Riep T, Thinh NT, Song le H, Dung TT, Van Truong L, Van Don L, Ky TD, Pan D, Shaheen M, Ghoneum M. Arabinoxylan rice bran (MGN-3) enhances the effects of interventional therapies for the treatment of hepatocellular carcinoma: a three-year randomized clinical trial. *Anticancer Res* 2010; **30**: 5145-5151 [PMID: 21187503]

P- Reviewers Vivarelli M, Andrisani OM, Uversky V
S- Editor Gou SX **L- Editor** A **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i3.331

World J Gastroenterol 2013 January 21; 19(3): 331-338
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

REVIEW

Clinical significance of heterotopic gastric mucosal patch of the proximal esophagus

Vui Heng Chong

Vui Heng Chong, Endoscopy Unit, Department of Medicine, RIPAS Hospital, Bandar Seri Begawan BA 1710, Brunei Darussalam
Author contributions: Chong VH conceived the idea and collated data through literature search, drafted the article for intellectual content and approved the final version.
Correspondence to: Vui Heng Chong, MD, FAMS, FRCP, Endoscopy Unit, Department of Medicine, RIPAS Hospital, Bandar Seri Begawan BA 1710,
Brunei Darussalam. chongvuih@yahoo.co.uk
Telephone: +673-2-242424 Fax: +673-2-242690
Received: May 23, 2012 Revised: July 30, 2012
Accepted: August 16, 2012
Published online: January 21, 2013

Abstract

Heterotopic gastric mucosa of the proximal esophagus (HGMPE), also referred to as "inlet patch" or "cervical inlet patch", is a salmon colored patch that is usually located just distal to the upper esophageal sphincter. HGMPE is uncommon with endoscopic studies reporting a prevalence ranging from less than one percent to 18%. Most HGMPE are asymptomatic and are detected incidentally during endoscopy for evaluations of other gastrointestinal complaints. Most consider HGMPE as clinically irrelevant entity. The clinical significance of HGMPE is mainly acid related or neoplastic transformation. The reported prevalence of laryngopharyngeal reflux symptoms varies from less than 20% to as high as 73.1%. However, most of these symptoms are mild. Clinically significant acid related complications such as bleeding, ulcerations, structure and fistulization have been reported. Although rare, dysplastic changes and malignancies in association with HGMPE have also been reported. Associations with Barrett's esophagus have also been reported but the findings so far have been conflicting. There are still many areas that are unknown or not well understood and these include the natural history of HGMPE, risk factors for complications, role of *Helicobacter pylori* infection and factors associated with malignant transformations. Follow-up may need to be considered for patients with complications of HGMPE and surveillance if biopsies show intestinal metaplasia or dysplastic changes. Despite the overall low incidence of clinically relevant manifestations reported in the literature, HGMPE is a clinically significant entity but further researches are required to better understand its clinical significance.

© 2013 Baishideng. All rights reserved.

Key words: Cervical inlet patch; Laryngopharyngeal reflux; Globus pharyngeus; Neoplasms; Barrett's esophagus

Chong VH. Clinical significance of heterotopic gastric mucosal patch of the proximal esophagus. *World J Gastroenterol* 2013; 19(3): 331-338 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i3/331.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i3.331

INTRODUCTION

Heterotopic gastric mucosa of the esophagus (HGMPE), also commonly referred to as "inlet patch" or "cervical inlet patch", is an island of ectopic gastric mucosa that is found in the proximal esophagus[1,2]. Rarely, they can also been found in the other part of the esophagus[2,3]. HGMPE is widely considered to be congenital in nature. However, it has also been proposed to be an acquired condition[1,4,5].

The reported incidence varies from less than one percent[6] to 13.8% in endoscopic studies[7]. Autopsy study has reported higher incidence of up to 70%[1,2]. Commonly reported symptoms include laryngopharyngeal reflux (LPR) symptoms and the prevalence has been reported to be as high as 73.1%[8]. However, most of these symptoms are mild and the management depends on the severity of symptoms. A clinico-pathologic classification has been proposed which categorized HGMPE into five distinct groups based on their clinical, endoscopic and

histological characteristics (Table 1)[1]. Serious and significant complications of HGMPE have been reported in both adults and the pediatric population[1,2,9]. Interestingly, other common upper aero-digestive disorders have also been linked to HGMPE[2,9]. The clinical significance of HGMPE remains debated due to the limited number of publications. This article reviews the literature and discusses the clinical manifestations and significance of HGMPE in clinical practice.

Table 1 Clinico-pathological classification for heterotopic gastric mucosa of the proximal esophagus

Category	Description	Symptoms/findings
I	Asymptomatic	None
II	Symptomatic	Laryngopharyngeal reflux
III	Symptomatic with benign complications	Strictures/webs/fistula/bleeding
IV	Intra-epithelial dysplasia	None/non-specific
V	Malignant transformation	Asymptomatic/dysphagia

BACKGROUND

Pathogenesis

Currently, there are three proposed theories on the development of HGMPE[1,2,4,5]. The most widely accepted theory is that HGMPE is congenital in origin. During the development of the esophagus and at 24 wk of gestation (equivalent to 90 mm crown rump length during fetal development), squamous lining replaces the columnar lining starting from the mid esophagus moving in both directions. The proximal esophagus is usually the last part to get stratification and this account for the common finding of heterotopic gastric mucosa in the proximal esophagus[1,2]. Although less common, ectopic mucosa can also be found in the other parts including the distal esophagus[2,3]. Another proposed theory involves metaplastic transformation of the squamous lining to columnar from chronic acid injury as seen in Barrett's esophagus[4]. Proliferation of remnant pluripotent cells as result of acid injury has been proposed as the underlying pathogenesis. The third theory involves rupture of proximal esophageal retention cystic glands[5].

The most common histological type is the cardia or oxyntic type mucosa followed by the antral mucosa. Depending on the mucosa type, some HGMPE can produce acid[10-12], and given their close proximity to the laryngopharyngeal complex can easily reflux into this area to cause symptoms. The laryngeal mucosa is also particularly susceptible to acid injury, even with mildly acidic secretion. Non-acidic secretions such as mucus have also been shown to induce laryngopharyngeal symptoms[13].

HGMPE can be colonized by *Helicobacter pylori* (*H. pylori*) and the prevalence have been reported to be as high as 82%[14]. The infection rates probably correlate with the prevalence of *H. pylori* infection in the general population. Although the role of *H. pylori* in HGMPE remains uncertain, one can probably surmise that it can cause changes similar to those seen in the stomach. Inflammatory and histological changes such as atrophy, metaplasia, dysplasia and carcinoma of the HGMPE have also been reported[2,15].

Endoscopic characteristics

HGMPE is often missed during endoscopy as the proximal esophagus is often neglected or briefly examined during routine endoscopic examination. On endoscopy, HGMPE appears as a salmon colored ovoid or round patch that is usually distinct from the surrounding esophageal squamous mucosa (Figure 1A and B). It can be flat, elevated or depressed and the surface can be smooth or nodular. Most HGMPE are located on the lateral walls typically a few centimetres distal to the upper esophageal sphincter (16 and 21 cm from the incisors)[1,2]. The sizes can vary from microscopic to as large as three to five centimeters. Most patients have a single patch while those with multiple patches, the patches tend to be small and are usually found within close proximity of other patches. On specialized imaging such as narrow band imaging, the HGMPE is clearly more visible (Figure 1C).

CLINICAL MANIFESTATIONS

The clinical manifestations of HGMPE can be broadly divided into non-neoplastic and neoplastic. The majority of patients found to have HGMPE are asymptomatic and the HGMPE are detected incidentally during evaluation for other gastrointestinal complaints. These patients are categorized to have type I HGMPE. The non-neoplastic manifestations (type II and III) such as LPR symptoms and strictures and bleeding are probably related to the acid produced by the patch. The least common manifestations are the histological or neoplastic changes (type IV and V)[1,2]. With the exception of malignancy in the pediatric population, all these have been reported both in the adult[1,2] and pediatric population[16-20]. Acid related symptoms are often seen in younger patients whereas neoplastic manifestations have been reported mainly in the elderly population.

Acid-related manifestations

Symptoms associated with HGMPE include LPR symptoms such as regurgitation, dysphagia, hoarseness, globus, throat discomfort and chronic cough[1,2]. Often dysphagia is located in the proximal esophagus or throat level. Associations with chronic ear or sinus problem have also been reported.

Symptoms in adult population

To date, there have only been a few studies that have reported comparisons of symptoms between patients with and without HGMPE. Published studies have so far reported mixed findings, some showing significantly higher LPR symptoms[8,21-24] whilst others reported no significant difference[15,25] (Table 2). The symptoms enquired and reported were not consistent between studies. Some reported the overall prevalence or limited to only

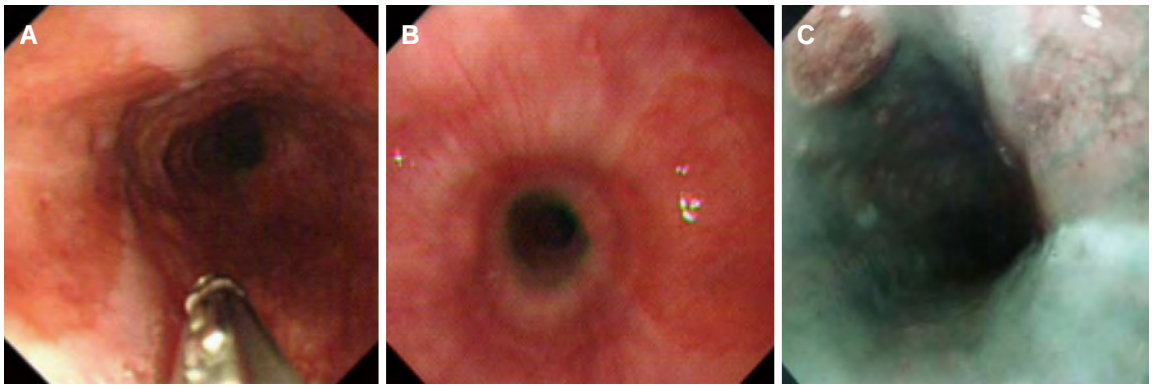


Figure 1 Endoscopic images of heterotopic gastric mucosa of the proximal esophagus. A: A large heterotopic gastric mucosa of the proximal esophagus (HGMPE) on the left lateral wall; B: A large HGMPE located on the right lateral wall; C: Small patch with a brownish hue located on the left lateral wall on neutral beam injection.

Table 2 Prevalence of symptoms reported to be associated with heterotopic gastric mucosa of the proximal esophagus in adult

Ref.	Symptoms reported	Prevalence of LPR symptoms (%)		*P* value
		HGMPE (+ve)	HGMPE (-ve)	
Chong *et al*[8]	Overall LPR symptoms	73.1	25.9	< 0.001
	Chronic cough	29.2	10.6	< 0.01
	Sore throat/hoarseness	54.2	11.7	< 0.01
	Globus	23.1	7.1	< 0.01
	Regurgitation	42.3	13.1	< 0.01
	Heartburn	50.0	22.5	< 0.01
Akbayir *et al*[15]	Upper esophageal and laryngopharyngeal symptoms	45	21.5	= 0.07
Baudet *et al*[21]	Dysphagia	21	4.0	< 0.001
Poyrazoglu *et al*[22]	Dysphagia	39.4	0	< 0.05
Alagozlu *et al*[23]	Globus	78.6	0	< 0.05
	Dyspepsia	88.2	97.8	NS
[1]Neumann *et al*[24]	Dysphagia/odynophagia	20.8	16.4	< 0.001
	Upper respiratory symptoms	2.5	0.9	< 0.001
	Globus	1.6	0.3	< 0.001
Weickert *et al*[25]	Recurrent hoarseness	9.1	5.6	= 0.5
	Dysphagia (any grade upper and lower)	15.2	9.4	NS
	Heartburn (any grade upper and lower)	15.2	9.4	NS

[1]In the study by Neumann *et al*, the symptoms presented are symptom recorded as indications for endoscopy. HGMPE: Heterotopic gastric mucosa of the proximal esophagus; LPR: Laryngopharyngeal reflux; NS: Not significant.

one or few symptoms. However, most of the symptoms reported were mild. Prevalence rate of LPR symptoms as high as 73.1% has been reported[8]. The largest study to date based on a database of 487 229 endoscopies carried out in non-referral centers in the United States showed that dysphagia or odynphagia, upper respiration symptoms suggestive of LPR and globus were significantly more common in patients with HGMPE[24]. However, this study was limited by its retrospective nature and the assessment of only referral indications. The other studies were mainly from referral or specialized centers.

Symptoms in pediatric population

Currently, there are only a few studies on HGMPE in the pediatric population and are usually limited by small sample sizes[9,16,18-20]. Most are in the form of case reports[16-20]. To date, there is no endoscopic study that has assessed HGMPE in this population. Clinical manifestations are different from the adult population. Common manifestations include laryngospasm[16], respiratory symptoms[17] and dysphagia from reflux or stricture[9,20]. In one multicentre study that had looked at 15 patients with a median age 9.5 years (range: 3.3-15 years) found that five patients had esophageal atresia, nine with gastroesophageal reflux disease (GERD) and asymptomatic in one. Dysphagia and food impaction were the most common manifestations reported in 14 patients. Six patients had respiratory or ear, nose, and throat symptoms[9]. An autopsy study in the pediatric population showed that HGMPE was more common in younger age and was more significantly associated with unexplained death. Variend *et al*[26] speculated that pulmonary aspiration of esophageal contents may have been the cause of death in some of the children.

Barrett's esophagus

Similarly to clinical symptoms, the association between

Table 3 Endoscopic studies reporting the associations between heterotopic gastric mucosa of the proximal esophagus and other endoscopic findings with special attention to Barrett's esophagus *n* (%)

Ref.	Prevalence of HGMPE	Findings
Positive association		
Avidan *et al*[4]	53 (1.1)	Significantly more reflux esophagitis (77 *vs* 50, $P = 0.023$), Barrett's esophagus (34 *vs* 9, $P < 0.001$), hiatus hernia (49 *vs* 30, $P < 0.05$) and gastric ulcer ($P < 0.05$) On multivariate analysis, hiatus hernia, gastric ulcer and Barrett's esophagus remained significant
Alagozlu *et al*[23]	68 (1)	Significantly more ($P < 0.05$) endoscopic Barrett's esophagus in patients with HGM (13.2 *vs* 2.4) but not with reflux esophagitis (10.3 *vs* 9.5) Hiatus hernia and duodenal ulcer were reported in 13.2% and 10.3% respectively but no comparisons were made
[1]Neumann *et al*[24]	870 (0.18)	Significantly more Barrett's mucosa on biopsy (9.7 *vs* 6.5, $P < 0.001$), adenocarcinoma arising from Barrett's mucosa (3.6 *vs* 0.7, $P < 0.01$) and reflux esophagitis (41.8 *vs* 49.7, $P < 0.001$)
Yuksel *et al*[27]	171 (1.8)	Significantly more reflux esophagitis (25.1 *vs* 5.6, $P < 0.001$) and histologically proven Barrett's esophagus (3.5 *vs* 0.5, $P < 0.000$) No difference in hiatus hernia
No association		
Borhan-Monesh *et al*[3]	64 (10)	No significant difference (all P = NS) between reflux esophagitis (34.3 *vs* 38.1) and Barrett's esophagus
Chong *et al*[8]	26 (5.6)	No significant difference (all P = NS) between esophageal, gastric and duodenal findings including Barrett's esophagus (3.8 *vs* 3.7), hiatus hernia (15.4 *vs* 12.2) and ulcers
Akbayir *et al*[15]	11 (1.67)	No significant difference (all P = NS): Barrett's esophagus (0 *vs* 0.9), hiatus hernia (0 *vs* 10), reflux esophagitis (27 *vs* 16) and duodenal ulcer (9 *vs* 7)
[2]Poyrazoglu *et al*[22]	33 (3.6)	No significant difference (all P = NS): Barrett's esophagus (0 *vs* 0.8), hiatus hernia (3 *vs* 9.1), reflux esophagitis (36.4 *vs* 34.8), gastric ulcer (3 *vs* 3) and duodenal ulcer (6.1 *vs* 6.8)
Weickert *et al*[25]	33 (11)	Overall prevalence: hiatus hernia (n = 92, 30.7%), reflux esophagitis (n = 41, 13.7%), Barrett's esophagus (n = 3, 1%), gastric ulcer (n = 24, 8%) and duodenal ulcer (n = 22, 7%), all P = NS
Jacobs *et al*[28]	33 (4.9)	Significant difference for reflux esophagitis (27.3 *vs* 11.4) but not for hiatus hernia (15.2 *vs* 12.5), Barrett's esophagus (6.1 *vs* 1.1) and any gastric or duodenal ulcer (15.2 *vs* 6.1)

Studies included are those with more than 10 patients with heterotopic gastric mucosa of the proximal esophagus (HGMPE). [1]The study by Neumann *et al* was based on analysis clinic-pathologic records of patients who had undergone upper gastrointestinal endoscopy with biopsies between January 2008 and December 2010 in private outpatient clinics and endoscopy centres in the United States; [2]The study by Poyrazoglu *et al* compared the prevalence of other endoscopic findings between 33 patients with HGMPE with 132 matched controls without HGMPE. NS: Not significant.

HGMPE and Barrett's esophagus remains debated. Studies have reported conflicting results (Table 3). Avidan *et al*[4] showed that patients with HGMPE had up to fivefold higher risk of Barrett's esophagus compared to patients without HGMPE. Three other studies[23,24,27] have reported significant positive correlations while five studies that included more than 20 patients with HGMPE have not found any correlations[3,8,22,25,28]. Two histological studies reported similar mucin staining patterns in Barrett's esophagus and HGMPE suggesting possible shared pathogenesis[29,30]. However, Fuerle *et al*[31] found some differences and postulated that the specialized columnar epithelium of Barrett's esophagus originates from a very immature multipotent gastrointestinal stem cell whereas HGMPE originates from remnant embryonic gastric mucosa located in the proximal esophagus

Non-laryngopharyngeal symptoms

Reports on symptoms associated with HGMPE are mainly on LPR symptoms[2]. There is currently little information to suggest whether HGMPE is associated with non-LPR symptoms. Heartburn has been shown to be common in patients with HGMPE, even in those not found to have any erosive esophagitis[4,8,32]. Explanations offered included symptoms induced by acid secretion flowing downward or increased sensitivity as seen in non-erosive esophagitis. Apart from proximal acid reflux independent of distal acid reflux, Korkut *et al*[11] also showed that some patients with HGMPE have signs of esophageal motor dysfunction based on manometry and 24 h dual probe pH study. They concluded that these abnormalities may be responsible for some of the symptoms experienced by patients with HGMPE. In another study, Rosztoczy *et al*[32] reported several differences between patients with and without HGMPE. Patients with HGMPE had more and prolonged acid exposure in both proximal and distal esophagus, longer bile exposure time in the distal esophagus, reduced lower esophageal sphincter pressure with prolonged relaxation and decreased peristaltic wave amplitude. There was also increased number of simultaneous contractions in the esophageal body of patients with HGMPE. These suggest that patients with HGMPE also have motility disorders contributing to their symptoms.

Other non-neoplastic complications

Complications of HGMPE reported in the literature range from formations of strictures[33-35], web[36-38], ulceration with bleeding[39], fistula formation with or without subcutaneous abscesses[19,40-42], perforation[43,44] and polyps[45-47] (Table 4). HGMPE have been reported to be the possible cause of Plummer Vinson or Paterson Kelly Brown syndrome[1,2,38]. The underlying pathogenesis is probably similar to those seen in acid-related gastric pathologies. In the pediatric population, HGMPE has also been associated with unexplained infant deaths[26], dysphagia from strictures in the

Table 4 Complications of heterotopic gastric mucosa of the proximal esophagus reported in the literature

Clinico-pathological classification	Conditions	Status	Numbers based on PubMed literature search
Type Ⅲ	Stricture	Reported	6
	Web	Reported	4
	Bleeding	Reported	1
	Fistula	Reported	4
	Perforation	Reported	2
	Polyp[1]	Reported	4
Type Ⅳ[2]	Dysplasia		
	Low grade	Reported	None
	High grade	Reported	3[32-34]
Type Ⅴ[2]	Adenocarcinoma		
	Early (pT1 tumor)	Reported	13[35-48]
	Advanced	Reported	19[33,49-54]

References for type Ⅲ cases reported in the literature not included in the reference list. [1]Not included in the original classification proposed by von Rahden *et al*[1]; [2]Number of cases of neoplastic transformation identified through literature searches up till December 2011.

proximal esophagus[9,18,20] or spasm[16] and recurrent neck abscesses secondary to fistula[19].

Histology progressions or neoplastic transformation

Overall, significant histological non-malignant changes or malignancies in HGMPE are extremely rare. Histological changes such as chronic inflammation[3,14], atrophy, intestinal metaplasia[14], and dysplasia[48-50] as seen in the stomach have been reported mainly in the adult population. However intestinal metaplasia has also been reported in children[17]. Macha *et al*[17] reported that 8.3% (n = 2/24) of their pediatric patients with HGMPE had intestinal metaplasia detected on histology. Neoplastic transformations have only been reported in the adult population[2,36-39]. A summary of the reported significant histological or malignancies in association with HGMPE are shown in Table 4.

Since the first case reported by Carrie *et al*[51] in 1950, there have only been 43 cases of adenocarcinoma[52-54] in association with HGMPE reported in the literature to date (Table 4). To date, Neumann *et al*[24] in the largest series of endoscopies done in non-specialised centers did not encounter any cases of HGMPE with malignancy whereas Alagozlu *et al*[23] encountered a case each of adenocarcinoma and low grade dysplasia among 64 patients with HGMPE. Based on these two studies, it can be estimated that the incidence of malignancies among patients with HGMPE ranges between 0 and 1.56%[23,24]. However, the incidence is likely to be much lower than the estimate from the latter study.

Based on a review of 43 cases reported in the literatures by Akanamu *et al*[54], majority of those affected were men (88.4%) with a median age of 60.4 years (range 35 to 85 years). Most of the lesions were advanced at diagnosis especially in the earlier reported. Early lesions (T1 lesion) accounted for 14 cases[54]. The lesions were ulcerated in 31.2%, protruding in 30.2% and polypoidal in 18.6%. Dysphagia was the most common compliant (74.4%) with smaller proportion detected through surveillance. Interestingly, acid-related symptoms were not common. Smoking appeared to be a risk factor.

Associations with extra-esophageal neoplasms

To date, association with extra-esophageal neoplasms have only been reported twice[55,56]. Basseri *et al*[55] reported the case of a 22-year-old lady with asthma and GERD diagnosed with laryngeal adenocarcinoma. She later developed a stricture proximal to a HGMPE (type Ⅲ). In another report, Satoh *et al*[56] reported the case of a 44-year-old lady with hypopharyngeal carcinoma with a HGMPE (2.0 cm × 1.5 cm) bordering the tumor. Chronic irritation from acid was proposed as the contributing cause to the development of the tumors.

OVERVIEW

HGMPE was first reported by Schmidt *et al*[1] as an aberrant gastric fundus-type epithelium located in the proximal esophagus. Despite this, the number of publications on this entity has remained low compared to the other esophageal disorders. Therefore it is not surprising that controversies remain regarding the clinical significance of this entity. Despite the lack of data, HGMPE is clinically significant and the clinical significance is related to acid-related manifestations, mucosal changes and malignant transformations.

The clinical manifestations of HGMPE are best summarized by the classification proposed by von Rahden *et al*[1]. Clinically significant manifestations which have been reported in only a small proportion of patients are categorized as type Ⅱ, Ⅲ, Ⅳ and Ⅴ HGMPE. The most common is the type Ⅱ with clinical symptoms of LPR[1,8]. However, some do not consider type Ⅱ HGMPE as significant as the symptoms experienced are mostly mild[8,13]. On the other hand, these symptoms are chronic and very troublesome for a small minority of patients.

It is currently uncertain if the symptoms are acid-related given that studies have shown that only a small proportion of symptomatic patient have documented acid secretion from the HGMPE[10,31]. However, one can probably surmise that there is a causal association. Similar to the stomach or heterotopic gastric mucosa found in Meckel's diverticulum, excess acid production can lead to acid-related symptoms and complications. Patients with HGMPE have also been shown to have motility disorders and this may contribute to the symptomology of HGMPE[11,32].

Symptomatic patients with HGMPE typically reports LPR symptoms; globus pharyngeus, sore throat, hoarseness, chronic cough, throat clearing and dysphagia[57]. This is not surprising given the close proximity of HGMPE with the laryngopharyngeal complex. The laryngopharyngeal complex is particularly sensitive and even a small amount of weakly acidic secretion can cause symptoms[57,58]. Symptoms can also occur with non-acidic secretions[4]. Presence of gastric enzymes such as pepsin

also contributes to irritations[58].

The prevalence of the various LPR symptoms in association with HGMPE varies from less than 20% to as high as 73.1%[2]. Comparisons of published studies are difficult due to differences in the study methodologies. Symptoms enquired were not always consistent with some studies enquiring on limited number of symptoms or not specifying the symptoms enquired. Furthermore, most studies had small sample sizes.

In clinical practice, we also often encounter patients with LPR symptoms without any heartburn. Such patients are categorized as having extra-esophageal manifestations of GERD. Some of these patients clearly do not have acid exposure in the distal esophagus and as such, LPR symptoms in association with HGMPE should be considered a separate entity separate from GERD.

Associations with neoplastic changes and importantly malignant transformation are reported and these represent the most significant associations of HGMPE. However, the numbers reported is still very low and are considered extremely rare. Interestingly, a large number of the reported cases especially in the non-English literatures have been from Japan[54]. Whether, HGMPE with malignant transformations is more common in the Japanese population is unknown. It may be related to better screening program and increased detection rate. Most of the reported cases were advanced at diagnosis especially in the earlier studies. However, there were more early cancers (T1 lesions) reported in the latter part. Men seem to be more at risk and smoking appears to be a risk factor[54]. Dysphagia was the main complaint leading to detection of tumors.

Association with Barrett's esophagus is another area that remains debated. A widely accepted theory is based on a shared pathogenesis with both Barrett's esophagus and HGMPE being congenital in origin but with differences in pathogenesis in the later part of the development. However, this had been based on western population where the prevalence of Barrett's esophagus is much higher compared to the East. Further large population studies will be required and help shade some lights into this controversy. Associations with other acid related complications such as erosions, ulcerations, bleeding, perforation, fistula and stricture formations have been documented[1,8]. Possible link between HGMPE and unexplained death in children have also been reported[26].

Currently, there are still many issues regarding HGMPE that remain unresolved or unknown. These ranges from the origin of HGMPE, exact correlation with Barrett's esophagus, role of *H. pylori* infection in symptoms manifestations, lack of natural history data and significance of mucosal changes in the HGMPE and its relation to neoplastic transformations.

Given that HGMPE mainly manifests with upper autodigestive symptoms, it is not unexpected that only clinicians from certain specialties such as the gastroenterologists, otorhinolaryngologists and rarely upper gastrointestinal surgeons will encounter such patients. However, it is important that other clinicians from the other specialties to be aware of this entity especially when they come across patients with troublesome LPR symptoms. Establishing a diagnosis is important as it can provide reassurance. This may also lead to symptoms improvement and avoid any further unnecessary investigations or consultations. As the awareness of HGMPE increase, more asymptomatic or symptomatic cases will be diagnosed and with the ageing population, it is very likely the number of cases of neoplastic transformations will also increase, albeit a small increase.

CONCLUSION

The clinical significance of HGMPE lies mainly with its capacity to produce acid and mucosal progressions to dysplasia and frank neoplastic transformation. Currently, there are still many areas of HGMPE that are not well understood and further research are required. Symptomatic patients should to be treated and those found to have metaplasia or dysplasia may need to be considered for surveillance.

REFERENCES

1 **von Rahden BH**, Stein HJ, Becker K, Liebermann-Meffert D, Siewert JR. Heterotopic gastric mucosa of the esophagus: literature-review and proposal of a clinicopathologic classification. *Am J Gastroenterol* 2004; **99**: 543-551 [PMID: 15056100 DOI: 10.1111/j.1572-0241.2004.04082.x]

2 **Chong VH**. Heterotopic gastric mucosal patch of the proximal esophagus. In: Pascu O, editor. Gastrointestinal Endoscopy. Croatia: InTech Publishing, 2011: 125-148

3 **Borhan-Manesh F**, Farnum JB. Incidence of heterotopic gastric mucosa in the upper oesophagus. *Gut* 1991; **32**: 968-972 [PMID: 1916499 DOI: 10.1136/gut.32.9.968]

4 **Avidan B**, Sonnenberg A, Chejfec G, Schnell TG, Sontag SJ. Is there a link between cervical inlet patch and Barrett's esophagus? *Gastrointest Endosc* 2001; **53**: 717-721 [PMID: 11375577 DOI: 10.1067/mge.2001.114782]

5 **Meining A**, Bajbouj M. Erupted cysts in the cervical esophagus result in gastric inlet patches. *Gastrointest Endosc* 2010; **72**: 603-605 [PMID: 20801291 DOI: 10.1016/j.gie.2010.05.008]

6 **Maconi G**, Pace F, Vago L, Carsana L, Bargiggia S, Bianchi Porro G. Prevalence and clinical features of heterotopic gastric mucosa in the upper oesophagus (inlet patch). *Eur J Gastroenterol Hepatol* 2000; **12**: 745-749 [PMID: 10929900]

7 **Ohara M**. Incidence of heteroptopic gastric mucosa in the upper esophagus in first time narrow banding image endoscopy of consecutive 900 patients. *Gastrointest Endosc* 2010; **71**: AB316-317

8 **Chong VH**, Jalihal A. Heterotopic gastric mucosal patch of the esophagus is associated with higher prevalence of laryngopharyngeal reflux symptoms. *Eur Arch Otorhinolaryngol* 2010; **267**: 1793-1799 [PMID: 20437050 DOI: 10.1007/s00405-010-1259-2]

9 **Georges A**, Coopman S, Rebeuh J, Molitor G, Rebouissoux L, Dabadie A, Kalach N, Lachaux A, Michaud L. Inlet patch: clinical presentation and outcome in children. *J Pediatr Gastroenterol Nutr* 2011; **52**: 419-423 [PMID: 21240021 DOI: 10.1097/MPG.0b013e3181f2a913]

10 **Kim EA**, Kang DH, Cho HS, Park DK, Kim YK, Park HC, Kim JH. Acid secretion from a heterotopic gastric mucosa in the upper esophagus demonstrated by dual probe 24-hour ambulatory pH monitoring. *Korean J Intern Med* 2001; **16**:

14-17 [PMID: 11417299]
11 **Korkut E**, Bektaş M, Alkan M, Ustün Y, Meco C, Ozden A, Soykan I. Esophageal motility and 24-h pH profiles of patients with heterotopic gastric mucosa in the cervical esophagus. *Eur J Intern Med* 2010; **21**: 21-24 [PMID: 20122608 DOI: 10.1016/j.ejim.2009.10.009]
12 **Galan AR**, Katzka DA, Castell DO. Acid secretion from an esophageal inlet patch demonstrated by ambulatory pH monitoring. *Gastroenterology* 1998; **115**: 1574-1576 [PMID: 9834287 DOI: 10.1016/S0016-5085(98)70038-1]
13 **Bajbouj M**, Becker V, Eckel F, Miehlke S, Pech O, Prinz C, Schmid RM, Meining A. Argon plasma coagulation of cervical heterotopic gastric mucosa as an alternative treatment for globus sensations. *Gastroenterology* 2009; **137**: 440-444 [PMID: 19410576 DOI: 10.1053/j.gastro.2009.04.053]
14 **Gutierrez O**, Akamatsu T, Cardona H, Graham DY, El-Zimaity HM. Helicobacter pylori and hetertopic gastric mucosa in the upper esophagus (the inlet patch). *Am J Gastroenterol* 2003; **98**: 1266-1270 [PMID: 12818267 DOI: 10.1111/j.1572-0241.2003.07488.x]
15 **Akbayir N**, Alkim C, Erdem L, Sökmen HM, Sungun A, Başak T, Turgut S, Mungan Z. Heterotopic gastric mucosa in the cervical esophagus (inlet patch): endoscopic prevalence, histological and clinical characteristics. *J Gastroenterol Hepatol* 2004; **19**: 891-896 [PMID: 15242492 DOI: 10.1111/j.1440-1746.2004.03474.x]
16 **di Palmo E**, Cazzato S, Tursini S, Salfi NC, Mazzotta A, Di Silverio Carulli C, Lima M, Bergamaschi R, Bernardi F, Cicognani A. A rare association of inlet patch with laryngospasm: a report of two children and literature review. *Pediatr Pulmonol* 2011; **46**: 934-938 [PMID: 21462362 DOI: 10.1002/ppul.21457.]
17 **Macha S**, Reddy S, Rabah R, Thomas R, Tolia V. Inlet patch: heterotopic gastric mucosa--another contributor to supraesophageal symptoms? *J Pediatr* 2005; **147**: 379-382 [PMID: 16182679 DOI: 10.1016/j.jpeds.2005.03.002]
18 **Tanpowpong P**, Katz AJ. Heterotopic gastric mucosa causing significant esophageal stricture in a 14-year-old child. *Dis Esophagus* 2011; **24**: E32-E34 [PMID: 21489041 DOI: 10.1111/j.1442-2050.2011.01192.x]
19 **Daher P**, Francis E, Raffoul L, Riachy E. Ectopic gastric mucosa in the cervical esophagus presenting as a recurrent neck abscess: a case report. *J Pediatr Surg* 2010; **45**: e15-e17 [PMID: 20620294 DOI: 10.1016/j.jpedsurg.2010.03.022]
20 **Karnak I**, Senocak ME, Akçören Z, Büyükpamukçu N, Hiçsönmez A. Ectopic gastric mucosa causing dysphagia due to strictures in a boy. *Eur J Pediatr Surg* 1999; **9**: 413-415 [PMID: 10661855 DOI: 10.1055/s-2008-1072295]
21 **Baudet JS**, Alarcón-Fernández O, Sánchez Del Río A, Aguirre-Jaime A, León-Gómez N. Heterotopic gastric mucosa: a significant clinical entity. *Scand J Gastroenterol* 2006; **41**: 1398-1404 [PMID: 17101570 DOI: 10.1080/00365520600763094]
22 **Poyrazoglu OK**, Bahcecioglu IH, Dagli AF, Ataseven H, Celebi S, Yalniz M. Heterotopic gastric mucosa (inlet patch): endoscopic prevalence, histopathological, demographical and clinical characteristics. *Int J Clin Pract* 2009; **63**: 287-291 [PMID: 17535303 DOI: 10.1111/j.1742-1241.2006.01215.x]
23 **Alagozlu H**, Simsek Z, Unal S, Cindoruk M, Dumlu S, Dursun A. Is there an association between Helicobacter pylori in the inlet patch and globus sensation? *World J Gastroenterol* 2010; **16**: 42-47 [PMID: 20039447 DOI: 10.3748/wjg.v16.i1.42]
24 **Neumann WL**, Luján GM, Genta RM. Gastric heterotopia in the proximal oesophagus ("inlet patch"): Association with adenocarcinomas arising in Barrett mucosa. *Dig Liver Dis* 2012; **44**: 292-296 [PMID: 22222950 DOI: 10.1016/j.dld.2011.11.008]
25 **Weickert U**, Wolf A, Schröder C, Autschbach F, Vollmer H. Frequency, histopathological findings, and clinical significance of cervical heterotopic gastric mucosa (gastric inlet patch): a prospective study in 300 patients. *Dis Esophagus* 2011; **24**: 63-68 [PMID: 20626446 DOI: 10.1111/j.1442-2050.2010.01091.x]
26 **Variend S**, Howat AJ. Upper oesophageal gastric heterotopia: a prospective necropsy study in children. *J Clin Pathol* 1988; **41**: 742-745 [PMID: 3410970 DOI: 10.1136/jcp.41.7.742]
27 **Yüksel I**, Usküdar O, Köklü S, Başar O, Gültuna S, Unverdi S, Oztürk ZA, Sengül D, Arikök AT, Yüksel O, Coban S. Inlet patch: associations with endoscopic findings in the upper gastrointestinal system. *Scand J Gastroenterol* 2008; **43**: 910-914 [PMID: 19086275 DOI: 10.1080/00365520801986619]
28 **Jacobs E**, Dehou MF. Heterotopic gastric mucosa in the upper esophagus: a prospective study of 33 cases and review of literature. *Endoscopy* 1997; **29**: 710-715 [PMID: 9427488 DOI: 10.1055/s-2007-1004294]
29 **Lauwers GY**, Mino M, Ban S, Forcione D, Eatherton DE, Shimizu M, Sevestre H. Cytokeratins 7 and 20 and mucin core protein expression in esophageal cervical inlet patch. *Am J Surg Pathol* 2005; **29**: 437-442 [PMID: 15767795]
30 **Bogomoletz WV**, Geboes K, Feydy P, Nasca S, Ectors N, Rigaud C. Mucin histochemistry of heterotopic gastric mucosa of the upper esophagus in adults: possible pathogenic implications. *Hum Pathol* 1988; **19**: 1301-1306 [PMID: 3181950]
31 **Feurle GE**, Helmstaedter V, Buehring A, Bettendorf U, Eckardt VF. Distinct immunohistochemical findings in columnar epithelium of esophageal inlet patch and of Barrett's esophagus. *Dig Dis Sci* 1990; **35**: 86-92 [PMID: 2295298 DOI: 10.1007/BF01537228]
32 **Rosztóczy A**, Izbéki F, Németh IB, Dulic S, Vadászi K, Róka R, Gecse K, Gyökeres T, Lázár G, Tiszlavicz L, Wittmann T. Detailed esophageal function and morphological analysis shows high prevalence of gastroesophageal reflux disease and Barrett's esophagus in patients with cervical inlet patch. *Dis Esophagus* 2012; **25**: 498-504 [PMID: 22107367 DOI: 10.1111/j.1442-2050.2011.01281.x]
33 **Powell RW**, Luck SR. Cervical esophageal obstruction by ectopic gastric mucosa. *J Pediatr Surg* 1988; **23**: 632-634 [PMID: 3204461]
34 **Steadman C**, Kerlin P, Teague C, Stephenson P. High esophageal stricture: a complication of "inlet patch" mucosa. *Gastroenterology* 1988; **94**: 521-524 [PMID: 3335324]
35 **Ward EM**, Achem SR. Gastric heterotopia in the proximal esophagus complicated by stricture. *Gastrointest Endosc* 2003: **57**; 131-133 [PMID 12518154 DOI: 10.1067/mge.2003.44]
36 **Buse PE**, Zuckerman GR, Balfe DM. Cervical esophageal web associated with a patch of heterotopic gastric mucosa. *Abdom Imaging* 1993; **18**: 227-228 [PMID: 8508079 DOI: 10.1007/BF00198108]
37 **Waring JP**, Wo JM. Cervical esophageal web caused by an inlet patch of gastric mucosa. *South Med J* 1997; **90**: 554-555 [PMID: 9160081]
38 **Weaver GA**. Upper esophageal web due to a ring formed by a squamocolumnar junction with ectopic gastric mucosa (another explanation of the Paterson-Kelly, Plummer-Vinson syndrome). *Dig Dis Sci* 1979; **24**: 959-963 [PMID: 510098]
39 **Bataller R**, Bordas JM, Ordi J, Llach J, Elizalde JI, Mondelo F. Upper gastrointestinal bleeding: a complication of "inlet patch mucosa" in the upper esophagus. *Endoscopy* 1995; **27**: 282 [PMID: 7664713 DOI: 10.1055/s-2007-1005690]
40 **Katsanos KH**, Christodoulou DK, Kamina S, Maria K, Lambri E, Theodorou S, Tsampoulas K, Vasiliki M, Tsianos EV. Diagnosis and endoscopic treatment of esophago-bronchial fistula due to gastric heterotopy. *World J Gastrointest Endosc* 2010; **2**: 138-142 [PMID: 21160729 DOI: 10.4253/wjge.v2.i4.138]
41 **Kohler B**, Köhler G, Riemann JF. Spontaneous esophagotracheal fistula resulting from ulcer in heterotopic gastric mucosa. *Gastroenterology* 1988; **95**: 828-830 [PMID: 3396826]
42 **García AO**, Mazzadi SA, Raffo L, Bonfanti M, Salis GB, Arra A, Chiocca JC. Heterotopic gastric mucosa in the upper esopha-

gus: report of a case with a fistula. *Dis Esophagus* 2002; **15**: 262-265 [PMID: 12445003]
43 **Righini CA**, Faure C, Karkas A, Schmerber S, Reyt E. Spontaneous perforation in the upper oesophagus resulting from ulcer in heterotopic gastric mucosa. *Rev Laryngol Otol Rhinol* (Bord) 2007; **128**: 197-200 [PMID: 18323333]
44 **Sánchez-Pernaute A**, Hernando F, Díez-Valladares L, González O, Pérez Aguirre E, Furió V, Remezal M, Torres A, Balibrea JL. Heterotopic gastric mucosa in the upper esophagus ("inlet patch"): a rare cause of esophageal perforation. *Am J Gastroenterol* 1999; **94**: 3047-3050 [PMID: 10520868 DOI: 10.1111/j.1572-0241.1999.01458.x]
45 **Rana SS**, Panda N, Sinha SK, Nada R, Nagi B, Bhasin DK. Heterotopic gastric mucosa in the upper esophagus manifesting as a polypoidal mass causing dysphagia. *Gastrointest Endosc* 2006; **63**: 185-187 [PMID: 16377348 DOI: 10.1016/j.gie.2005.07.042]
46 **Oguma J**, Ozawa S, Omori T, Kitagawa Y, Saikawa Y, Mikami S, Kitajima M. EMR of a hyperplastic polyp arising in ectopic gastric mucosa in the cervical esophagus: case report. *Gastrointest Endosc* 2005; **61**: 335-338 [PMID: 15729260]
47 **Schmulewitz N**, Tobias J, Singh P. Hyperplastic polyp arising from heterotopic gastric epithelium in the esophagus. *Gastrointest Endosc* 2007; **66**: 1221-1222; discussion 1222 [PMID: 17719036 DOI: 10.1016/j.gie.2007.06.025]
48 **Mion F**, Lambert R, Partensky C, Cherkaoui M, Berger F. High-grade dysplasia in an adenoma of the upper esophagus developing on heterotopic gastric mucosa. *Endoscopy* 1996; **28**: 633-635 [PMID: 8911811 DOI: 10.1055/s-2007-1005561]
49 **Klaase JM**, Lemaire LC, Rauws EA, Offerhaus GJ, van Lanschot JJ. Heterotopic gastric mucosa of the cervical esophagus: a case of high-grade dysplasia treated with argon plasma coagulation and a case of adenocarcinoma. *Gastrointest Endosc* 2001; **53**: 101-104 [PMID: 11154502 DOI: 10.1067/mge.2001.111394]
50 **Sauvé G**, Croué A, Denez B, Boyer J. High-grade dysplasia in heterotopic gastric mucosa in the upper esophagus after radiotherapy: successful eradication 2 years after endoscopic treatment by argon plasma coagulation. *Endoscopy* 2001; **33**: 732 [PMID: 11490394 DOI: 10.1055/s-2001-16221]
51 **Carrie A**. Adenocarcinoma of the upper end of the oesophagus arising from ectopic gastric epithelium. *Br J Surg* 1950; **37**: 474 [PMID: 15414304 DOI: 10.1002/bjs.18003714810]
52 **Yoshida T**, Shimizu Y, Kato M. Image of the month. Use of magnifying endoscopy to identify early esophageal adenocarcinoma in ectopic gastric mucosa of the cervical esophagus. *Clin Gastroenterol Hepatol* 2010; **8**: e91-e93 [PMID: 20347051 DOI: 10.1016/j.cgh.2010.02.025]
53 **Komori S**, Osada S, Tanaka Y, Takahashi T, Nagao N, Yamaguchi K, Asano N, Yoshida K. A case of esophageal adenocarcinoma arising from the ectopic gastric mucosa in the thoracic esophagus. *Rare Tumors* 2010; **2**: e5 [PMID: 21139950 DOI: 10.4081/rt.2010.e5]
54 **Akanuma N**, Hoshino I, Akutsu Y, Shuto K, Shiratori T, Kono T, Uesato M, Sato A, Isozaki Y, Maruyama T, Takeshita N, Matsubara H. Primary esophageal adenocarcinoma arising from heterotopic gastric mucosa: report of a case. *Surg Today* 2012 Jun 17; Epub ahead of print [PMID: 22706784 DOI: 10.1007/s00595-012-0206-9]
55 **Basseri B**, Conklin JL, Mertens RB, Lo SK, Bellack GS, Shaye OA. Heterotopic gastric mucosa (inlet patch) in a patient with laryngopharyngeal reflux (LPR) and laryngeal carcinoma: a case report and review of literature. *Dis Esophagus* 2009; **22**: E1-E5 [PMID: 19473208 DOI: 10.1111/j.1442-2050.2008.00915.x]
56 **Satoh S**, Nakashima T, Watanabe K, Toda S, Kuratomi Y, Sugihara H, Inokuchi A. Hypopharyngeal squamous cell carcinoma bordering ectopic gastric mucosa "inlet patch" of the cervical esophagus. *Auris Nasus Larynx* 2007; **34**: 135-139 [PMID: 16949237 DOI: 10.1016/j.anl.2006.05.014]
57 **Ford CN**. Evaluation and management of laryngopharyngeal reflux. *JAMA* 2005; **294**: 1534-1540 [PMID: 16189367 DOI: 10.1001/jama.294.12.1534]
58 **Hammer HF**. Reflux-associated laryngitis and laryngopharyngeal reflux: a gastroenterologist's point of view. *Dig Dis* 2009; **27**: 14-17 [PMID: 19439955 DOI: 10.1159/00021009]

P- Reviewers Quesada BM, Morelli L **S- Editor** Gou SX
L- Editor A **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i3.339

World J Gastroenterol 2013 January 21; 19(3): 339-346
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

ORIGINAL ARTICLE

Diagnostic value of AFP-L3 and PIVKA-Ⅱ in hepatocellular carcinoma according to total-AFP

Jong Young Choi, Seung Won Jung, Hee Yeon Kim, Myungshin Kim, Yonggoo Kim, Dong Goo Kim, Eun-Jee Oh

Jong Young Choi, Hee Yeon Kim, Department of Internal Medicine, School of Medicine, The Catholic University of Korea, Seoul 137-701, South Korea
Seung Won Jung, Myungshin Kim, Yonggoo Kim, Eun-Jee Oh, Department of Laboratory Medicine and Laboratory Development Evaluation Center, School of Medicine, The Catholic University of Korea, Seoul 137-701, South Korea
Dong Goo Kim, Department of Surgery, School of Medicine, The Catholic University of Korea, Seoul 137-701, South Korea
Author contributions: Choi JY and Jung SW contributed equally to the work; Choi JY and Oh EJ designed the research; Jung SW performed the majority of experiments; Kim HY, Kim M and Kim Y provided analytical tools and were also involved in editing the manuscript; Choi JY and Kim DG provided the collection of all the human material for this work; Choi JY and Oh EJ wrote the manuscript.
Supported by The Industrial Core Technology Development Program funded by the Ministry of Knowledge Economy, No. 10033183
Correspondence to: Dr. Eun-Jee Oh, Department of Laboratory Medicine and Laboratory Development Evaluation Center, School of Medicine, The Catholic University of Korea, 505 Banpo-Dong, Seocho-Ku, Seoul 137-701, South Korea. ejoh@catholic.ac.kr
Telephone: +82-2-22581641 Fax: +82-2-22581719
Received: July 16, 2012 Revised: October 25, 2012
Accepted: November 6, 2012
Published online: January 21, 2013

Abstract

AIM: To evaluate diagnostic value of α-fetoprotein (AFP)-L3 and prothrombin induced by vitamin K absence-Ⅱ (PIVKA-Ⅱ) in hepatocellular carcinoma (HCC).

METHODS: One hundred and sixty-eight patients during routine HCC surveillance were included in this study. Of the 168 patients, 90 (53.6%) had HCC including newly developed HCC (n = 82) or recurrent HCC after treatment (n = 8). Sera were obtained during their first evaluation for HCC development and at the time of HCC diagnosis before commencing HCC treatment. HCC was diagnosed by histological examination, appropriate imaging characteristics-computed tomography or magnetic resonance imaging. Control sera were collected from 78 patients with benign liver disease (BLD), which were obtained during routine surveillance with a suspicion of HCC. AFP, AFP-L3 and PIVKA-Ⅱ were measured in the same serum by microchip capillary electrophoresis and liquid-phase binding assay on a micro-total analysis system Wako i30 auto analyzer. The performance characteristics of three tests and combined tests for the diagnosis of HCC were obtained using receiver operating characteristic curves in all populations and subgroups with AFP < 20 ng/mL.

RESULTS: Of 90 HCC patients, 38 (42.2%) patients had AFP < 20 ng/mL, 20 (22.2%) patients had AFP 20-200 ng/mL and 32 (35.6%) patients had AFP > 200 ng/mL. Of the 78 BLD patients, 74 (94.9%) patients had AFP < 20 ng/mL. After adjustment for age and HBV infection status, AFP-L3 levels were higher in HCC than in BLD among patients with low AFP levels (< 20 ng/mL) (P < 0.001). In a total of 168 patients, areas under the curve (AUC) for HCC were 0.879, 0.887, 0.801 and 0.939 for AFP, AFP-L3, PIVKA-Ⅱ and the combined markers, respectively. The combined AUC for three markers showed higher value than the AUCs of individual marker (P < 0.05). AFP-L3 had higher AUC value than PIVKA-Ⅱ for HCC detection in entire patients (P = 0.043). With combination of AFP-L3 (cut-off > 5%) and PIVKA-Ⅱ (cut-off > 40 AU/L), the sensitivity were 94.4% and specificity were 75.6% in all patients. In 112 patients with low AFP levels (< 20 ng/mL), AUCs of AFP-L3, PIVKA-Ⅱ and combine AFP-L3 and PIVKA-Ⅱ tests were 0.824, 0.774 and 0.939, respectively. AFP-L3 with a cut-off value of 5% showed sensitivity of 71.1% and specificity of 83.8%, and PIVKA-Ⅱ with a cut-off value of 40 AU/L had sensitivity of 57.9% and specificity of 95.9% in patients with low AFP levels. The combination of AFP-L3 and PIVKA-Ⅱ increased the sensitivity and specificity up to 92.1% and 79.7%, respectively, in low AFP group. Combined markers detected 81.8% of early stage HCC (Union for Inter-

national Cancer Control stage Ⅰ), 86.7% of small sized tumor (< 2 cm) and 91.7% of single tumor of HCC in the low AFP group. In multivariate analysis, AFP-L3 was correlated with AFP and tumor size, and PIVKA-Ⅱ was correlated with laboratory tests including serum aspartate aminotransferase, total bilirubin, platelets and albumin levels. PIVKA-Ⅱ had no correlation with AFP, AFP-L3 or tumor characteristics.

CONCLUSION: Combined determination of AFP-L3 and PIVKA-Ⅱ could improve the diagnostic value for HCC detection in patients with or without increased AFP levels.

© 2013 Baishideng. All rights reserved.

Key words: α-fetoprotein; Prothrombin induced by vitamin K absence-Ⅱ; Hepatocellular carcinoma; Diagnosis; Tumor marker

Choi JY, Jung SW, Kim HY, Kim M, Kim Y, Kim DG, Oh EJ. Diagnostic value of AFP-L3 and PIVKA-Ⅱ in hepatocellular carcinoma according to total-AFP. *World J Gastroenterol* 2013; 19(3): 339-346 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i3/339.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i3.339

INTRODUCTION

Early detection of hepatocellular carcinoma (HCC) is important as the treatment of HCC with surgical resection, liver transplantation or percutaneous ablation can be curative at early stage. Currently, the recommended screening strategy includes measurement of serum α-fetoprotein (AFP) levels and an abdominal ultrasound every 3-6 mo for the detection of HCC at an earlier stage[1-4]. Usually, a serum AFP level of 20 ng/mL is considered as a cut-off value to differentiate HCC from non-HCC[5]. However, serum AFP level has a high false-negative rate for the detection of small or early stage tumors[2,6-9], and it is often markedly elevated in patients with either cirrhosis or those with exacerbated chronic hepatitis without HCC[10,11].

Therefore, additional tumor markers including fucosylated fraction of AFP (AFP-L3) fraction[12,13] and prothrombin induced by vitamin K absence-Ⅱ (PIVKA-Ⅱ) or Des-γ-carboxyprothrombin[14-16] have been suggested, and the surveillance program with these markers have been mostly organized by Japanese study groups[1,17]. The lens culinaris agglutinin-reactive, AFP-L3 has been reported to be highly specific for HCC[5,18-20] and in predicting prognosis[21-24]. Recently, a highly sensitive analytical system using the technically advanced microfluidics-based separation system has been introduced[25]. With this micro-total analysis system (μTAS), AFP-L3 can be measured accurately at very low AFP concentrations under 10 ng/mL with high sensitivity[7,19,25,26]. PIVKA-Ⅱ is an abnormal protein and some reports have indicated the improved specificity of PIVKA-Ⅱ over AFP in the diagnosis of HCC[2,5,27-29]. The combination of AFP-L3 and PIVKA-Ⅱ seems to improve the diagnostic value for HCC patients, and these additive assays are especially recommended for patients with low AFP levels according to previous Japanese studies[19,26,27]. However, diagnostic values of these markers are controversial and different cut-off values have been suggested depending on the study design[5,20,26]. In addition, the clinical values of these markers in combination are not conclusive yet, and the clinical and laboratory factors which possibly effect the measured values of AFP-L3 and PIVKA-Ⅱ have not been sufficiently investigated.

In this study, we compared the levels of AFP, AFP-L3 and PIVKA-Ⅱ in benign liver diseases (BLD) patients and HCC patients, and evaluated the individual and combined diagnostic values of AFP-L3 and PIVKA-Ⅱ for HCC detection according to the total AFP levels and tumor characteristics. The clinical and laboratory factors affecting AFP-L3 and PIVKA-Ⅱ were also analyzed.

MATERIALS AND METHODS

Patients

Serum samples were obtained from 168 patients during routine HCC surveillance at the Seoul St. Mary's Hospital, Seoul, South Korea, during the period between April and November 2011. This study was approved by the Institutional Review Board of Seoul St. Mary's Hospital. Of the 168 patients, 90 (53.6%) had HCC including newly developed HCC (n = 82) or recurrent HCC after treatment (n = 8). Sera were obtained during their first evaluation for HCC development and at the time of HCC diagnosis before commencing HCC treatment. HCC was diagnosed by histological examination, appropriate imaging characteristics-computed tomography or magnetic resonance imaging. Tumor stage was assessed by the Union for International Cancer Control (UICC) staging system, which is based on tumor number, size, vascular invasion and metastasis[4,30]. Control sera were collected from 78 patients with BLD, which were obtained during routine surveillance with a suspicion of HCC. All patients were diagnosed by imaging study and were followed up for 7-16 mo after sampling to confirm non-HCC.

Measurement of AFP, AFP-L3 and PIVKA-Ⅱ

AFP, AFP-L3 and PIVKA-Ⅱ were measured in the same serum by microchip capillary electrophoresis and liquid-phase binding assay on a μTAS Wako i30 auto analyzer (Wako Pure Chemical Industries, Ltd, Osaka, Japan). The measuring range was 0.3-1000 ng/mL for AFP and 5-50 000 AU/L for PIVKA-Ⅱ. AFP-L3 levels were calculated in sera with AFP levels over 0.3 ng/mL. The total imprecision for two levels of the three markers were less than 5% CVs in 20 d of evaluation. Serum samples were stored at -80 ℃ until tested. All processes were preformed automatically and followed the manufacturer's instructions.

Table 1 Demographics and laboratory test values of patients with benign liver disease and with hepatocellular carcinoma *n* (%)

	BLD (*n* = 78)	HCC (*n* = 90)	*P* value
Age (mean ± SD), yr	55.6 ± 12.5	59.7 ± 10.1	0.020
Male	51 (65.4)	64 (71.1)	0.506
Cause of disease			
HBV	34 (43.6)	61 (67.8)	0.002
HCV	9 (11.5)	9 (10.0)	0.749
Alcohol	5 (6.4)	12 (13.3)	0.139
NANBNC	1 (1.3)	8 (8.9)	0.038
Benign mass	10 (12.8)		
Others	19 (24.4)		
AFP < 20 ng/mL	74 (94.9)	38 (42.2)	
AFP 20-200 ng/mL	4 (5.1)	20 (22.2)	
AFP > 200 ng/mL	0 (0)	32 (35.6)	
Tumor stage			
Ⅰ / Ⅱ / Ⅲ / Ⅳa / Ⅳb		16 (17.8)/31 (34.4)/ 19 (21.1)/18 (20.0)/6 (6.7)	
Tumor size			
< 2/2-5/> 5 cm		25 (27.8)/33 (36.7)/32 (35.6)	
Tumor number			
Single/multiple		40 (44.4)/50 (55.6)	
AFP level (mean ± SD, ng/mL)	6.9 ± 13.5	473.9 ± 746.0	< 0.001
AFP-L3 level (mean ± SD, %)	2.3 ± 4.9	40.0 ± 35.7	< 0.001
PIVKA-Ⅱ level (mean ± SD, AU/L)	20.0 ± 31.2	4469 ± 11 553.8	< 0.001

HBV: Hepatitis B virus; HCV: Hepatitis C virus; NANBNC: Non-hepatitis A, non-hepatitis B, non-hepatitis C virus; AFP: α-fetoprotein; PIVKA-Ⅱ: Prothrombin induced by vitamin K absence-Ⅱ; BLD: Benign liver disease; HCC: Hepatocellular carcinoma.

Statistical analysis

Statistical analysis were performed with SPSS version 12.0 (SPSS, Chicago, IL) and Med-Calc version 12. 2. 1. 0 (MedCalc Software, Mariakerke, Belgium). Comparisons were made using *t* test. The performance characteristics of three tests and combined tests for the diagnosis of HCC were obtained using receiver operating characteristic (ROC) curves in all populations and subgroups with AFP < 20 ng/mL. The sensitivity and specificity of these three markers for the diagnosis of HCC were calculated in all the groups and subgroups according to the total AFP levels. A logistic regression model was performed in SPSS on the three markers and ROC curves analysis was performed for area under the curve (AUC) values for the combined markers. All *P* values were 2-tailed with $P < 0.05$ considered statistically significant.

RESULTS

Patient characteristics

Table 1 shows the demographics of the study population. The most common cause of liver disease was hepatitis B virus (HBV) infection in both BLD and HCC patients. HCC patients had a higher frequency of males and HBV infection than that of BLD patients ($P < 0.05$). Of the 90 HCC patients, 38 (42.2%) patients had AFP < 20 ng/mL, 20 (22.2%) patients had AFP 20-200 ng/mL and 32 (35.6%) patients had AFP > 200 ng/mL. Of the 78 BLD patients, 74 (94.9%) patients had AFP < 20 ng/mL. In four patients with non-HCC and increased AFP levels (45.2-72.8 ng/mL), PIVKA-Ⅱ levels were less than 40 AU/L, but AFP-L3 levels were above 10%. The levels of AFP, AFP-L3 and PIVKA-Ⅱ in BLD and HCC patients are plotted with respect to the AFP levels (Figure 1). In patients with AFP < 20 ng/mL, AFP-L3 levels were higher in HCC than that in BLD ($P < 0.001$). Regarding the patients with total AFP levels of 20-200 ng/mL, not AFP-L3 but PIVKA-Ⅱ was able to discriminate HCC from BLD ($P = 0.010$). After adjustment for age (> 50 and < 50 years) and HBV infection status, AFP-L3 levels were still higher in HCC than in BLD among patients with low AFP levels (< 20 ng/mL) ($P < 0.001$).

ROC curve analysis of three markers for the detection of HCC

The ROC curves of AFP, AFP-L3 and PIVKA-Ⅱ for the diagnosis of HCC in all of 168 patients are shown in Figure 2. The maximum AUCs for distinguishing between HCC and BLD were 0.879, 0.887 and 0.801 for AFP, AFP-L3 and PIVKA-Ⅱ, respectively. AFP-L3 had higher AUC than PIVKA-Ⅱ for HCC ($P = 0.043$). Using logistic regression model, the combined AUC for three markers was 0.939 and showed higher AUC values than the individual markers ($P < 0.05$).

In 112 patients with AFP < 20 ng/mL (HCC, $n = 38$; BLD, $n = 74$), the AUC for HCC detection was 0.824 for AFP-L3, 0.774 for PIVKA-Ⅱ and 0.939 for the two combined markers. Combined AUC was significantly higher than each AUC ($P < 0.05$), and the AUC value was not different between AFP-L3 and PIVKA-Ⅱ ($P = 0.516$) (Figure 3).

Sensitivity and specificity of three markers for HCC diagnosis

The sensitivities and specificities of the three markers for all patients and subgroups are shown in Table 2. In all patients, the total AFP showed a sensitivity of 78.9% and a specificity of 84.6% with a cut-off of 10 ng/mL. AFP-L3 with a cut-off value of 5% increased the sensitivity up to 82.2%. When the cut-off value for AFP-L3 and PIVKA-Ⅱ were set at 10% and 40 AU/L, the specificities were improved up to 93.6% and 94.9%, respectively.

In the subgroups according to the total AFP levels (AFP < 20 ng/mL, $n = 112$; AFP < 200 ng/mL, $n = 136$), the sensitivity and specificity of AFP-L3 were close to 70% and 80% respectively with a cut-off of 5%, and there was no significant difference compared to that of the entire group. The sensitivity and specificity of PIVKA-Ⅱ was approximately 60% and 95% respectively with a cut-off of 40 AU/L in all patients and subgroups.

To increase the diagnostic value for HCC detection, we combined the AFP-L3 and PIVKA-Ⅱ results. If either AFP-L3 or PIVKA-Ⅱ level was above the cut-off value, the combined result was defined as positive. With combination of AFP-L3 (cut-off > 5%) and PIVKA-Ⅱ

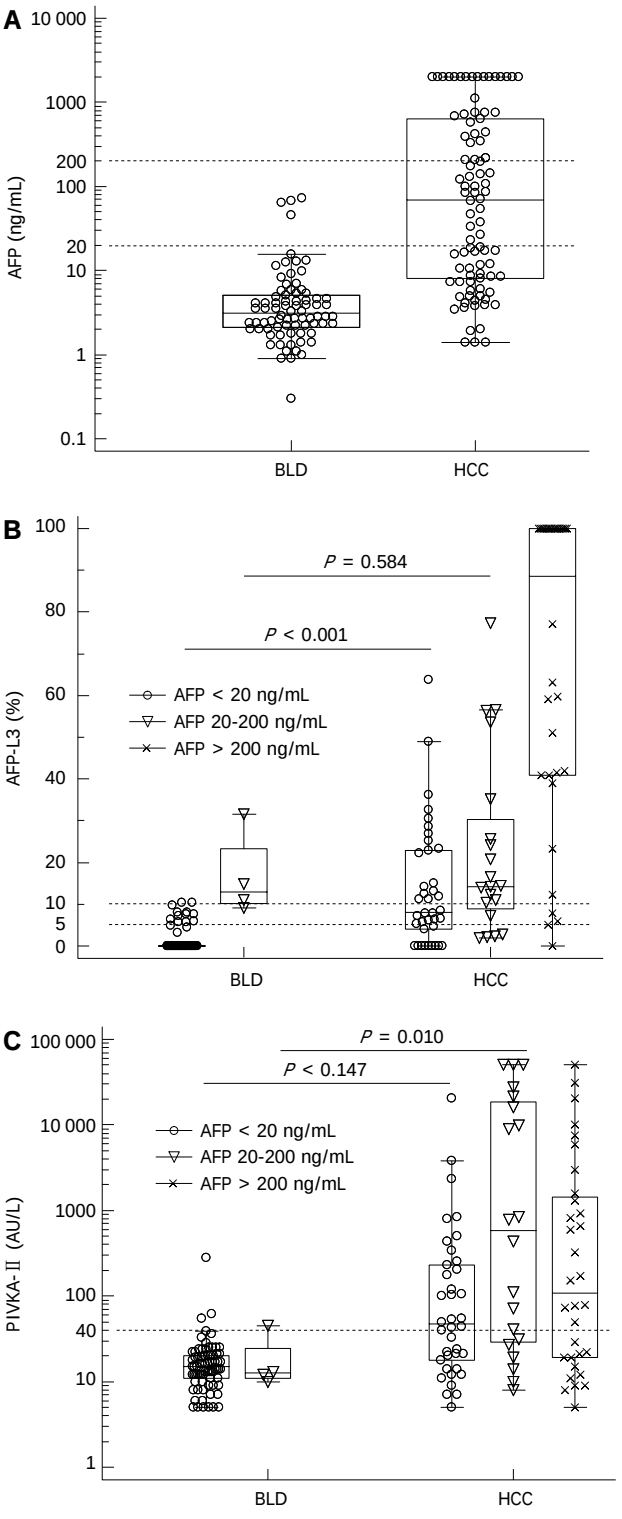


Figure 1 α-fetoprotein (A), α-fetoprotein-L3 (B) and prothrombin induced by vitamin K absence-Ⅱ (C) values in benign liver disease and hepatocellular carcinoma patients. α-fetoprotein (AFP)-L3 and prothrombin induced by vitamin K absence (PIVKA)-Ⅱ levels are plotted by AFP concentrations (AFP < 20 ng/mL, AFP 20-200 ng/mL and AFP > 200 ng/mL). In patients with AFP < 20 ng/mL, AFP-L3 levels were higher in hepatocellular carcinoma (HCC) (P < 0.001) (B). In patients with AFP 20-200 ng/mL, PIVKA-Ⅱ showed higher levels in HCC (P = 0.010) (C). The horizontal dotted lines are cut-off values used for performance analysis in this study. BLD: Benign liver disease.

(cut-off > 40 AU/L), the sensitivities were 92.1%-94.4%

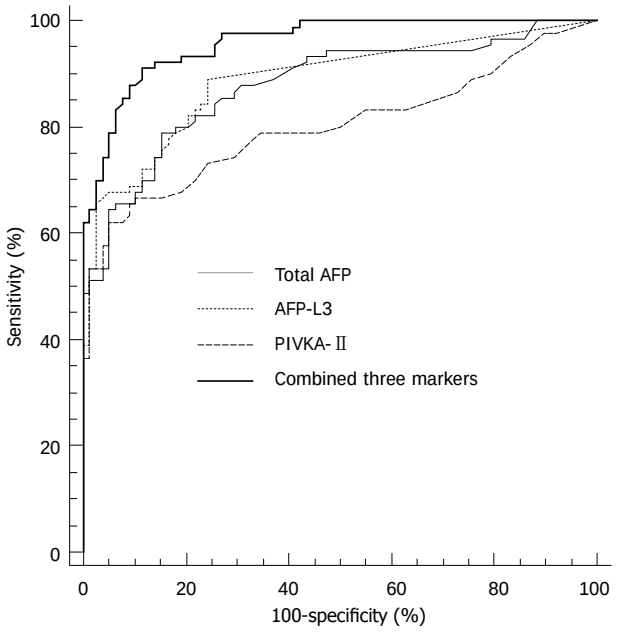


Figure 2 Receiver operating characteristic curves of total α-fetoprotein, α-fetoprotein-L3, prothrombin induced by vitamin K absence-Ⅱ and three combined markers for the diagnosis of hepatocellular carcinoma in all patients. The area under the curve values were 0.879 for total α-fetoprotein (AFP), 0.887 for AFP-L3, 0.801 for prothrombin induced by vitamin K absence (PIVKA)-Ⅱ and 0.959 for the three combined markers.

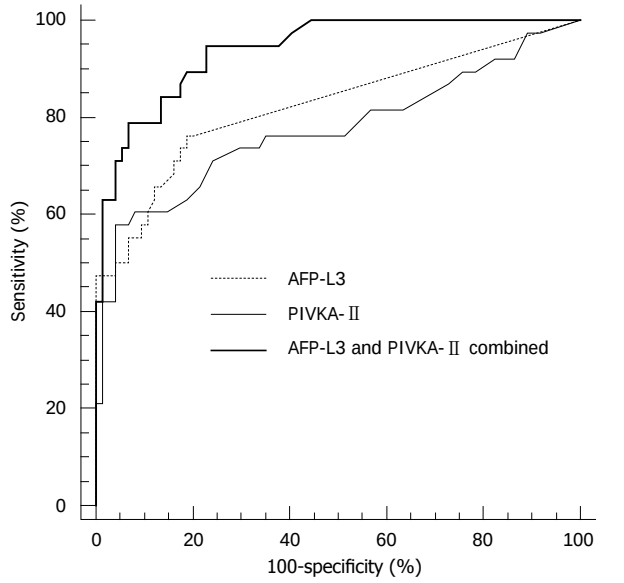


Figure 3 Receiver operating characteristic curves of α-fetoprotein-L3 and prothrombin induced by vitamin K absence-Ⅱ for the diagnosis of hepatocellular carcinoma in patients with total α-fetoprotein < 20 ng/mL. The area under the curve values were 0.824 for α-fetoprotein (AFP)-L3, 0.774 for prothrombin induced by vitamin K absence (PIVKA)-Ⅱ and 0.939 for the two combined markers.

and specificities were 75.6%-79.7% in all patients and subgroups. When a cut-off value was set at 10% for AFP-L3 and combined with PIVKA-Ⅱ, the sensitivities were approximately 80% (78.9%-84.4%) and the specificities were maintained close to 90%.

Table 2 Performance of α-fetoprotein, α-fetoprotein-L3 and prothrombin induced by vitamin K absence-Ⅱ in detecting hepatocellular carcinoma in all patients and in subgroups according to total α-fetoprotein levels: Total α-fetoprotein

		Cut-off value	Sensitivity	Specificity
Entire group	AFP	10 ng/mL	78.9%	84.6%
(*n* = 168, 90 HCC)	AFP-L3	5%	82.2%	79.5%
		10%	67.8%	93.6%
	PIVKA-Ⅱ	40 AU/L	62.2%	94.9%
	AFP-L3 or PIVKA-Ⅱ	5% or 40 AU/L	94.4%	75.6%
		10% or 40 AU/L	84.4%	89.7%
AFP < 20 ng/mL	AFP-L3	5%	71.1%	83.8%
(*n* = 112, 38 HCC)		10%	47.4%	97.3%
	PIVKA-Ⅱ	40 AU/L	57.9%	95.9%
	AFP-L3 or PIVKA-Ⅱ	5% or 40 AU/L	92.1%	79.7%
		10% or 40 AU/L	78.9%	93.2%
AFP < 200 ng/mL	AFP-L3	5%	74.1%	79.5%
(*n* = 136, 58 HCC)		10%	56.9%	93.6%
	PIVKA-Ⅱ	40 AU/L	62.1%	94.9%
	AFP-L3 or PIVKA-Ⅱ	5% or 40 AU/L	93.1%	75.6%
		10% or 40 AU/L	82.8%	89.7%

AFP: α-fetoprotein; PIVKA-Ⅱ: Prothrombin induced by vitamin K absence-Ⅱ; HCC: Hepatocellular carcinoma.

Sensitivity of AFP-L3 and PIVKA-Ⅱ according to the tumor characteristics in patients with AFP < 20 ng/mL

To establish the sensitivity of AFP-L3 (cut-off 5%) and PIVKA-Ⅱ (cut-off 40 AU/L) for the detection of small tumors and early stage HCC especially in 38 HCC patients with AFP < 20 ng/mL, we subgrouped the HCC patients according to the UICC stage (Ⅰ, Ⅱ, Ⅲ, Ⅳa, Ⅳb), tumor size (< 2, 2-5, > 5 cm) and tumor number (single and multiple tumor) (Table 3). The sensitivity of AFP-L3 and PIVKA-Ⅱ in UICC stage Ⅰ-Ⅱ (n = 27) was 63.6%-75.0% and 45.5%-56.3%. The combination of AFP-L3 and PIVKA-Ⅱ increased the sensitivity up to 81.8%-100% (Table 3). In HCC patients with tumor size < 2 (n = 15) and 2-5 cm (n = 17), the sensitivity of AFP-L3 was 46.7% and 47.1%, respectively. The sensitivity of PIVKA-Ⅱ for the detection of HCC with tumor size < 2 and 2-5 cm was 40.0% and 64.7%, and combination of AFP-L3 and PIVKA-Ⅱ resulted in an enhancement of sensitivity up to 86.7% and 94.1%, respectively. With a combined test (AFP-L3 and PIVKA-Ⅱ), 22 (91.7%) of 24 HCCs with a single tumor were detected.

Correlation analysis

To investigate the clinical and laboratory factors which may affect the measured values of AFP-L3 and PIVKA-Ⅱ, we performed univariate and multivariate analyses. In univariate correlation analysis, there was a correlation between AFP-L3 and PIVKA-Ⅱ. AFP-L3 and PIVKA-Ⅱ levels were correlated with tumor stage (UICC), tumor size, aspartate aminotransferase (AST) and albumin. Tumor number and AFP levels were correlated with only AFP-L3 levels.

In multivariate analysis, only AFP and tumor size were significant correlates of AFP-L3 (Table 4). In terms of PIVKA-Ⅱ, laboratory tests including serum AST, total bilirubin, platelets and albumin levels were correlated with the PIVKA-Ⅱ results. However, PIVKA-Ⅱ had no correlation with AFP, AFP-L3 or tumor characteristics.

Table 3 Sensitivity of α-fetoprotein-L3 and prothrombin induced by vitamin K absence-Ⅱ according to the tumor characteristics in 38 hepatocellular carcinoma patients with α-fetoprotein < 20 ng/mL *n* (%)

	n	AFP-L3 (> 5%)	PIVKA-Ⅱ (> 40 AU/L)	AFP-L3 and PIVKA-Ⅱ combined (> 5%, > 40 AU/L)
Tumor stage (UICC)				
Ⅰ	11	7 (63.6)	5 (45.5)	9 (81.8)
Ⅱ	16	12 (75.0)	9 (56.3)	16 (100)
Ⅲ	9	6 (66.7)	6 (66.7)	8 (88.9)
Ⅳa	2	2 (100)	2 (100)	2 (100)
Tumor size				
< 2 cm	15	7 (46.7)	6 (40.0)	13 (86.7)
2-5 cm	17	8 (47.1)	11 (64.7)	16 (94.1)
> 5 cm	6	3 (50.0)	5 (83.3)	6 (100)
Tumor number				
Single	24	16 (66.7)	12 (50.0)	22 (91.7)
Multiple	14	11 (78.6)	10 (71.4)	13 (92.7)

AFP: α-fetoprotein; PIVKA-Ⅱ: Prothrombin induced by vitamin K absence-Ⅱ; UICC: International Union Against Cancer; HCC: Hepatocellular carcinoma.

Table 4 Stepwise multiple linear regression analysis using dependent variables α-fetoprotein-L3 and prothrombin induced by vitamin K absence-Ⅱ

Variable	AFP-L3		PIVKA-Ⅱ	
	β coefficient	*P* value	β coefficient	*P* value
AFP	0.839	< 0.001		NS
Tumor size	0.190	0.002		NS
AST		NS	0.550	< 0.001
Total bilirubin		NS	0.336	< 0.001
Platelet		NS	0.566	0.001
Albumin		NS	-0.674	< 0.001

AFP: α-fetoprotein; PIVKA-Ⅱ: Prothrombin induced by vitamin K absence-Ⅱ; AST: Aspartate aminotransferase; NS: Not significant.

DISCUSSION

In patients at risk for developing HCC, systematic screening is required for detection of small tumors at an early stage. Although the diagnostic strategy for early detection of HCC has been changed with the technical development in imaging diagnosis[3], measurement of tumor markers for HCC at regular intervals is still a common practice. AFP is the most widely used tumor marker for HCC. However, recent studies reported that AFP lacks adequate sensitivity and specificity for effective surveillance[6,9-11]. Our data showed that 38 (42.2%) of 90 HCC patients had AFP < 20 ng/mL, and 4 (5.1%) of 78 non-HCC patients had AFP > 20 ng/mL, which support the inadequacy of AFP as a tumor marker shown by previous reports. Therefore, we examined the individual and combined diagnostic values of AFP-L3 and PIVKA-Ⅱ for HCC according to the AFP levels (< 20 ng/mL and

< 200 ng/mL), and investigated the clinical and laboratory factors that influence the measured values of AFP-L3 and PIVKA-Ⅱ.

The AFP-L3 fraction has been reported to be more sensitive than AFP for small sized or early stage HCC[20,31]. AFP-L3 is also known to be highly specific for HCC and reflect tumor characteristics including poor differentiation or malignant invasion[32-35]. The present data of all patients showed that the AUC value of AFP-L3 for HCC detection was 0.887, which was not different with the AUC value of AFP (0.879), but was higher than the AUC of PIVKA-Ⅱ. In focusing on the patients with AFP < 20 ng/mL, AFP-L3 levels were significantly higher in HCC than in non-HCC, and the sensitivity of AFP-L3 for HCC was 71.1% and 47.4% with cut-offs of 5% and 10%, respectively. These values are higher than those reported in previous studies showing sensitivity of 25%-50% with cut-offs between 5% and 10%[19,20]. The high specificity for HCC is an important advantage of AFP-L3 measurement and higher sensitivity without a concomitant reduction in specificity is useful in clinical settings. In the present study, the specificities of AFP-L3 for HCC were 79.5%-97.3% in all patients or in patients with low AFP levels. The values are similar to those in previous reports showing the specificities of AFP-L3 for HCC as over 85% among patients with low total AFP-levels[19,20]. Overall, in patients with low AFP levels, AFP-L3 (cut-off 5%) had reasonable AUC value, sensitivity and specificity (0.824, 71.1% and 83.8%, respectively) which are similar or improved results compare to previously reported data[19,36].

In the present study, PIVKA-Ⅱ alone had sensitivities and specificities close to 60% and 95% in all patients and patients with low AFP levels. PIVKA-Ⅱ was shown to have a higher specificity in previous studies[2,5,27], and all four patients with non-HCC and increased AFP/AFP-L3 levels showed lower values of PIVKA-Ⅱ in the present study. This suggests that the PIVKA-Ⅱ test may be useful in detecting false-positive AFP results.

The levels of AFP-L3 and PIVKA-Ⅱ had no correlation between them and these two may be compensative markers reflecting a different developmental form of HCC[37]. In multivariate analysis, AFP-L3 correlated with total AFP and tumor size. In contrast, PIVKA-Ⅱ had no correlation with AFP or tumor characteristics, but was correlated with some liver function tests including serum AST, tuberculosis, platelet and albumin.

In our study, the combination of PIVKA-Ⅱ and AFP-L3 improved the sensitivity close to 90% and showed significantly increased AUC (0.939) even in patients with low AFP. In addition, the combined tests showed approximately 90% sensitivity for the detection of early stage, small sized or single HCC tumors in patients with low AFP levels. These results suggest that these two biomarkers should be measured simultaneously and in combination with imaging tests to improve the diagnostic accuracy.

Potential limitations of our study include the small number of newly diagnosed HCC patients, lack of randomization and relative short-term follow-up. Our results should be validated with a larger number of patients with age- and sex-matched control subjects. Since AFP-L3 has been suggested as a prognostic marker in patients with HCC[22,23,26], future studies using longitudinal AFP-L3 data analysis or studies regarding the effect of AFP-L3 effect on the survival rate of HCC patients after treatment are needed. Despite these limitations, the present study indicates that AFP-L3 and PIVKA-Ⅱ are useful additive tumor markers for the diagnosis of HCC.

In conclusion, combined determination of AFP-L3 and PIVKA-Ⅱ improved the diagnostic value for HCC detection in patients with or without increased AFP levels. The utility of improved surveillance protocol based on these tumor markers needs to be investigated.

COMMENTS

Background

In patients at risk for developing hepatocellular carcinoma (HCC), systematic screening is required for detection of small tumors at an early stage. Although, α-fetoprotein (AFP) is the most widely used tumor marker for HCC, AFP lacks adequate sensitivity and specificity for effective surveillance. Therefore, additional tumor markers including fucosylated fraction of AFP (AFP-L3) fraction and prothrombin induced by vitamin K absence-Ⅱ (PIVKA-Ⅱ) have been suggested.

Research frontiers

The clinical values of AFP, AFP-L3 and PIVKA-Ⅱ in combination are not conclusive yet, and the clinical and laboratory factors which possibly affect the measured values of AFP-L3 and PIVKA-Ⅱ have not been sufficiently investigated. In this study, the authors evaluated the individual and combined diagnostic values of AFP-L3 and PIVKA-Ⅱ for HCC detection according to the total AFP levels and tumor characteristics. The clinical and laboratory factors affecting AFP-L3 and PIVKA-Ⅱ were also analyzed.

Innovations and breakthroughs

In the study, the combination of PIVKA-Ⅱ and AFP-L3 improved the sensitivity close to 90% and showed significantly increased areas under the curve (0.939) even in patients with low AFP. In addition, the combined tests showed approximately 90% sensitivity for the detection of early stage, small sized or single HCC tumors in patients with low AFP levels. In multivariate analysis, AFP-L3 correlated with total AFP and tumor size. In contrast, PIVKA-Ⅱ had no correlation with AFP or tumor characteristics, but was correlated with some liver function tests including serum aspartate aminotransferase, tuberculosis, platelet and albumin. The present study indicates that AFP-L3 and PIVKA-Ⅱ are useful additive tumor markers for the diagnosis of HCC.

Applications

Combined determination of AFP-L3 and PIVKA-Ⅱ could improve the diagnostic value for HCC detection in patients with or without increased AFP levels. The utility of improved surveillance protocol based on these tumor markers needs to be investigated.

Peer review

This study confirmed that the combined determination of AFP-L3 and PIVKA-Ⅱ improved the diagnostic value for HCC detection in patients with or without increased AFP levels. The authors performed the multivariate analysis and revealed that AFP-L3 and PIVKA-Ⅱ had no correlation between them and might be useful additive and compensate tumor markers for the diagnosis of HCC. The results are interesting and can be used to elucidate the different development form of HCC.

REFERENCES

1 **Izumi N**. Diagnostic and treatment algorithm of the Japanese society of hepatology: a consensus-based practice guideline. *Oncology* 2010; **78** Suppl 1: 78-86 [PMID: 20616588 DOI: 10.1159/000315234]

2 **Saffroy R**, Pham P, Reffas M, Takka M, Lemoine A, Debuire B.

New perspectives and strategy research biomarkers for hepatocellular carcinoma. *Clin Chem Lab Med* 2007; **45**: 1169-1179 [PMID: 17635075 DOI: 10.1515/CCLM.2007.262]
3 **Bruix J**, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]
4 **Korean Liver Cancer Study Group and National Cancer Center, Korea**. Practice guidelines for management of hepatocellular carcinoma 2009. *Korean J Hepatol* 2009; **15**: 391-423 [PMID: 19783891 DOI: 10.3350/kjhep.2009.15.3.391]
5 **Durazo FA**, Blatt LM, Corey WG, Lin JH, Han S, Saab S, Busuttil RW, Tong MJ. Des-gamma-carboxyprothrombin, alpha-fetoprotein and AFP-L3 in patients with chronic hepatitis, cirrhosis and hepatocellular carcinoma. *J Gastroenterol Hepatol* 2008; **23**: 1541-1548 [PMID: 18422961 DOI: 10.1111/j.1440-1746.2008.05395.x]
6 **Sherman M**, Peltekian KM, Lee C. Screening for hepatocellular carcinoma in chronic carriers of hepatitis B virus: incidence and prevalence of hepatocellular carcinoma in a North American urban population. *Hepatology* 1995; **22**: 432-438 [PMID: 7543434 DOI: 10.1002/hep.1840220210]
7 **Toyoda H**, Kumada T, Tada T. Highly sensitive Lens culinaris agglutinin-reactive α-fetoprotein: a new tool for the management of hepatocellular carcinoma. *Oncology* 2011; **81** Suppl 1: 61-65 [PMID: 22212938 DOI: 10.1159/000333263]
8 **Matsuda M**, Asakawa M, Amemiya H, Fujii H. Lens culinaris agglutinin-reactive fraction of AFP is a useful prognostic biomarker for survival after repeat hepatic resection for HCC. *J Gastroenterol Hepatol* 2011; **26**: 731-738 [PMID: 21155886 DOI: 10.1111/j.1440-1746.2010.06532.x]
9 **Cui R**, He J, Zhang F, Wang B, Ding H, Shen H, Li Y, Chen X. Diagnostic value of protein induced by vitamin K absence (PIVKAII) and hepatoma-specific band of serum gamma-glutamyl transferase (GGTII) as hepatocellular carcinoma markers complementary to alpha-fetoprotein. *Br J Cancer* 2003; **88**: 1878-1882 [PMID: 12799630 DOI: 10.1038/sj.bjc.6601018]
10 **Bae JS**, Park SJ, Park KB, Paik SY, Ryu JK, Choi CK, Hwang TJ. Acute exacerbation of hepatitis in liver cirrhosis with very high levels of alpha-fetoprotein but no occurrence of hepatocellular carcinoma. *Korean J Intern Med* 2005; **20**: 80-85 [PMID: 15906959 DOI: 10.3904/kjim.2005.20.1.80]
11 **Chu CW**, Hwang SJ, Luo JC, Lai CR, Tsay SH, Li CP, Wu JC, Chang FY, Lee SD. Clinical, virologic, and pathologic significance of elevated serum alpha-fetoprotein levels in patients with chronic hepatitis C. *J Clin Gastroenterol* 2001; **32**: 240-244 [PMID: 11246354 DOI: 10.1097/00004836-200103000-00014]
12 **Taketa K**, Sekiya C, Namiki M, Akamatsu K, Ohta Y, Endo Y, Kosaka K. Lectin-reactive profiles of alpha-fetoprotein characterizing hepatocellular carcinoma and related conditions. *Gastroenterology* 1990; **99**: 508-518 [PMID: 1694805]
13 **Taketa K**, Endo Y, Sekiya C, Tanikawa K, Koji T, Taga H, Satomura S, Matsuura S, Kawai T, Hirai H. A collaborative study for the evaluation of lectin-reactive alpha-fetoproteins in early detection of hepatocellular carcinoma. *Cancer Res* 1993; **53**: 5419-5423 [PMID: 7693340]
14 **Liebman HA**. Isolation and characterization of a hepatoma-associated abnormal (des-gamma-carboxy)prothrombin. *Cancer Res* 1989; **49**: 6493-6497 [PMID: 2555045]
15 **Okuda H**, Obata H, Nakanishi T, Furukawa R, Hashimoto E. Production of abnormal prothrombin (des-gamma-carboxy prothrombin) by hepatocellular carcinoma. A clinical and experimental study. *J Hepatol* 1987; **4**: 357-363 [PMID: 3036940 DOI: 10.1016/S0168-8278(87)80546-9]
16 **Yano Y**, Yamashita F, Kuwaki K, Fukumori K, Kato O, Yamamoto H, Ando E, Tanaka M, Sata M. Clinical features of hepatitis C virus-related hepatocellular carcinoma and their association with alpha-fetoprotein and protein induced by vitamin K absence or antagonist-II. *Liver Int* 2006; **26**: 789-795 [PMID: 16911460 DOI: 10.1111/j.1478-3231.2006.01310.x]
17 **Kudo M**. Early detection and curative treatment of early-stage hepatocellular carcinoma. *Clin Gastroenterol Hepatol* 2005; **3**: S144-S148 [PMID: 16234064]
18 **Hirai Y**, Waki I, Momose A, Fukazawa T, Aida T, Takagi K, Hirano T. Increase of O 2p unoccupied electronic states within the ab plane of YBa2Cu3O6.8 due to a superconducting transition. *Phys Rev B Condens Matter* 1992; **45**: 2573-2576 [PMID: 10001800 DOI: 10.1103/PhysRevB.45.2573]
19 **Toyoda H**, Kumada T, Tada T, Kaneoka Y, Maeda A, Kanke F, Satomura S. Clinical utility of highly sensitive Lens culinaris agglutinin-reactive alpha-fetoprotein in hepatocellular carcinoma patients with alpha-fetoprotein & lt; 20 ng/mL. *Cancer Sci* 2011; **102**: 1025-1031 [PMID: 21244578 DOI: 10.1111/j.1349-7006.2011.01875.x]
20 **Tamura Y**, Igarashi M, Kawai H, Suda T, Satomura S, Aoyagi Y. Clinical advantage of highly sensitive on-chip immunoassay for fucosylated fraction of alpha-fetoprotein in patients with hepatocellular carcinoma. *Dig Dis Sci* 2010; **55**: 3576-3583 [PMID: 20407827 DOI: 10.1007/s10620-010-1222-5]
21 **Yamashita F**, Tanaka M, Satomura S, Tanikawa K. Prognostic significance of Lens culinaris agglutinin A-reactive alpha-fetoprotein in small hepatocellular carcinomas. *Gastroenterology* 1996; **111**: 996-1001 [PMID: 8831594 DOI: 10.1016/S0016-5085(96)70067-7]
22 **Kobayashi M**, Kuroiwa T, Suda T, Tamura Y, Kawai H, Igarashi M, Fukuhara Y, Aoyagi Y. Fucosylated fraction of alpha-fetoprotein, L3, as a useful prognostic factor in patients with hepatocellular carcinoma with special reference to low concentrations of serum alpha-fetoprotein. *Hepatol Res* 2007; **37**: 914-922 [PMID: 17610501]
23 **Toyoda H**, Kumada T, Kaneoka Y, Osaki Y, Kimura T, Arimoto A, Oka H, Yamazaki O, Manabe T, Urano F, Chung H, Kudo M, Matsunaga T. Prognostic value of pretreatment levels of tumor markers for hepatocellular carcinoma on survival after curative treatment of patients with HCC. *J Hepatol* 2008; **49**: 223-232 [PMID: 18571271 DOI: 10.1016/j.jhep.2008.04.013]
24 **Saito Y**, Shimada M, Utsunomiya T, Morine Y, Imura S, Ikemoto T, Mori H, Hanaoka J, Yamada S, Asanoma M. Prediction of recurrence of hepatocellular carcinoma after curative hepatectomy using preoperative Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein. *Hepatol Res* 2012; **42**: 887-894 [PMID: 22524419]
25 **Kagebayashi C**, Yamaguchi I, Akinaga A, Kitano H, Yokoyama K, Satomura M, Kurosawa T, Watanabe M, Kawabata T, Chang W, Li C, Bousse L, Wada HG, Satomura S. Automated immunoassay system for AFP-L3% using on-chip electrokinetic reaction and separation by affinity electrophoresis. *Anal Biochem* 2009; **388**: 306-311 [PMID: 19250915 DOI: 10.1016/j.ab.2009.02.030]
26 **Hanaoka T**, Sato S, Tobita H, Miyake T, Ishihara S, Akagi S, Amano Y, Kinoshita Y. Clinical significance of the highly sensitive fucosylated fraction of α-fetoprotein in patients with chronic liver disease. *J Gastroenterol Hepatol* 2011; **26**: 739-744 [PMID: 21083609 DOI: 10.1111/j.1440-1746.2010.06585.x]
27 **Wang CS**, Lin CL, Lee HC, Chen KY, Chiang MF, Chen HS, Lin TJ, Liao LY. Usefulness of serum des-gamma-carboxy prothrombin in detection of hepatocellular carcinoma. *World J Gastroenterol* 2005; **11**: 6115-6119 [PMID: 16273636]
28 **Ishii M**, Gama H, Chida N, Ueno Y, Shinzawa H, Takagi T, Toyota T, Takahashi T, Kasukawa R. Simultaneous measurements of serum alpha-fetoprotein and protein induced by vitamin K absence for detecting hepatocellular carcinoma. South Tohoku District Study Group. *Am J Gastroenterol* 2000; **95**: 1036-1040 [PMID: 10763956 DOI: 10.1111/j.1572-0241.2000.01978.x]
29 **Marrero JA**, Su GL, Wei W, Emick D, Conjeevaram HS, Fontana RJ, Lok AS. Des-gamma carboxyprothrombin can differentiate hepatocellular carcinoma from nonmalignant chronic liver disease in american patients. *Hepatology* 2003; **37**: 1114-1121 [PMID: 12717392 DOI: 10.1053/jhep.2003.50195]
30 **Ueno S**, Tanabe G, Nuruki K, Hamanoue M, Komorizono

Y, Oketani M, Hokotate H, Inoue H, Baba Y, Imamura Y, Aikou T. Prognostic performance of the new classification of primary liver cancer of Japan (4th edition) for patients with hepatocellular carcinoma: a validation analysis. *Hepatol Res* 2002; **24**: 395-403 [PMID: 12479938]

31 **Shiraki K**, Takase K, Tameda Y, Hamada M, Kosaka Y, Nakano T. A clinical study of lectin-reactive alpha-fetoprotein as an early indicator of hepatocellular carcinoma in the follow-up of cirrhotic patients. *Hepatology* 1995; **22**: 802-807 [PMID: 7544756 DOI: 10.1002/hep.1840220317]

32 **Khien VV**, Mao HV, Chinh TT, Ha PT, Bang MH, Lac BV, Hop TV, Tuan NA, Don LV, Taketa K, Satomura S. Clinical evaluation of lentil lectin-reactive alpha-fetoprotein-L3 in histology-proven hepatocellular carcinoma. *Int J Biol Markers* 2001; **16**: 105-111 [PMID: 11471892]

33 **Hagiwara S**, Kudo M, Kawasaki T, Nagashima M, Minami Y, Chung H, Fukunaga T, Kitano M, Nakatani T. Prognostic factors for portal venous invasion in patients with hepatocellular carcinoma. *J Gastroenterol* 2006; **41**: 1214-1219 [PMID: 17287901 DOI: 10.1007/s00535-006-1950-7]

34 **Tada T**, Kumada T, Toyoda H, Kiriyama S, Sone Y, Tanikawa M, Hisanaga Y, Kitabatake S, Kuzuya T, Nonogaki K, Shimizu J, Yamaguchi A, Isogai M, Kaneoka Y, Washizu J, Satomura S. Relationship between Lens culinaris agglutinin-reactive alpha-fetoprotein and pathologic features of hepatocellular carcinoma. *Liver Int* 2005; **25**: 848-853 [PMID: 15998436 DOI: 10.1111/j.1478-3231.2005.01111.x]

35 **Oka H**, Saito A, Ito K, Kumada T, Satomura S, Kasugai H, Osaki Y, Seki T, Kudo M, Tanaka M. Multicenter prospective analysis of newly diagnosed hepatocellular carcinoma with respect to the percentage of Lens culinaris agglutinin-reactive alpha-fetoprotein. *J Gastroenterol Hepatol* 2001; **16**: 1378-1383 [PMID: 11851836 DOI: 10.1046/j.1440-1746.2001.02643.x]

36 **Choi J**, Park Y, Kim JH, Kim HS. Evaluation of revisited fucosylated alpha-fetoprotein (AFP-L3) with an autoanalyzer μTAS in a clinical laboratory. *Clin Chim Acta* 2012; **413**: 170-174 [PMID: 21946051 DOI: 10.1016/j.cca.2011.09.015]

37 **Hayashi K**, Kumada T, Nakano S, Takeda I, Sugiyama K, Kiriyama S, Sone Y, Miyata A, Shimizu H, Satomura S. Usefulness of measurement of Lens culinaris agglutinin-reactive fraction of alpha-fetoprotein as a marker of prognosis and recurrence of small hepatocellular carcinoma. *Am J Gastroenterol* 1999; **94**: 3028-3033 [PMID: 10520864 DOI: 10.1016/S0002-9270(99)00434-7]

P- Reviewers Aspinall RJ, Yang SF, Kataoka H
S- Editor Wen LL **L- Editor** A **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i3.347

World J Gastroenterol 2013 January 21; 19(3): 347-354
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Epidemiology of perforated peptic ulcer: Age- and gender-adjusted analysis of incidence and mortality

Kenneth Thorsen, Jon Arne Søreide, Jan Terje Kvaløy, Tom Glomsaker, Kjetil Søreide

Kenneth Thorsen, Jon Arne Søreide, Tom Glomsaker, Kjetil Søreide, Department of Surgery, Stavanger University Hospital, N-4068 Stavanger, Norway
Jan Terje Kvaløy, Department of Mathematics and Natural Science, University of Stavanger, 4036 Stavanger, Norway
Kenneth Thorsen, Jon Arne Søreide, Kjetil Søreide, Department of Surgical Science, University of Bergen, 5020 Bergen, Norway
Author contributions: Thorsen K contributed to conception, design, acquisition of data, analysis and interpretation of data, drafting the manuscript, evising it critically for important intellectual content and final approval of the version to be published; Søreide JA, Kvaløy JT and Glomsaker T contributed to analysis and interpretation of data, drafting the manuscript, revising it critically for important intellectual content and final approval of the version to be published; Søreide K contributed to conception and design, analysis and interpretation of data, drafting the manuscript, revising it critically for important intellectual content and final approval of the version to be published.
Correspondence to: Kjetil Søreide, MD, PhD, Professor of Surgery, Department of Surgery, Stavanger University Hospital, POB 8100, N-4068 Stavanger, Norway. ksoreide@mac.com
Telephone: +47-5151-8330 Fax: +47-5151-9919
Received: October 24, 2012 Revised: December 12, 2012
Accepted: December 20, 2012
Published online: January 21, 2013

Abstract

AIM: To investigate the epidemiological trends in incidence and mortality of perforated peptic ulcer (PPU) in a well-defined Norwegian population.

METHODS: A retrospective, population-based, single-center, consecutive cohort study of all patients diagnosed with benign perforated peptic ulcer. Included were both gastric and duodenal ulcer patients admitted to Stavanger University Hospital between January 2001 and December 2010. Ulcers with a malignant neoplasia diagnosis, verified by histology after biopsy or resection, were excluded. Patients were identified from the hospitals administrative electronic database using pertinent ICD-9 and ICD-10 codes (K25.1, K25.2, K25.5, K25.6, K26.1, K26.2, K26.5, K26.6). Additional searches using appropriate codes for relevant laparoscopic and open surgical procedures (e.g., JDA 60, JDA 61, JDH 70 and JDH 71) were performed to enable a complete identification of all patients. Patient demographics, presentation patterns and clinical data were retrieved from hospital records and surgical notes. Crude and adjusted incidence and mortality rates were estimated by using national population demographics data.

RESULTS: In the study period, a total of 172 patients with PPU were identified. The adjusted incidence rate for the overall 10-year period was 6.5 per 100 000 per year (95%CI: 5.6-7.6) and the adjusted mortality rate for the overall 10-year period was 1.1 per 100 000 per year (95%CI: 0.7-1.6). A non-significant decline in adjusted incidence rate from 9.7 to 5.6 occurred during the decade. The standardized mortality ratio for the whole study period was 5.7 (95%CI: 3.9-8.2), while the total 30-d mortality was 16.3%. No difference in incidence or mortality was found between genders. However, for patients $\geqslant$ 60 years, the incidence increased over 10-fold, and mortality more than 50-fold, compared to younger ages. The admission rates outside office hours were high with almost two out of three (63%) admissions seen at evening/night time shifts and/or during weekends. The observed seasonal variations in admissions were not statistically significant.

CONCLUSION: The adjusted incidence rate, seasonal distribution and mortality rate was stable. PPU frequently presents outside regular work-hours. Increase in incidence and mortality occurs with older age.

© 2013 Baishideng. All rights reserved.

Key words: Perforated peptic ulcer; Epidemiology; Incidence; Mortality; Seasonal variation

Thorsen K, Søreide JA, Kvaløy JT, Glomsaker T, Søreide K. Epidemiology of perforated peptic ulcer: Age- and gender-adjusted analysis of incidence and mortality. *World J Gastroenterol* 2013; 19(3): 347-354 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i3/347.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i3.347

INTRODUCTION

Each year peptic ulcer disease (PUD) affects 4 million people around the world[1]. Complications are encountered in 10%-20% of these patients and 2%-14% of the ulcers will perforate[2,3]. Perforated peptic ulcer (PPU) is a quite rare, but life threatening disease and the mortality varies from 10%-40%[2,4-6]. Females account for more than half the cases, they are older and have more comorbidity than their male counterparts[6]. Main etiologic factors include use of non-stereoidal anti-inflammatory drugs (NSAIDs), steroids, smoking, *Helicobacter pylori* (*H. pylori*) and a diet high in salt[3,7]. All these factors have in common that they affect acid secretion in the gastric mucosa. Defining the exact etiological factor in any given patient may often be difficult, as more than one risk factor may be present and they tend to interact[8]. While previous reports have shown a seasonal variation in the incidence of PPU, others have failed to find such a pattern[9-11].

The incidence rate of PPU has, with some fluctuations, been fairly stable in Northern Europe for decades with reported annual incidence rates of about 4-11 per 100 000 per year[12,13]. Several studies have investigated this up to the beginning of the 21st century, however there is hardly any data on incidence reports from this area the last decade. Due to the high mortality and morbidity rates from PPU, it is of importance to understand the epidemiology to, if possible, enable preventive measures.

The aim of this study was to investigate the recent epidemiological incidence trends and presentation of benign perforated gastroduodenal peptic ulcer in a well-defined Norwegian population.

MATERIALS AND METHODS

Study population

In this retrospective study all patients diagnosed with benign perforated peptic ulcer (either gastric ulcer or duodenal ulcer) admitted to Stavanger University Hospital (SUH) between January 2001 and December 2010 were included. Ulcers with a malign neoplasia diagnosis, verified by histology after biopsy or resection, were excluded.

SUH serves as the only hospital in the greater Stavanger area and has a primary mixed urban and rural catchment area of about 330 000 (per January, 2012). The "twin cities" of Stavanger and Sandnes are together the third most populated area in Norway, and Stavanger is the densest populated city in Norway.

Patients were identified from the hospitals administrative electronic database using pertinent ICD-9 and ICD-10 codes (K25.1, K25.2, K25.5, K25.6, K26.1, K26.2, K26.5, K26.6). Additional searches using appropriate codes for various surgical procedures (JDA 60 gastroraphy, JDA 61 laparoscopic gastroraphy, JDH 70 duodenoraphy, JDH 71 laparoscopic duodenoraphy) were done to enable a complete identification of all patients. Patient demographics and clinical data were retrieved from hospital records and surgical notes. Crude and adjusted mortality rates were estimated.

Definitions

Incidence calculations: The crude incidence is the number of new cases of perforated peptic ulcer in our region per 100 000 persons per year. The adjusted incidencerates are the crude rates adjusted for age and gender *vs* the total Norwegian population using Statistics Norway. The crude death rate is the total number of deaths in the study population per 100 000 persons per year. The adjusted mortality rate is the crude death rate adjusted for age and gender *vs* the total Norwegian population using Statistics Norway.

The standardized mortality ratio is the number of observed deaths within 30 d of hospital admission in the study group divided by the number of expected deaths in a general population with the same age and sex distribution.

Ulcer localisation was regarded as gastric when present anywhere in the stomach, including prepyloric and pyloric ulcers. Postpyloric ulcers were classified as duodenal. One ulcer located in an anastomosis after a gastrectomy was regarded as a gastric ulcer. The diagnosis and exact localisation was made at operation in most cases, while some were verified at postoperative endoscopy.

Comorbidity was defined as any concomitant disease at the time of admittance for PPU, including cardiovascular disease, pulmonary disease, autoimmune disorders and known or previous cancer disease in patient history.

NSAIDs included acetylic acid as part of the NSAID group in this study.

Seasons were classified as spring (March, April and May); late spring/summer (May, June, July and August); summer (June, July and August); autumn (September, October and November); and winter (December, January and February).

Evening/night time was defined as the hours between 16:30 and 07:30 all days of the week, including Saturday and Sunday.

Study ethics

The study was approved as a quality control assurance according to general guidelines provided by the Regional Ethics Committee Vest (REK Vest).

Statistical analysis

PASW Statistics 19.0 for Mac (SPSS Inc., Chicago, IL) was used for statistical analysis. Descriptive analyses were performed using χ^2 or Fisher's exact test where appropri-

Table 1 Patient characteristics according to ulcer localisation *n* (%)

	Duodenal ulcer *n* = 60	Gastric ulcer *n* = 112	Total *n* = 172	*P* value
Age				
< 60 yr	18 (30)	37 (33)	55 (32)	0.68
≥ 60 yr	42 (70)	75 (67)	117 (68)	
Gender				
Female	27 (45)	62 (55)	89 (52)	0.20
Male	33 (55)	50 (45)	83 (48)	
ASA status				0.99
1	0	0	0	
2	2 (3)	3 (3)	5 (3)	
3	34 (57)	63 (56)	97 (56)	
4	20 (33)	39 (35)	59 (34)	
5	4 (7)	7 (6)	11 (6)	
Social status				0.32
Married	37 (62)	58 (52)	95 (55)	
Divorced	3 (5)	8 (7)	11 (6)	
Widowed	9 (15)	28 (25)	37 (22)	
Single	6 (10)	7 (6)	13 (8)	
Unknown	5 (8)	11 (10)	16 (9)	
Smokers[1]	26 (54)	60 (69)	86 (64)	0.09
NSAID-use	26 (43)	50 (45)	76 (44)	0.87
Steroid-use	5 (8)	11 (10)	16 (9)	0.73
Comorbidity	45 (75)	97 (87)	142 (82)	0.06
Cardiovascular disease	27 (45)	50 (45)	77 (45)	0.89
Pulmonary disease	8 (13)	19 (17)	27 (16)	0.53
Autoimmune disease	13 (22)	18 (16)	31 (18)	0.36
Malignancy (past or current)	6 (10)	23 (21)	29 (17)	0.08
Past ulcer history	8 (13)	18 (16)	26 (15)	0.36
30-d mortality	14 (23)	14 (13)	28 (16)	0.07

P values are for trends across groups. Percentages may not add up due to rounding. [1]Data on smoking was missing in 37 (22%) of patients. Percentages are of patients with reported data (*n* = 135). ASA: American Society of Anesthesiology; NSAIDs: Non-steroidal anti-inflammatory drugs.

ate for dichotomous data, and Mann-Whitney *U* test for continuous data, where applicable. Poisson regression analysis was used to test for differences in crude rates between different periods and between different population groups. Time-trends were also tested by a Poisson time series model. Logistic regression was used to test for association with gender and comorbidity. All tests are two sided and *P* values < 0.050 were regarded as statistically significant.

RESULTS

Patient characteristics

A total of 172 patients with a perforated peptic ulcer were identified between 2001 and 2010. Median age was 68 years ranging from 18 to 100 years. There was an equal gender distribution (52% were women), but women were significantly older than men (median age of 73 years *vs* 62 years, respectively, $P < 0.001$). The number of women affected increased significantly with age across age groups, with only one in four patients aged < 50 years of age being female, compared to two-thirds of those > 70 years of age being female (26% *vs* 65% women, respectively, $P = 0.002$). Further patient characteristics are given in Table 1.

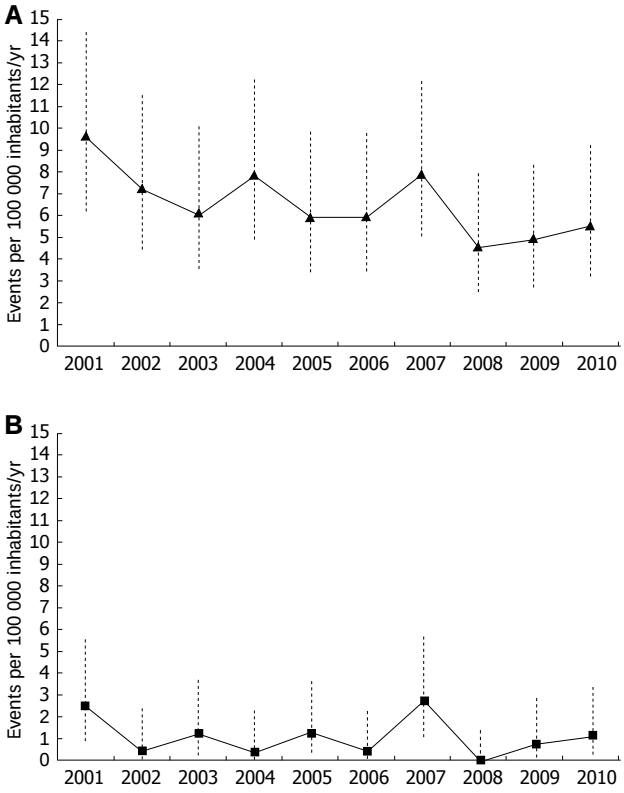


Figure 1 Incidence and mortality of perforated peptic ulcer from 2001-2010. A: The incidence rates of perforated peptic ulcer (PPU) per 100 000 inhabitants per year, adjusted for age and gender; B: The mortality rate from PPU per 100 000 inhabitants per year, adjusted for age and gender.

Crude and adjusted incidence trends

The crude incidence rate of PPU during the study period was 5.6 per 100 000 per year and declined from 8.5 in 2001 to 4.6 in 2010. The adjusted incidence rate for the overall 10-year period was 6.5 per 100 000 per year (95%CI: 5.6-7.6) and declined from 9.7 to 5.6 over the decade, but neither crude nor adjusted incidence rate decline were statistically significant (Figure 1A). Patients ≥ 60 years of age accounted for 117 of 172 (68%), and remained the dominant age group throughout the decade.

When considering the incidence in specific age- and gender-groups, the adjusted incidence rate for men demonstrated considerable differences, with men < 60 years at 2.7 per 100 000 per year (95%CI: 1.9-3.8) compared to 22.1 (95%CI: 16.3-29.4) for men ≥ 60 years of age. A similar pattern was seen in women, with women < 60 years at 1.5 per 100 000 per year (95%CI: 0.9-2.4), while it was 26.1 for those aged ≥ 60 (95%CI: 20.3-32.9) for the whole period. This corresponds to an almost 10-fold increase for men and a corresponding 17-fold increase for women in incidence for those aged ≥ 60 years.

An indicated decline in PPU numbers seen in Figure 1A is evident (but not statistically significant) in those aged > 70 years when the decade was divided in two peri-

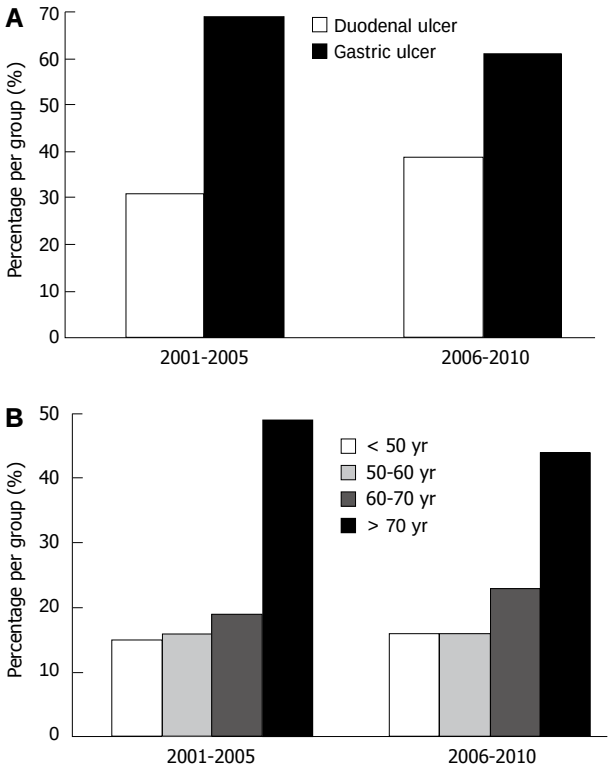


Figure 2 Localisation of ulcers and distribution of age-groups. A: Perforated peptic ulcer (PPU) localisation with admission rates over time. The decade is divided in two periods from 2001-2005 and 2006-2010; B: PPU presentation according to age groups (age in years; < 50, 50-59, 60-69, > 70) divided into the two time periods.

ods, as illustrated in Figure 2.

Crude and adjusted mortality trends

The crude mortality rate for the study period was 0.9 and the adjusted mortality rate for the overall 10-year period was 1.1 per 100 000 per year (95%CI: 0.7-1.6) and was stable during the whole period (Figure 1B). The standardized mortality ratio for the whole study period was 5.7 (95%CI: 3.9-8.2), while the total 30-d mortality was 16.3%.

The adjusted mortality rate for men < 60 years was 0.1 per 100 000 per year (95%CI: 0.0-0.43) and 4.6 (95%CI: 2.21-8.48) for men aged ⩾ 60 years. For women the adjusted mortality rate for the whole decade for those aged < 60 years was 0.1 (95%CI: 0.0-0.45) while it was 5.6 (95%CI: 3.13-9.22) for those aged ⩾ 60 years. This demonstrated negligible differences between genders, but an almost 50 to 60 times increase in mortality among the elderly age group.

Ulcer localisation

Gastric ulcers predominated (Figure 2A) and accounted for 112 of 172 (65%) patients in this study, but declined during the latter years of the period, while the frequency of duodenal ulcers remained stable, but increased somewhat the latter period (Figure 2). Prepyloric ulcers represented 61 of 112 (54%) of gastric ulcers and 21 of 112 (19%) were located in the pylorus. In the corpus/fundus area 12 of 112 (11%) ulcers were observed, while 8 of 112 (7%) were located in the antrum. One ulcer was located in an anastomosis and 9 of 112 (8%) ulcers were missing exact localisation in the stomach, but being classified as gastric ulcers at operation.

Age and comorbidity

Of those aged > 60 years of age 105 of 117 (90%) had comorbidity compared to 37 of 55 (67%) of those aged ⩽ 60 years ($P < 0.001$). Women also had significantly higher rate of comorbidity compared to men (91% *vs* 74%, respectively, $P = 0.002$), also when adjusted for age ($P = 0.036$).

Associated etiologic factors

Data on smoking was obtained in 135 of 172 patients. Of the 135 with confirmed information on smoking habits, 86 (64%) were smokers. Consequently, at least 50% of the total 172 patients were smokers, but likely this is an underestimate. Of those aged < 60 years of age 37 of 44 (84%) were smokers, while 49 of 91 (54%) of those aged ⩾ 60 were smokers ($P = 0.001$). The number of smokers registered was stable during the decade.

The number of NSAID users was stable during the decade studied and were used by 47 of 89 women and 29 of 83 men (53% *vs* 35%, respectively, $P = 0.018$). Also, NSAID-use was more common in those aged ⩾ 60 years (55 of 117; 47%), compared to the younger patients aged < 60 years (20 of 55; 36%), but this was not statistically significant.

Patterns of seasonal and circadian presentation

Patients were admitted at all hours and days during the week. While 70 of 172 patients (41%) were admitted during weekends (Friday, Saturday and Sunday), more than half (91 of 172; 53%) of the patients were admitted during evening/night time shifts and 109 of 172 (63%) patients were admitted either during evening/night time and/or on Saturday/Sunday. Notably, during this time a lower staff: patient ratio than during regular office hours is present.

PPU was more frequent in patients ⩾ 60 years of age (117 of 172; 68%) than < 60 years of age (55 of 172; 32%). This was particularly evident during the four months of late spring/summer. Poisson regression and time series analyses revealed no statistical significant differences regarding monthly variation or seasonal variation for PPU admittance patterns (Figure 3).

DISCUSSION

In this single-institution, population based study we found an adjusted incidence rate of 6.5 (95%CI: 5.5-7.5) per 100 000/year during this period. This is in concordance to several Northern European studies, most of them reporting an incidence between 4-11 per 100 000/year[13-17].

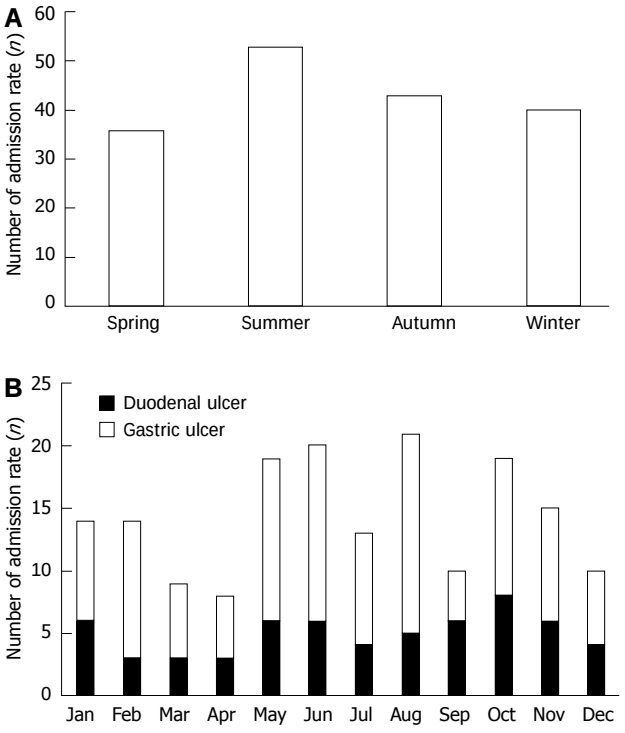


Figure 3 Seasonal and monthly variation in admissions. A: Seasonal variation in admissions overall; B: The monthly variation in admissions with numbers for gastric and duodenal ulcers, respectively.

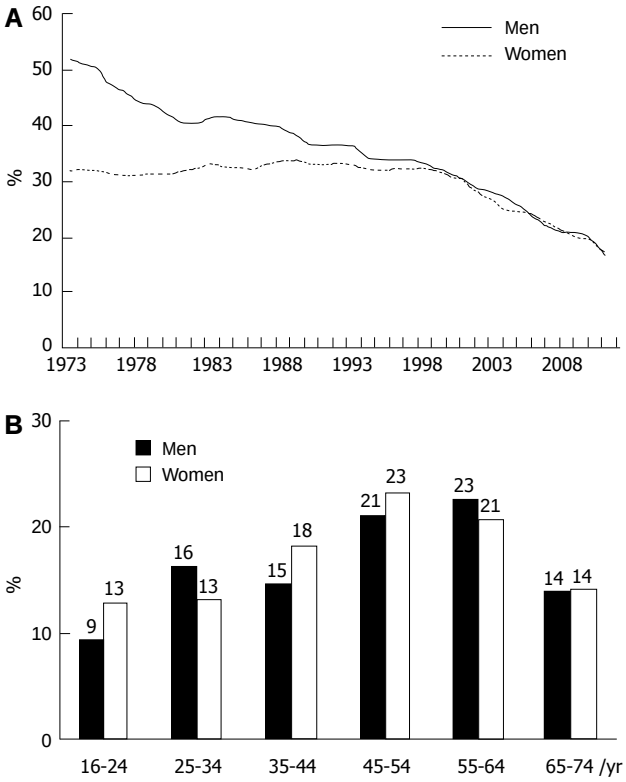


Figure 4 Change in prevalence of daily smokers in Norway. A: The time trend of daily smokers over time in the Norwegian population aged between 16-74 years, showing a decline over time for each gender; B: The distribution of smokers according to age groups and gender for the fiscal year 2011. Data are reproduced and presented from the Norwegian Directorate of Health (http://www.helsedirektoratet.no, accessed October 5, 2012).

Several factors may influence the incidence rates of PPU. For one, epidemiological studies have demonstrated that birth-cohorts born up to the 1930s were at higher risk of acquiring PPU than later birth cohorts[15,18]. The reason for this is not known, but speculation of an overriding influence of *H. pylori* infection on the population, has been postulated as the main cause[18]. However, *H. pylori* infection has been deemed of less importance in perforated peptic ulcer disease compared to that of uncomplicated peptic ulcer disease[1,7]. However, the association between specific birth-cohorts and mortality from PUD has been quite convincing, and a decline in the incidence of PPU may thus be expected when the cohorts at risk disappear with time[4,18]. This may have affected the incidence rates also in this study, which are rates comparable to other epidemiological studies from the turn of the century[19], and which may reflect a stable scenario with a relatively low population at risk for PPU in the current century.

Another etiologic factor for perforated peptic ulcer is smoking[20,21]. Prospective data from SUH between 1987 and 1993 observed that 58 of 104 (56%) of all PPU patients were smokers[22], reflecting the current data. The percentage of smokers in the PPU population may reflect this as a known risk for ulcer disease and perforation. Notably, in 2011 the number of daily smokers in Norway (Figure 4) is reduced to half the number compared to 2001 data[23], and consequently this risk factor has decreased dramatically over just a decade. The decrease in smoking habits is seen in all the age groups from 16-64 years, but most evident in the age group from 16-44 years[23]. As previously stated, PPU is much more common in older than younger people, and the declining number of smokers in the population should influence incidence rates of PPU with time. However, a decade of decline in smoking in the population may not be enough time to reveal this. Also, an increasing age-population and preserved smoking habits in the older may contribute to a stable incidence still.

Medications may influence PPU incidence and occurrence in several ways, both as risk factors and as preventive means. NSAID-use is associated to peptic ulcers and to peptic ulcer complications, however, the majority of PPUs are not related to NSAIDs[24]. In recent years an increase of NSAID-use has been seen and according to numbers from the Norwegian pharmaceutical association: both acetylic acid (14% increase), diclofenac (35% increase) and ibuprofen (26% increase) usage increased from 2006 to 2010 (over the counter sales included), while the usage of the most common PPI (Esomeprazol) was stable[25]. Previous data from Australia has also shown increasing use of NSAIDs in the population without increasing incidence rates of PPU[26]. A study of peptic ulcer complications in the Swedish population over three decades up to 2002, found declining incidence rates after 1988, when PPI were introduced in Sweden. The authors concluded that the reasons for this most likely were multifactorial, but then

including an effect of PPI[14]. However, few studies have found an effect of PPI or H2 blockers on the PPU incidence or mortality rates[13].

The overall mortality at 16.3% is in comparison to other contemporary European studies from Denmark, the United Kingdom and the Netherlands ranging from 12% to 27%[27-29], but differs from a recent South Korean study reporting mortality at 3%[30]. The difference may be explained by methodology to retrieve data, as the European studies are hospital-based cohorts, and the South Korean study is based on a national health insurance claims database. In one Danish national cohort[31], the overall mortality was 27%, and reduced to 17% for patients entered in a pre-specified perioperative protocol to reduce mortality[31]. In the current population under study with an overall mortality of 16%, there was no pre-specified protocol in use, but institutional trend towards an increased use of pre-operative computed tomography for diagnosis and laparoscopy as mode of intervention during the study period[6].

Ulcer site definition may be confusing, since some classify prepyloric and pyloric ulcers as duodenal[10], while others classify those ulcers as part of the stomach and hence gastric ulcers[13]. Further, extensive perioperative and inflammatory tissue changes may make it difficult to distinguish between the duodenum and the pyloric area during the operation, hence clinical misclassification can obviously occur. Nevertheless, gastric ulcers predominated in this study and this is in accordance to similar reports from Norway, The Netherlands and Iceland[32-34]. However, a non-significant decline of perforated gastric ulcers was seen in the latter years of the decade, while the frequency of duodenal ulcers was stable. Decreasing incidence of gastric ulcers in those aged > 70 years have also been shown in a large study from England and Wales[17]. We did not identify any associations according to ulcer site, but a trend towards younger men having more duodenal ulcers and older women having more gastric ulcers were seen. This has been shown in similar studies before and may represent a somewhat different aetiology, with older, female patients more often having NSAIDs exposure prior to PPU episodes[17,35]. The latter was also seen in this study.

The off-hour admission rates were high with almost two of three patients admitted at either evening/night time shifts or during weekends (i.e., Saturday/Sunday). Consequently, a high proportion of potentially very sick patients requiring an acute operation will be seen when senior staff may not be immediately available or in-house, which may potentially delay a correct work-up and timely diagnosis and consequently operative intervention, than if admitted at office hours. However, we do not have data to substantiate this potential association in the current study.

Several studies have addressed seasonal variation of perforated peptic ulcer[9,11,36,37]. In this study a peak of perforated peptic ulcers was observed during the four months of late spring/summer, but this pattern faded when seasons where divided in three months. However a previous study from Western Norway over 5.5 decades found similar seasonal variations and those variations were consistent over time[38]. However, no statistical significance was found for variations over time, and this is in line with previous reports that failed to find such patterns[9,10].

The retrospective nature of this study limits the accuracy of data quality, which would have been obtained by a prospective manner for some variables, such as exposure to risk factors (i.e., smoking status; medications used, *etc.*) which may be subject to bias by underreporting of past history. However, with a population-based catchment area and a well-developed health system of general physicians and a universal health insurance program for all citizens in Norway, patients are usually admitted with admission notes containing past history, although this may obviously be more prone to failure outside office hours and for emergency referrals. Due to restrictions of the number of patients, we are cautious of performing unwarranted subgroup analysis, and may thus not have been able to confirm significant trends found in other studies.

In conclusion, the adjusted incidence rates in the first decade of the 21st century was stable and reflected the decline seen towards the end of the 20th century. Smoking cessation in the general population and a reduction in the population represented by birth cohorts at higher risk are two important factors that may influence the current low incidence of PPU, compared to that in past decades. Perforated peptic ulcer continues to present outside regular work-hours in over half the time and frequently during weekends, with little difference in seasonal distribution. Mortality is unchanged and stable and is most considerable in the aged population. This may be further subject to change with an increasingly elderly population and should be followed by population-based monitoring for this disease over time.

COMMENTS

Background

Perforated peptic ulcer (PPU) is a life threatening disease with historically reported high morbidity and mortality rates. Disease epidemiology has changed during the last century, but current data on epidemiological trends in PPU is lacking. The aim of this study is to investigate epidemiological trends in a well-defined Norwegian population over a decade.

Research frontiers

Central research in perforated peptic ulcer the latter years has focused on safety of laparoscopy as primary operation and regarding short term mortality after operation. Few studies have presented data regarding epidemiology over the last decade. This study demonstrates a stable incidence and mortality rate over a decade.

Innovations and breakthroughs

A change in peptic ulcer disease epidemiology the last decades came with the discovery of *Helicobacter pylori* with the subsequent eradication therapy with antibiotica, and the introduction of proton pump inhibitors. For perforated peptic ulcers little has changed except the use of laparoscopy as a surgical alternative for repair. The mortality remains high and the incidence has been stable.

Applications

The epidemiology of perforated peptic ulcer appears to be stable with few changes compared to the very recent past. A better understanding of the aetiol-

ogy may be warranted for better prevention and reduction of incidence. Understanding factors contributing to mortality will be important to further improve outcomes.

Peer review

The study is an interesting, well designed and well written one. It will help to shed light on the current situation regarding peptic ulcer disease.

REFERENCES

1 **Zelickson MS**, Bronder CM, Johnson BL, Camunas JA, Smith DE, Rawlinson D, Von S, Stone HH, Taylor SM. Helicobacter pylori is not the predominant etiology for peptic ulcers requiring operation. *Am Surg* 2011; **77**: 1054-1060 [PMID: 21944523]

2 **Bertleff MJ**, Lange JF. Perforated peptic ulcer disease: a review of history and treatment. *Dig Surg* 2010; **27**: 161-169 [PMID: 20571260 DOI: 10.1159/000264653]

3 **Lau JY**, Sung J, Hill C, Henderson C, Howden CW, Metz DC. Systematic review of the epidemiology of complicated peptic ulcer disease: incidence, recurrence, risk factors and mortality. *Digestion* 2011; **84**: 102-113 [PMID: 21494041]

4 **Svanes C**. Trends in perforated peptic ulcer: incidence, etiology, treatment, and prognosis. *World J Surg* 2000; **24**: 277-283 [PMID: 10658061 DOI: 10.1007/s002689910045]

5 **Møller MH**, Adamsen S, Wøjdemann M, Møller AM. Perforated peptic ulcer: how to improve outcome? *Scand J Gastroenterol* 2009; **44**: 15-22 [PMID: 18752147 DOI: 10.1080/00365520802307997]

6 **Thorsen K**, Glomsaker TB, von Meer A, Søreide K, Søreide JA. Trends in diagnosis and surgical management of patients with perforated peptic ulcer. *J Gastrointest Surg* 2011; **15**: 1329-1335 [PMID: 21567292 DOI: 10.1007/s11605-011-1482-1]

7 **Gisbert JP**, Legido J, García-Sanz I, Pajares JM. Helicobacter pylori and perforated peptic ulcer prevalence of the infection and role of non-steroidal anti-inflammatory drugs. *Dig Liver Dis* 2004; **36**: 116-120 [PMID: 15002818 DOI: 10.1016/j.dld.2003.10.011]

8 **Kurata JH**, Nogawa AN. Meta-analysis of risk factors for peptic ulcer. Nonsteroidal antiinflammatory drugs, Helicobacter pylori, and smoking. *J Clin Gastroenterol* 1997; **24**: 2-17 [PMID: 9013343]

9 **Manfredini R**, De Giorgio R, Smolensky MH, Boari B, Salmi R, Fabbri D, Contato E, Serra M, Barbara G, Stanghellini V, Corinaldesi R, Gallerani M. Seasonal pattern of peptic ulcer hospitalizations: analysis of the hospital discharge data of the Emilia-Romagna region of Italy. *BMC Gastroenterol* 2010; **10**: 37 [PMID: 20398297]

10 **Janik J**, Chwirot P. Perforated peptic ulcer--time trends and patterns over 20 years. *Med Sci Monit* 2000; **6**: 369-372 [PMID: 11208340]

11 **Svanes C**, Sothern RB, Sørbye H. Rhythmic patterns in incidence of peptic ulcer perforation over 5.5 decades in Norway. *Chronobiol Int* 1998; **15**: 241-264 [PMID: 9653578]

12 **Paimela H**, Tuompo PK, Peräkyl T I, Höckerstedt K, Kivilaakso E. Peptic ulcer surgery during the H2-receptor antagonist era: a population-based epidemiological study of ulcer surgery in Helsinki from 1972 to 1987. *Br J Surg* 1991; **78**: 28-31 [PMID: 1671826]

13 **Christensen A**, Bousfield R, Christiansen J. Incidence of perforated and bleeding peptic ulcers before and after the introduction of H2-receptor antagonists. *Ann Surg* 1988; **207**: 4-6 [PMID: 2892468]

14 **Hermansson M**, Ekedahl A, Ranstam J, Zilling T. Decreasing incidence of peptic ulcer complications after the introduction of the proton pump inhibitors, a study of the Swedish population from 1974-2002. *BMC Gastroenterol* 2009; **9**: 25 [PMID: 19379513 DOI: 10.1186/1471-230X-9-25]

15 **Svanes C**, Lie RT, Kvåle G, Svanes K, Søreide O. Incidence of perforated ulcer in western Norway, 1935-1990: cohort- or period-dependent time trends? *Am J Epidemiol* 1995; **141**: 836-844 [PMID: 7717360]

16 **Paimela H**, Oksala NK, Kivilaakso E. Surgery for peptic ulcer today. A study on the incidence, methods and mortality in surgery for peptic ulcer in Finland between 1987 and 1999. *Dig Surg* 2004; **21**: 185-191 [PMID: 15249752 DOI: 10.1159/000079654]

17 **Higham J**, Kang JY, Majeed A. Recent trends in admissions and mortality due to peptic ulcer in England: increasing frequency of haemorrhage among older subjects. *Gut* 2002; **50**: 460-464 [PMID: 11889062 DOI: 10.1136/gut.50.4.460]

18 **Sonnenberg A**. Time trends of ulcer mortality in Europe. *Gastroenterology* 2007; **132**: 2320-2327 [PMID: 17570207 DOI: 10.1053/j.gastro.2007.03.108]

19 **Lassen A**, Hallas J, Schaffalitzky de Muckadell OB. Complicated and uncomplicated peptic ulcers in a Danish county 1993-2002: a population-based cohort study. *Am J Gastroenterol* 2006; **101**: 945-953 [PMID: 16573778]

20 **Svanes C**, Søreide JA, Skarstein A, Fevang BT, Bakke P, Vollset SE, Svanes K, Søreide O. Smoking and ulcer perforation. *Gut* 1997; **41**: 177-180 [PMID: 9301495 DOI: 10.1136/gut.41.2.177]

21 **Andersen IB**, Jørgensen T, Bonnevie O, Grønbaek M, Sørensen TI. Smoking and alcohol intake as risk factors for bleeding and perforated peptic ulcers: a population-based cohort study. *Epidemiology* 2000; **11**: 434-439 [PMID: 10874551]

22 **Søreide JA**, Søndenaa K, Ulvestad E. Helicobacter pylori Status after Treatment for Perforated Gastroduodenal Ulcer: A Cross-Sectional Study Utilizing Serology. *Dig Surg* 1997; **14:** 366-370

23 Statistics Norway. Available from: URL: http://www.ssb.no (Accessed September 6, 2012)

24 **Svanes C**, Øvrebø K, Søreide O. Ulcer bleeding and perforation: non-steroidal anti-inflammatory drugs or Helicobacter pylori. *Scand J Gastroenterol Suppl* 1996; **220**: 128-131 [PMID: 8898450]

25 Norwegian Pharmacy Association. Available from: URL: http: //www.apotek.no (Accessed July 6, 2012)

26 **Henry D**, Robertson J. Nonsteroidal anti-inflammatory drugs and peptic ulcer hospitalization rates in New South Wales. *Gastroenterology* 1993; **104**: 1083-1091 [PMID: 8462796]

27 **Møller MH**, Engebjerg MC, Adamsen S, Bendix J, Thomsen RW. The Peptic Ulcer Perforation (PULP) score: a predictor of mortality following peptic ulcer perforation. A cohort study. *Acta Anaesthesiol Scand* 2012; **56**: 655-662 [PMID: 22191386 DOI: 10.1111/j.1399-6576.2011.02609.x]

28 **Hemmer PH**, de Schipper JS, van Etten B, Pierie JP, Bonenkamp JJ, de Graaf PW, Karsten TM. Results of surgery for perforated gastroduodenal ulcers in a Dutch population. *Dig Surg* 2011; **28**: 360-366 [PMID: 22086121 DOI: 10.1159/000331320]

29 **Critchley AC**, Phillips AW, Bawa SM, Gallagher PV. Management of perforated peptic ulcer in a district general hospital. *Ann R Coll Surg Engl* 2011; **93**: 615-619 [PMID: 22041238 DOI: 10.1308/003588411X13165261994030]

30 **Bae S**, Shim KN, Kim N, Kang JM, Kim DS, Kim KM, Cho YK, Jung SW. Incidence and short-term mortality from perforated peptic ulcer in Korea: a population-based study. *J Epidemiol* 2012; **22**: 508-516 [PMID: 22955110 DOI: 10.2188/jea.JE20120056]

31 **Møller MH**, Adamsen S, Thomsen RW, Møller AM. Multicentre trial of a perioperative protocol to reduce mortality in patients with peptic ulcer perforation. *Br J Surg* 2011; **98**: 802-810 [PMID: 21442610 DOI: 10.1002/bjs.7429]

32 **Forsmo HM**, Glomsaker T, Vandvik PO. Perforated peptic ulcer--a 12-year material. *Tidsskr Nor Laegeforen* 2005; **125**:

1822-1824 [PMID: 16012551]
33 **Eiriksson K**, Oddsdottir M, Magnusson J. Peptic ulcer perforations, University Hospital of Iceland 1989-1995. *Laeknabladid* 1998; **84**: 466-473 [PMID: 19667452]
34 **Bertleff MJ**, Halm JA, Bemelman WA, van der Ham AC, van der Harst E, Oei HI, Smulders JF, Steyerberg EW, Lange JF. Randomized clinical trial of laparoscopic versus open repair of the perforated peptic ulcer: the LAMA Trial. *World J Surg* 2009; **33**: 1368-1373 [PMID: 19430829 DOI: 10.1007/s00268-009-0054-y]
35 **Wysocki A**, Budzyński P, Kulawik J, Drożdż W. Changes in the localization of perforated peptic ulcer and its relation to gender and age of the patients throughout the last 45 years. *World J Surg* 2011; **35**: 811-816 [PMID: 21267567 DOI: 10.1007/s00268-010-0917-2]
36 **Adler J**, Ingram D, House T. Perforated peptic ulcer--a seasonal disease? *Aust N Z J Surg* 1984; **54**: 59-61 [PMID: 6586169]
37 **Christensen A**, Hansen CP, Thagaard C, Lanng C. Seasonal periodicity of perforated gastric ulcer. *Dan Med Bull* 1988; **35**: 281-282 [PMID: 3168554]
38 **Svanes C**, Salvesen H, Stangeland L, Svanes K, Søreide O. Perforated peptic ulcer over 56 years. Time trends in patients and disease characteristics. *Gut* 1993; **34**: 1666-1671 [PMID: 8282252 DOI: 10.1136/gut.34.12.1666]

P- Reviewer Abdel-Salam OMElS **S- Editor** Song XX
L- Editor A **E- Editor** Li JY



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i3.355

World J Gastroenterol 2013 January 21; 19(3): 355-361
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Biliary fistula after treatment for hydatid disease of the liver: When to intervene

Nazif Zeybek, Hakan Dede, Deniz Balci, Ali Kagan Coskun, Ismail Hakki Ozerhan, Subutay Peker, Yusuf Peker

Nazif Zeybek, Hakan Dede, Ali Kagan Coskun, Ismail Hakki Ozerhan, Subutay Peker, Yusuf Peker, Department of Surgery, Gulhane Military Medical Academy, 06010 Ankara, Turkey
Deniz Balci, Department of Surgery, Ankara University School of Medicine, 06410 Ankara, Turkey
Author contributions: Zeybek N, Dede H and Peker Y designed the research; Dede H, Coskun AK and Ozerhan IH performed the research; Balci D and Peker S analyzed the data; Zeybek N and Balci D wrote the paper.
Correspondence to: Deniz Balci, MD, Department of Surgery, Ankara University School of Medicine, 06410 Ankara, Turkey. deniz.balci@medicine.ankara.edu.tr
Telephone: +90-532-5981276 Fax: +90-312-2879687
Received: May 6, 2012 Revised: November 12, 2012
Accepted: November 24, 2012
Published online: January 21, 2013

Abstract

AIM: To determine the outcome of patients with biliary fistula (BF) after treatment for hydatid disease of the liver.

METHODS: Between January 2000 and December 2010, out of 301 patients with a diagnosis of hydatid cyst of the liver, 282 patients who underwent treatment [either surgery or puncture, aspiration, injection and reaspiration (PAIR) procedure] were analysed. Patients were grouped according to the presence or absence of postoperative biliary fistula (PBF) (PBF *vs* no-PBF groups, respectively). Preoperative clinical, radiological and laboratory characteristics, operative characteristics including type of surgery, peroperative detection of BF, postoperative drain output, morbidity, mortality and length of hospital stays of patients were compared amongst groups. Multivariate analysis was performed to detect factors predictive of PBF. Receiver operative characteristics (ROC) curve analysis were used to determine ideal cutoff values for those variables found to be significant. A comparison was also made between patients whose fistula closed spontaneously (CS) and those with intervention in order to find predictive factors associated with spontaneous closure.

RESULTS: Among 282 patients [median (range) age, 23 (16-78) years; 77.0% male]; 210 (74.5%) were treated with conservative surgery, 33 (11.7%) radical surgery and 39 (13.8%) underwent percutaneous drainage with PAIR procedure A PBF developed in 46 (16.3%) patients, all within 5 d after operation. The maximum cyst diameter and preoperative alkaline phosphatase levels (U/L) were significantly higher in the PBF group than in the no-PBF group [10.5 ± 3.7 U/L *vs* 8.4 ± 3.5 U/L (P < 0.001) and 40.0 ± 235.1 U/L *vs* 190.0 ± 167.3 U/L (P = 0.02), respectively]. Hospitalization time was also significantly longer in the PBF group than in the no-PBF group [37.4 ± 18.0 d *vs* 22.4 ± 17.9 d (P < 0.001)]. A preoperative high alanine aminotransferase level (> 40 U/L) and a peroperative attempt for fistula closure were significant predictors of PBF development (P = 0.02, 95%CI: -0.03-0.5 and P = 0.001, 95%CI: 0.1-0.4), respectively. Comparison of patients whose PBF CS or with biliary intervention (BI) revealed that the mean diameter of the cyst was not significantly different between CS and BI groups however maximum drain output was significantly higher in the BI group (81.6 ± 118.1 cm *vs* 423.9 ± 298.4 cm, P < 0.001). Time for fistula closure was significantly higher in the BI group (10.1 ± 3.7 d *vs* 30.7 ± 15.1 d, P < 0.001). The ROC curve analysis revealed cut-off values of a maximum bilious drainage < 102 mL and a waiting period of 5.5 postoperative days for spontaneous closure with the sensitivity and specificity values of (83.3%-91.1%, AUC: 0.90) and (97%-91%, AUC: 0.95), respectively. The multivariate analysis demonstrated a PBF drainage volume < 102 mL to be the only statistically significant predictor of spontaneous closure (P < 0.001, 95%CI: 0.5-1.0).

CONCLUSION: Patients with PBF after hydatid surgery often have complicated postoperative course with serious morbidity. Patients who develop PBF with an output < 102 mL might be managed expectantly.

© 2013 Baishideng. All rights reserved.

Key words: Hydatid disease; Biliary fistula; Postoperative complications; Surgery

Zeybek N, Dede H, Balci D, Coskun AK, Ozerhan IH, Peker S, Peker Y. Biliary fistula after treatment for hydatid disease of the liver: When to intervene. *World J Gastroenterol* 2013; 19(3): 355-361 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i3/355.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i3.355

INTRODUCTION

Hydatid disease, which is caused by the larval form of *Echinococcus granulosus*, is endemic in Mediterranean countries, the Middle East, Australia and South America and has a worldwide distribution[1]. Despite a decreasing incidence, Turkey is an endemic country according to the Ministry of Health database with more than 14 000 cases recorded from 2001-2005[2]. Although this zoonosis is rare in the United States and Europe, it is a gradually increasing cause of morbidity and mortality in these regions, largely due to travel and ongoing immigration[3].

As a definitive treatment, surgery was considered to be relatively safe based on over 4 decades of accumulated experience[4], but a recent meta-analysis of 14 studies analyzing the outcomes of surgery in endemic areas reported minor and major complication rates of 33.0% and 25.1%, respectively, with a mortality rate of 0.7%[5]. Intrabiliary rupture (IBR), which involves a communication ofthe cyst cavity, which is under high pressure,with the biliary system, is the most common and troublesome complication[6]. IBR was reported to occur at a relatively high rate of 5%-30% of the cases, and it has a wide range of complications, such as biliary obstruction, fistula, infection and secondary biliary cirrhosis[7,8]. If not promptly diagnosed and treated, these biliary complications may result in serious postoperative morbidity and mortality[9]. However, most IBRs are occult in nature, and patients may remain clinically silent in most cases, without any specific radiologicalor laboratory findings being observed in the preoperative work-upand with the IBRs only being discovered during or after surgery. The presence of a postoperative biliary fistula (PBF) is believed to determine the prognosis in these patients[10].

In fact, there is limited information regarding the natural course and outcome of those patients who had an IBR that was not treated successfully and developed a PBF. We previously reported our experience with the treatment of liver hydatiddisease[11]. In this study, we aimed to report the clinical significance of PBF after treatment for hydatid disease in a separate cohort of patients and to identify clinical predictors associated with the closure of PBF.

MATERIALS AND METHODS

Between January 2000 and December 2010, 301 patients with a diagnosis of hydatid cyst of the liver were admitted to the Gulhane Military Medical Academy Hospital, Ankara, Turkey. The data were gathered from a prospectively collected database and the electronic medical records of the patients, including age, gender, medical history of hydatid disease, main symptoms and findings, abdominal ultrasonographic cyst characteristics (number of cysts, single, or multiple; presence or absence of other organs involved with the disease), chest radiography, presence of preoperative complications (jaundice, dilation of the biliary tree, intrabiliary, peritoneal or intrathoracic rupture), type of surgical procedure performed, postoperative daily follow-up of complications, duration of stay after the operation and mortality.

All of the patients underwent a complete blood cell count and liver function test determinations. An abdominal ultrasonographic examination (USG) was performed in all of the patients, but computed tomography (CT) was not used routinely. An indirect hemagglutination test was used for the serological confirmation of the diagnosis.

The liver cysts were classified into 5 types according to their ultrasonographic appearance[12]: type Ⅰ, a simple hydatid cyst with pure fluid collection; type Ⅱ, a cyst containing undulated hyperechogenic membranes that float in the cystic fluid (the detached germinative layer of the endocyst); type Ⅲ, a cyst containing secondary vesicles (daughter cysts); type Ⅳ, a cyst with a heterogeneous echopattern and filled with a matrix or amorphous mass; and type Ⅴ, a cyst with a thick and calcified wall. The presence of irregular linear echogenic structures without acoustic shadowing in the bile duct and/or the dilated biliary tract was accepted as a suggestive USG finding of IBR[13]. All of the patients with these USG findings were further evaluated with either abdominal CT or magnetic resonance cholangiopancreatography (MRCP).

In total, 19 patients were excluded from the analysis. This group included patients discharged without treatment (5 patients), patients with a preoperative diagnosis of cholangitis (the presence of 2 out of the following 3 symptoms or findings: fever > 38 ℃, right upper abdominal pain and jaundice) or patients with suggestive findings of IBR on USG (9 patients) who underwent further evaluation with endoscopic retrograde cholangiography (ERCP) preoperatively. Four additional patients with perioperative common bile duct exploration plus T-tube placement and a patient who died of anaphylactic shock after the operation due to free rupture of the hydatid cyst into the peritoneal cavity were excluded.

The technique of the procedure was mainly selected according to the World Health Organization guidelines on the management of the echinococcal disease, with criteria including the size, type and location of the cyst and the general medical condition of the patient[14]. In patients with significant comorbidities who refused surgery and had cysts > 5 cm that were Gharbi Types 1-2 on USG were treated with the puncture, aspiration, injection and reaspiration (PAIR) procedure[15]. Open surgical

Table 1 Preoperative characteristics of patients with hydatid disease of the liver (mean ± SD)

	Bile fistula		*P* value
	Absent	Present	
Age, yr	32.0	31.5	0.8
Gender (M/F)	179/57	38/8	0.3
Cholangitis	6 (3%)	24 (52%)	< 0.001
Albendazol treatment	118 (50%)	23 (50%)	0.7
AST (U/L)	221.0 ± 32.2	44.0 ± 36.0	0.5
ALT (U/L)	223.0 ± 41.2	44.0 ± 38.4	0.8
ALP (U/L)	40.0 ± 235.1	190.0 ± 167.3	0.02
GGT (U/L)	25.0 ± 71.7	151.0 ± 64.3	0.08
Total bilirubin (mg/dL)	0.9 ± 1.44	1.5 ± 3.0	0.05
White blood cell	8077.3 ± 2945.0	8802.2 ± 3222.7	0.1

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; ALP: Alkaline phosphatase; GGT: Gamma-glutamyltranspeptidase.

procedures were classified as radical for any type of liver resection and en-bloc cystectomy[16]. A partial cystectomy with tube drainage, capitonnage or omentoplasty was considered to be a conservative procedure[17]. In patients with multiple or communicating cysts, the same surgical procedures were used. The intraoperative detection of a biliary fistula (BF) was at the discretion of the attending surgeon. Uniformly, the cyst cavity was opened, explored for a BF or resection surface and carefully evaluated for a bile leak. Further measures included cholecystectomy and saline or methylene blue injection from the cystic duct stump. A PBF was defined as any drain output consistent with a biliary appearance and a bilirubin count higher than the synchronous serum bilirubin count within 5 d postoperatively.

The patients were grouped according to the presence or absence of PBF (PBF *vs* no-PBF groups, respectively). The preoperative clinical, radiological and laboratory characteristics, operative characteristics including the type of surgery, perioperative detection of BF, postoperative drain output, morbidity, mortality, and length of hospital stay of the patients were compared among the groups. A comparison was also performed between the patients whose fistula closed spontaneously and those who received intervention. The postoperative biliary interventions included ERCP with sphincterotomy and stent placement or nasobiliary tube drainage. Percutaneous drainage catheter insertions were performed under USG guidance.

The following variables were analyzed as potential predictors of PBF development: age, sex, findings on physical examination, leukocyte count, preoperative higher-than-normal liver function test results [alanine aminotransferase (ALT), aspartate aminotransferase, alkaline phosphatase (ALP), gamma-glutamyltranspeptidase, and total bilirubin levels], ultrasonographic cyst features (type, diameter, number, and localization), operation type and perioperative attempted fistula closure.

Statistical analysis

The quantitative data were compared using the *t* test, and the categorical data were compared using the Pearson χ^2 test or Fisher's exact test for the presence of a PBF. Receiver operating characteristic (ROC) curve analysis with calculations of the area under the curve (AUC) was used to determine the ideal cutoff values for those variables that were found to be significant. Logistic regression analysis was used for the multivariate analysis, with a $P < 0.20$ being entered into the model, and a $P < 0.05$ was considered to be significant. All of the statistics were performed using the SPSS 18.0 software (SPSS, Inc., Chicago, IL, United States). The study was approved by the local Ethics Committee of the Gulhane Military Medical Academy.

RESULTS

Of the 282 patients, 217 (77%) were male, and 65 (23%) were female. The median age was 23 years (range 16-78 years). A preoperative diagnosis of cholangitis was found in 30 (10.6%) of the patients. The duration of the symptoms ranged from 1 mo to 7 years (median, 3 mo) (Table 1).

Overall, 178 patients (63.1%) had a single cyst, and 104 (36.9%) had multiple cysts. The cysts were localized only in the right lobe of the liver in 150 (53.2%) patients and in the left in 39 (13.8%), and bilobar involvement was observed in 93 (33%). A total of 484 hepatic cysts were treated in the 282 patients. Thirty-two patients (11.3%) had concomitant cysts in the lung, and 17(6%) had cysts elsewhere.

According to the largest cyst diameter in the USG evaluation, 79 (28%) were type Ⅰ, 35 (12.4%) were type Ⅱ, 127 (45%) were type Ⅲ, 35 (12.4%) were type Ⅳ, and 3 (1.1%) were type Ⅴ. The cyst diameter ranged from 2 cm to 20 cm (median, 8.0 cm) (Table 2).

Operations and perioperative interventions

Of the 282 patients, 210 (74.5%) were treated with conservative surgery, 33 (11.7%) were treated with radical surgery, and 39 (13.8%) underwent percutaneous drainage with the PAIR procedure (Table 2). Three patients (9%) treated with radical surgery, 6 (15%) treated with the PAIR procedure and 37 (17.6%) treated with conservative surgery developed a PBF ($P = 0.44$). An infected cyst cavity was found in 13 (4.6%) patients, with 1 developing a PBF. Perioperative suture ligation of the detected cyst-biliary communication was attempted in 39 (13.8%) patients, with 11 (4.2%) developing a PBF.

Outcome of patients with PBF

In total, PBF developed in 46 (16.3%) patients, all of whom were diagnosed within 5 d after the operation. The maximum cyst diameter (cm) and preoperative ALP levels (U/L) were significantly higher in the PBF group than in the no PBF group (10.5 ± 3.7 U/L *vs* 8.4 ± 3.5 U/L, $P < 0.001$; and 40.0 ± 235.1 U/L *vs* 190.0 ± 167.3 U/L, $P = 0.02$, respectively). The hospitalization time was also significantly longer in the PBF group (37.4 ± 18.0 d *vs* 22.4 ± 17.9 d, $P < 0.001$) (Table 3).

The multivariate analysis revealed that high preopera-

Table 2 Characteritics of hydatid cysts in patients with hydatid disease of the liver (mean ± SD)

Cyst	Bile fistula		*P* value
	Absent *n* = 236	Present *n* = 46	
Size localisation (cm)			
Diameter	8.46 ± 3.52	10.59 ± 3.74	0.0001
Right lobe	127 ± 45.9	23 ± 8.2	0.2
Left lobe	36 ± 12.8	3 ± 1.1	
Bilobar	73 ± 25.9	20 ± 7.1	
Type			
I	68 ± 24.4	11 ± 3.9	0.7
II	30 ± 10.8	5 ± 1.8	
III	102 ± 36.6	102 ± 36.6	
IV	30 ± 10.8	5 ± 1.8	
V	3 ± 1.1	0	
Operation type, *n* (%)			
Conservative surgery	173 (61.3)	37 (13.1)	0.4
Radical surgery	30 (10.6)	3 (1.1)	
PAIR	33 (11.7)	6 (2.1)	

PAIR: Puncture, aspiration, injection and reaspiration.

tive ALT levels (> 40 U/L) and a perioperative attempted fistula closure were significant predictors of PBF development ($P = 0.02$, 95%CI: -0.03-0.5 and $P = 0.001$, 95%CI: 0.1-0.4, respectively).

Factors related to spontaneous fistula closure

The patients with PBF were further divided into 2 groups regarding fistula closure-either spontaneous (CS group, 12 patients, 4.6%) or with biliary intervention (BI group, 34 patients, 12.1%). The mean diameter of the cyst was not significantly different between the CS and BI groups (9.9 ± 4.1 cm *vs* 10.8 ± 3.6 cm). The maximum drain output was significantly higher in the BI group (81.6 ± 118.1 mL *vs* 423.9 ± 298.4 mL, $P < 0.001$). The time for the fistula closure was significantly higher in the BI group (10.1 ± 3.7 d *vs* 30.7 ± 15.1 d, $P < 0.001$).

The ROC curve analysis revealed cut-off values of a maximum bilious drainage < 102 mL and a waiting period of 5.5 postoperative days for spontaneous closure with sensitivity and specificity values of 83.3%-91.1% (AUC: 0.90) and 91%-97% (AUC: 0.95), respectively.

The multivariate analysis demonstrated a postoperative biliary drainage volume < 102 mL to be the only significant predictor of spontaneous closure ($P < 0.001$, 95%CI: 0.5-1.0), and the presence of multiple cysts on preoperative imaging approached significance ($P = 0.06$, 95%CI: 0.2-0.5).

In the BI group, 15 (5.3%) patients had an ERCP with sphincterotomy, and 19 (6.7%) had an ERCP and NBD. Two patients required repeated ERCPs and plastic stent placement. In addition, 5 (10.8%) patients required catheter placement with USG guidance to control the biliary collections that were not adequately drained.

There were 3 (1.1%) deaths in our series: 2 due to spontaneous rupture of the cyst with subsequent anaphylactic shock and 1 who underwent multiple biliary interventions due to PBF and died due to myocardial infarction.

Table 3 Postoperative characteristics of patients with postoperative biliary fistula (mean ± SD)

	Biliary fistula closed		*P* value
	Spontaneous *n* = 12	Intervention *n* = 34	
Maximum output (mL)	81.6 ± 118.1	423.9 ± 298.4	< 0.001
Drain removal (d)	13.0 ± 6.0	27.1 ± 12.8	0.001
Maximum cyst diameter (cm)	9.9 ± 4.1	10.8 ± 3.6	0.4
Time to fistula closure (d)	10.1 ± 3.7	30.7 ± 15.1	< 0.001
Time to intervention (d)	10.1 ± 3.7	11.4 ± 6.7	0.5
Hospitalization (d)	22.4 ± 17.9	11.4 ± 6.7	< 0.001

DISCUSSION

This study investigated the outcome of patients with PBF due to the IBR of a hepatic hydatid cyst. Whether minimally invasive techniques, conservative surgery or radical surgery were used, the goal of treatment was to eliminate the parasite completely and prevent complications, including pressure on adjacent structures, secondary infection or rupture of the cyst into the biliary system that results in PBF. In our series, PBF occurred in 46 (16%) patients, despite preoperative and operative efforts to prevent this complication. The fistula closed spontaneously in only 12 (26%) of the patients with PBF without further intervention, confirming that patients with PBF often have a complicated postoperative course, requiring multiple endoscopic and other interventional procedures with serious morbidity and mortality[18,19].

Although there are several surgical techniques described to control cyst-biliary communications intraoperatively, 10%-32% of cases eventually develop a PBF[20]. A cyst-biliary communication complicates the natural history of the disease and the treatment processes. Aktan *et al*[21] previously reported a median intracystic pressure of 25 mmHg (range 5-55 mmHg) and a positive correlation between cyst size and pressure in viable cysts. Manometric studies indicate that the sphincter of Oddi pressure (basal 10 mmHg, peak 124 mmHg) is higher than the normal common bile duct pressure (10 mmHg). According to the LaPlace Law, the expansion of the cyst due to increased intracystic pressure results in increased cyst diameter, which causes increased tension on the cyst wall that serves as the pericyst containing the neighboring bile ducts. The pressure dynamics arising from higher intracystic pressures than the resting bile duct pressure may explain how the cyst communicates with the biliary system as the pericyst becomes thinner and thinner, eventually eroding into the bile ducts and leading to the development of an IBR[22].

Several studies reported a history of cholangitis, high bilirubin, high ALP levels, a cyst larger than 10 cm and the presence of suggestive USG findings as clinical predictors of IBR, and an ERCP was suggested to delineate the presence of cyst-biliary communication in these cas-

es[8,13]. Our analysis also showed that a high preoperative ALP, history of cholangitis and larger cyst diameter (> 10 cm) were significantly more common in patients that developed PBF. However, the multivariate analysis revealed that a perioperative attempt at suture ligation and a high preoperative ALT level were significant predictors of PBF. Perioperative suture ligation was performed in 39 patients in this cohort, and 11 (28%) patients developed a PBF. We have not encountered any reports on the success rate of perioperative suture ligation attempts in the literature. Our data indicate that there might be a significant failure rate for an attempt to control a detected BF intraoperatively if that was used as the only method to control the cyst-biliary communication.

Our analysis showed no significant difference in the development of PBF based on the type of procedure. During the hydatid surgery in a given patient with a cyst-biliary communication, regardless of whether a conservative or radical approach (total pericystectomy) is taken, the same biliary pedicles will be encountered, and the fistula risk should be the same unless each communicating pedicle is individually detected and effectively controlled. In our experience, PBF developed in 3 patients [3 (9%) out of 33 patients in the radical surgery group) despite at least 1 attempt to control the fistula with suture ligation, suggesting that controlling all of the detected fistulae with suture ligation may not even be possible, especially when the fistula orifices are deeply located in the cyst cavity and liver. Furthermore, our data indicate that certain fistulae may not even be detected intraoperatively. Other than technical failure, such misses may be partially explained by the abovementioned fluctuating pressure dynamics in the biliary system arising from intermittent Oddi sphincter contractions, which may cause a cyst-biliary communication that is not visible at the time of the surgery unless further manipulation is performed. We agree with others that all interventions on liver hydatidosis should be considered to be potentially major operations and that further intraoperative intervention searching for a cyst-biliary communication is warranted in patients with preoperative risk factors[23]. Our results underline the importance of preoperative detection and effective treatment of the cyst-biliary communication. A preoperative MRCP for patients with clinical and laboratory risk factors could detect biliary complications, and an intervention with ERCP and sphincterotomy could decrease the pressure in the biliary tract, with the further benefit of providing useful anatomical information for the surgeon to select the best therapeutic approach[24]. This information may enable the surgeon to safely perform a conservative approach and avoid a possible cholecystectomy and/or biliary exploration procedure that potentially confers further morbidity, especially in patients with previous symptoms or a history of cholangitis. These patients have a cyst that communicates with the biliary system, resulting in an ascendant infection of the cyst contents. Although bacterial superinfection may kill the parasite, this phenomenon causes extensive inflammation with the subsequent adhesion of the duodenum and colon to the cyst, which renders biliary exploration potentially dangerous. There is a growing body of literature suggesting that it is not the type of surgery but rather the preoperative determination of cyst-biliary communication that is more important in avoiding PBF[25,26].

In contrast to post-cholecystectomy fistulae, which close rapidly after ERCP and sphincterotomy, hydatid fistulae were reported to be more resistant and rarely closed spontaneously without decompression of the biliary tract[27]. Endoscopic treatment has been advocated in high-output fistulae with a duration of more than 1 wk and no signs of reduction and in low-output fistulae with a duration of more than 3 wk and no signs of reduction[28]. The ROC curve analysis enabled the detection of a maximum biliary drainage < 102 mL and a waiting period of 5.5 postoperative days for spontaneous closure (sensitivity and specificity values of 83.3%-91.1%, AUC: 0.90 and 91%-97%, AUC: 0.95, respectively) as significant cut-off levels, and multivariate analysis demonstrated a postoperative biliary drainage volume < 102 mL to be the only significant predictor of spontaneous closure (P < 0.001, 95%CI: 0.5-1.0). This information might help with the decision of when to attempt further intervention in a patient with PBF to prevent delays in treatment.

There are several limitations of this study. In addition to the study's retrospective nature and the lack of a uniform treatment protocol, our results may also be influenced by patient selection bias for each type of surgical or PAIR procedure. Furthermore, there was intersurgeon variability in terms of technical experience, which might have an effect on operative outcomes.

This study showed that an aggressive approach to the detection of cyst-biliary communications, both preoperatively and intraoperatively, is warranted to prevent the development of PBF and thus to avoid serious morbidity and mortality in patients with hydatid disease of the liver. Patients who develop PBF with a fistula output < 102 mL may be managed expectantly, but timely endoscopic intervention and aggressive monitoring is necessary to prevent further complications.

COMMENTS

Background

Hydatid disease, which is caused by *Echinococcus granulosus*, has a worldwide distribution, resulting with tissues developing cysts containing the parasite located mainly in the liver in humans. There are several treatment options including, medical, interventional and surgical modalities. Intrabiliary rupture (IBR), which involves a communication of the cyst cavity with the biliary system is the most common and troublesome complication resulting with a biliary fistula (BF) Development of BF complicates the natural history and outcomes of the disease treatment.

Research frontiers

To date, there is limited information regarding to the development of BF after hydatid disease treatment and its outcomes. Several risk factors including the size and location of the cyst in the liver have been documented however, very few groups reported the outcome of the patients with BF. Furthermore, treat-

ment of BF after treatment of hydatid disease is seldom reported. In order to prevent BF complication; developing multimodality approaches for detecting patients with risk factors preoperatively and developing guidelines for selecting the most suitable surgical or conservative management for these patients as well as best postoperative treatment for this complication is an active field of research.

Innovations and breakthroughs

The authors found certain risk factors in preoperative radiology and biochemistry tests of patients for development of a BF. However, this study is different from others in two aspects. The authors' analysis revealed that certain amount of patients undergoing treatment for hydatid disease of the liver would eventually develop a BF regardless of the method chosen to control the disease. Secondly, the authors documented that detecting a BF intraoperatively and attempting to control by the current standard surgical means may not be enough to control the development of BF hence emphasizing the importance of preoperative detection and aggressive intervention. The authors were also able to find a cut-off value for postoperative BF drainage output in order to choose between expectant or interventional treatment.

Applications

The study results suggest that an aggressive approach to the detection of IBR both preoperatively and intraoperatively is warranted to prevent the development of a postoperative BF and thus to avoid serious morbidity and mortality in patients with hydatid disease of the liver. Patients who develop postoperative BF with a fistula output < 102 mL may be managed expectantly.

Peer review

The manuscript is well designed, with clear objective. The manuscript aim to determine the outcome of patients with BF after treatment for hydatid disease of the liver. The authors found certain risk factors in preoperative radiology and biochemistry tests of patients for development of a BF.

REFERENCES

1 **Moro P**, Schantz PM. Echinococcosis: a review. *Int J Infect Dis* 2009; **13**: 125-133 [PMID: 18938096 DOI: 10.1016/j.ijid.2008.03.037]

2 **Yazar S**, Ozkan AT, Hökelek M, Polat E, Yilmaz H, Ozbilge H, Ustün S, Koltaş IS, Ertek M, Sakru N, Alver O, Cetinkaya Z, Koç Z, Demirci M, Aktaş H, Parsak CK, Ozerdem D, Sakman G, Cengiz ZT, Ozer A, Keklik K, Yemenici N, Turan M, Daştan A, Kaya E, Tamer GS, Girginkardeşler N, Türk M, Sinirtaş M, Evci C, Kiliçturgay S, Mutlu F, Artiş T. [Cystic echinococcosis in Turkey from 2001-2005]. *Turkiye Parazitol Derg* 2008; **32**: 208-220 [PMID: 18985573]

3 **Khuroo MS**, Wani NA, Javid G, Khan BA, Yattoo GN, Shah AH, Jeelani SG. Percutaneous drainage compared with surgery for hepatic hydatid cysts. *N Engl J Med* 1997; **337**: 881-887 [PMID: 9302302 DOI: 10.1056/NEJM199709253371303]

4 **Saidi F**, Nazarian I. Surgical treatment of hydatid cysts by freezing of cyst wall and instillation of 0.5 per cent silver nitrate solution. *N Engl J Med* 1971; **284**: 1346-1350 [PMID: 5576454 DOI: 10.1056/NEJM197106172842403]

5 **Smego RA**, Bhatti S, Khaliq AA, Beg MA. Percutaneous aspiration-injection-reaspiration drainage plus albendazole or mebendazole for hepatic cystic echinococcosis: a meta-analysis. *Clin Infect Dis* 2003; **37**: 1073-1083 [PMID: 14523772 DOI: 10.1086/378275]

6 **Hamamci EO**, Besim H, Sonisik M, Korkmaz A. Occult intrabiliary rupture of hydatid cysts in the liver. *World J Surg* 2005; **29**: 224-226 [PMID: 15654660 DOI: 10.1007/s00268-004-7571-5]

7 **Langer JC**, Rose DB, Keystone JS, Taylor BR, Langer B. Diagnosis and management of hydatid disease of the liver. A 15-year North American experience. *Ann Surg* 1984; **199**: 412-417 [PMID: 6712316 DOI: 10.1097/00000658-198404000-00007]

8 **Atli M**, Kama NA, Yuksek YN, Doganay M, Gozalan U, Kologlu M, Daglar G. Intrabiliary rupture of a hepatic hydatid cyst: associated clinical factors and proper management. *Arch Surg* 2001; **136**: 1249-1255 [PMID: 11695968 DOI: 10.1001/archsurg.136.11.1249]

9 **Erzurumlu K**, Dervisoglu A, Polat C, Senyurek G, Yetim I, Hokelek M. Intrabiliary rupture: an algorithm in the treatment of controversial complication of hepatic hydatidosis. *World J Gastroenterol* 2005; **11**: 2472-2476 [PMID: 15832420]

10 **El Malki HO**, El Mejdoubi Y, Souadka A, Mohsine R, Ifrine L, Abouqal R, Belkouchi A. Predictive factors of deep abdominal complications after operation for hydatid cyst of the liver: 15 years of experience with 672 patients. *J Am Coll Surg* 2008; **206**: 629-637 [PMID: 18387467 DOI: 10.1016/j.jamcollsurg.2007.11.012]

11 **Yagci G**, Ustunsoz B, Kaymakcioglu N, Bozlar U, Gorgulu S, Simsek A, Akdeniz A, Cetiner S, Tufan T. Results of surgical, laparoscopic, and percutaneous treatment for hydatid disease of the liver: 10 years experience with 355 patients. *World J Surg* 2005; **29**: 1670-1679 [PMID: 16311852 DOI: 10.1007/s00268-005-0058-1]

12 **Gharbi HA**, Hassine W, Brauner MW, Dupuch K. Ultrasound examination of the hydatic liver. *Radiology* 1981; **139**: 459-463 [PMID: 7220891]

13 **Kilic M**, Yoldas O, Koc M, Keskek M, Karakose N, Ertan T, Gocmen E, Tez M. Can biliary-cyst communication be predicted before surgery for hepatic hydatid disease: does size matter? *Am J Surg* 2008; **196**: 732-735 [PMID: 18513700 DOI: 10.1016/j.amjsurg.2007.07.034]

14 Guidelines for treatment of cystic and alveolar echinococcosis in humans. WHO Informal Working Group on Echinococcosis. *Bull World Health Organ* 1996; **74**: 231-242 [PMID: 8789923]

15 **Ustünsöz B**, Akhan O, Kamiloğlu MA, Somuncu I, Uğurel MS, Cetiner S. Percutaneous treatment of hydatid cysts of the liver: long-term results. *AJR Am J Roentgenol* 1999; **172**: 91-96 [PMID: 9888746]

16 **Akbulut S**, Senol A, Sezgin A, Cakabay B, Dursun M, Satici O. Radical vs conservative surgery for hydatid liver cysts: experience from single center. *World J Gastroenterol* 2010; **16**: 953-959 [PMID: 20180233 DOI: 10.3748/wjg.v16.i8.953]

17 **Safioleas MC**, Misiakos EP, Kouvaraki M, Stamatakos MK, Manti CP, Felekouras ES. Hydatid disease of the liver: a continuing surgical problem. *Arch Surg* 2006; **141**: 1101-1108 [PMID: 17116803 DOI: 10.1001/archsurg.141.11.1101]

18 **Gollackner B**, Längle F, Auer H, Maier A, Mittlböck M, Agstner I, Karner J, Langer F, Aspöck H, Loidolt H, Rockenschaub S, Steininger R. Radical surgical therapy of abdominal cystic hydatid disease: factors of recurrence. *World J Surg* 2000; **24**: 717-721 [PMID: 10773125 DOI: 10.1007/s002689910115]

19 **Regev A**, Reddy KR, Berho M, Sleeman D, Levi JU, Livingstone AS, Levi D, Ali U, Molina EG, Schiff ER. Large cystic lesions of the liver in adults: a 15-year experience in a tertiary center. *J Am Coll Surg* 2001; **193**: 36-45 [PMID: 11442252 DOI: 10.1016/S1072-7515(01)00865-1]

20 **Galati G**, Sterpetti AV, Caputo M, Adduci M, Lucandri G, Brozzetti S, Bolognese A, Cavallaro A. Endoscopic retrograde cholangiography for intrabiliary rupture of hydatid cyst. *Am J Surg* 2006; **191**: 206-210 [PMID: 16442947 DOI: 10.1016/j.amjsurg.2005.09.014]

21 **Aktan AO**, Yalin R. Preoperative albendazole treatment for liver hydatid disease decreases the viability of the cyst. *Eur J Gastroenterol Hepatol* 1996; **8**: 877-879 [PMID: 8889454]

22 **Geenen JE**, Toouli J, Hogan WJ, Dodds WJ, Stewart ET, Mavrelis P, Riedel D, Venu R. Endoscopic sphincterotomy: follow-up evaluation of effects on the sphincter of Oddi. *Gastroenterology* 1984; **87**: 754-758 [PMID: 6468866]

23 **Agaoglu N**, Türkyilmaz S, Arslan MK. Surgical treatment of hydatid cysts of the liver. *Br J Surg* 2003; **90**: 1536-1541 [PMID: 14648733 DOI: 10.1002/bjs.4313]

24 **Dumas R**, Le Gall P, Hastier P, Buckley MJ, Conio M, Del-

mont JP. The role of endoscopic retrograde cholangiopancreatography in the management of hepatic hydatid disease. *Endoscopy* 1999; **31**: 242-247 [PMID: 10344429 DOI: 10.1055/s-1999-14209]

25 **Dziri C**, Haouet K, Fingerhut A. Treatment of hydatid cyst of the liver: where is the evidence? *World J Surg* 2004; **28**: 731-736 [PMID: 15457348 DOI: 10.1007/s00268-004-7516-z]

26 **Tagliacozzo S**, Miccini M, Amore Bonapasta S, Gregori M, Tocchi A. Surgical treatment of hydatid disease of the liver: 25 years of experience. *Am J Surg* 2011; **201**: 797-804 [PMID: 20832053 DOI: 10.1016/j.amjsurg.2010.02.011]

27 **Rodriguez AN**, Sánchez del Río AL, Alguacil LV, De Dios Vega JF, Fugarolas GM. Effectiveness of endoscopic sphincterotomy in complicated hepatic hydatid disease. *Gastrointest Endosc* 1998; **48**: 593-597 [PMID: 9852449 DOI: 10.1016/S0016-5107(98)70041-0]

28 **Skroubis G**, Vagianos C, Polydorou A, Tzoracoleftherakis E, Androulakis J. Significance of bile leaks complicating conservative surgery for liver hydatidosis. *World J Surg* 2002; **26**: 704-708 [PMID: 12053223 DOI: 10.1007/s00268-002-6259-y]

P- Reviewer Grande L **S- Editor** Lv S
L- Editor A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i3.362

World J Gastroenterol 2013 January 21; 19(3): 362-365
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Right recumbent position on gastric emptying of water evidenced by ^{13}C breath testing

Masaki Sanaka, Yoshihisa Urita, Takatsugu Yamamoto, Tsuguru Shirai, Satoshi Kimura, Hitoshi Aoyagi, Yasushi Kuyama

Masaki Sanaka, Department of Medical Pharmacy, Faculty of Pharmaceutical Sciences, Josai International University, Chiba 283-8555, Japan
Masaki Sanaka, Yoshihisa Urita, Department of General Medicine and Emergency Care, School of Medicine, Toho University, Tokyo 143-8540, Japan
Takatsugu Yamamoto, Tsuguru Shirai, Satoshi Kimura, Hitoshi Aoyagi, Yasushi Kuyama, Department of Internal Medicine, School of Medicine, Teikyo University, Tokyo 173-8605, Japan
Author contributions: Sanaka M analyzed the data and wrote the paper; Sanaka M and Urita Y designed the research study; Yamamoto T, Shirai T, Kimura S, Aoyagi H and Kuyama Y performed the research.
Correspondence to: Takatsugu Yamamoto, MD, PhD, Department of Internal Medicine, School of Medicine, Teikyo University, 2-11-1 Kaga, Itabashi-ku, Tokyo 173-8605, Japan. ymmtmze@zpost.plala.or.jp
Telephone: +81-3-39641211 Fax: +81-3-53751308
Received: February 3, 2012 Revised: December 11, 2012
Accepted: December 15, 2012
Published online: January 21, 2013

Abstract

AIM: To compare the impact of the right recumbent position with the sitting position on gastric emptying of water.

METHODS: In eight healthy male volunteers, the 13C acetate breath test was performed twice to assess gastric emptying of 100 mL tap water. Subjects were seated in one test and lying on their right side in the other. In both positions, pulmonary $^{13}CO_2$ exhalation curves were obtained by plotting breath data against time. Percent gastric retention curves were created by analyzing data using the Wagner-Nelson protocol.

RESULTS: No significant posture effect was found in pulmonary $^{13}CO_2$ output curves (P = 0.2150), whereas a significant effect was seen in gastric retention curves (P = 0.0315). The percent retention values at 10 min and 15 min were significantly smaller when subjects were in the right recumbent position compared with the seated position ($P < 0.05$). Our results verified the accelerating effect of the right recumbent position on gastric emptying of non-nutritive solutions. Concerning clinical implications, this study suggests that placing patients with acute pain on their right side after oral administration of analgesic drugs in solution is justified as an effective practice for rapid pain relief. For patients with gastrointestinal reflux symptoms, sleeping in the right recumbent position may reduce nocturnal symptoms, as delayed gastric emptying can cause reflux symptoms.

CONCLUSION: Gastric emptying of water occurs more quickly when a subject lies on the right side compared with sitting.

© 2013 Baishideng. All rights reserved.

Key words: Gastric emptying; Breath test; Right recumbent position; Water

Sanaka M, Urita Y, Yamamoto T, Shirai T, Kimura S, Aoyagi H, Kuyama Y. Right recumbent position on gastric emptying of water evidenced by ^{13}C breath testing. *World J Gastroenterol* 2013; 19(3): 362-365 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i3/362.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i3.362

INTRODUCTION

Gastric emptying of orally ingested contents is a great concern to clinicians because it is associated with gastrointestinal symptoms and can affect the efficacy of medications. Little absorption takes place in the stomach,

and any oral medication must therefore pass beyond the stomach before being absorbed within the small intestine. The rate of gastric emptying is determined by the balance between the propulsive force (fundic tonic pressure and antral contractions) and the outlet resistance (pyloric and duodenal contractions). For calorie-containing meals, the force-resistance balance is primarily regulated by the nutrient-evoked feedback loop that strictly controls duodenal entry of meals so as to optimize intestinal absorption of nutrients[1]. On the other hand, for non-caloric fluids (e.g., water and normal saline), the feedback system is barely activated and consequently, the stomach behaves like "a motionless bag" in which the fluids passively flow back and forth depending on gravity[2]. Considering this gravity-dependent flow, the idea has been proposed that, among the variety of body positions, the right lateral recumbent position makes emptying of non-nutritive fluids the fastest[3].

Positioning a patient has traditionally been regarded as an effective maneuver to modulate the absorption of orally administered drugs in solution. In the case of oral overdosing, for which drug absorption should be delayed, being recumbent on the left side ("pylorus up" status) is advisable.; In the case of acute pain, for which the rapid onset of analgesic effect is favored, being recumbent on the right side ("pylorus down" status) is recommended. Indeed, the delaying effect of the left recumbent position on gastric emptying of non-caloric liquids has been well documented. However, the evidence that the right recumbent position enhances liquid emptying is much less certain[4]. According to a recent review on the postural effect on gastric emptying[4], only one trial supports the enhanced emptying of non-nutritive liquids in the right recumbent position, showing that emptying is significantly faster than in the left recumbent position[2]. However, this result only suggests that emptying in the left recumbent position is slower than that in the right.

The present study was conducted to elucidate whether gastric emptying of water is faster when lying on the right side than when seated. The sitting position is regarded as "neutral" if the effect of the gravity over intragastric fluids is accounted for, and was thereby set as the reference posture. The ^{13}C-acetate breath test with Wagner-Nelson analysis was used to measure gastric emptying accurately without any invasive procedures.

MATERIALS AND METHODS

Subjects

Eight healthy male volunteers (age: 19-52 years, median 38 years; weight: 48.0-80.2 kg, median 64.0 kg) participated in this study. No subject had a history of, or symptoms referable to, gastrointestinal and pulmonary disease or chronic medical problems. Additionally, no subjects were receiving medication at the time of the study. Written informed consent was obtained from each volunteer. The study protocol was approved by the Ethics Committee of Toho University.

Protocol

The ^{13}C-acetate breath test was used to assess gastric emptying [5]. The assessment was carried out on two randomized occasions, at least 3 d apart, with subjects either seated or lying on their right side. On both occasions, subjects had fasted overnight, and they drank 100 mL tap water labeled with 100 mg ^{13}C-acetate in the sitting position. Breath samples were collected at baseline and, following ingestion of the water, at 5-min intervals for the first 20 min and at 10-min intervals thereafter up to 120 min. On one occasion, the subjects remained seated throughout the 120-min period. On the other occasion, immediately after drinking the test solution, they lay down on their right side and maintained the position until they exhaled the 15-min breath sample; thereafter they remained seated.

Data analysis

Breath $^{13}CO_2$ isotopic enrichment was determined using non-dispersive infrared isotope spectrometry (UbiT-IR 300; Otsuka Electronics, Osaka, Japan). The pulmonary recovery of $^{13}CO_2$ was expressed in %dose/h based on the body surface area and the assumed CO_2 production. The %dose/h recovery values were plotted against time to create the time-breath $^{13}CO_2$ excretion curve, which indirectly reflects gastric emptying. The time to the maximal recovery (Tmax), a semi-quantitative index of gastric emptying, was determined by visual inspection of the $^{13}CO_2$ output curve[6]. The breath data were further analyzed using the Wagner-Nelson method, which enables creation of a percent gastric retention-time curve that is as accurate as the scintigraphic emptying curve[7]. The Wagner-Nelson procedure has been detailed elsewhere[7,8]. In short, estimated fraction of the labeled test meal that has been emptied from the stomach by time t, F(t), is obtained from the conventional equation; F(t) = area under the concentration curve (AUC)(t)/AUC(∞), although the value can differ from those obtained using the scintigraphic method. Wagner-Nelson method allows accurate estimation of F(t) using the revised equation as follows: F(t) = [AUC(t) + C(t)/Kel]/AUC(∞). In the equation, Kel (1/h) is the first-order rate constant for total elimination of $^{13}CO_2$ from the human body, C(t) is the $^{13}CO_2$ excretion rate (%dose/h), AUC(t) is the area under the C(t) curve (%dose) (= the pulmonary recovery of $^{13}CO_2$), and AUC(∞) is the cumulative amount of $^{13}CO_2$ recovered in the breath at the infinite time (% dose).

The time by which half of the water has been emptied ($t_{1/2}$), which is used as the standard index in gastric emptying scintigraphy, was determined by interpolation from the retention-time curve[7].

Statistical analysis

Differences in the pulmonary $^{13}CO_2$ output and the percent gastric retention curves between the two postures were assessed with repeated measures of analysis of variance (ANOVA) using "posture" and "time" as factors. When the ANOVA showed a significant difference, it was

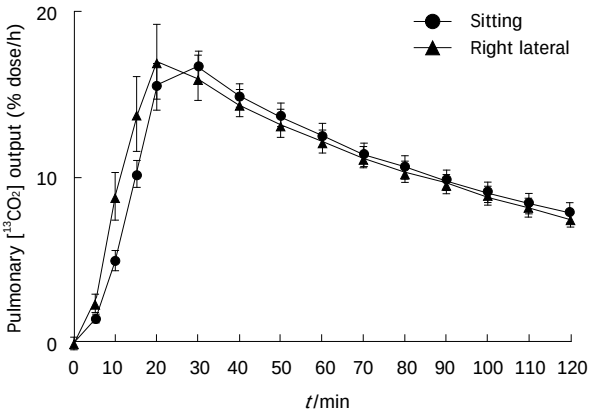


Figure 1 Pulmonary $^{13}CO_2$ recovery-time curves. Error bars indicate SE.

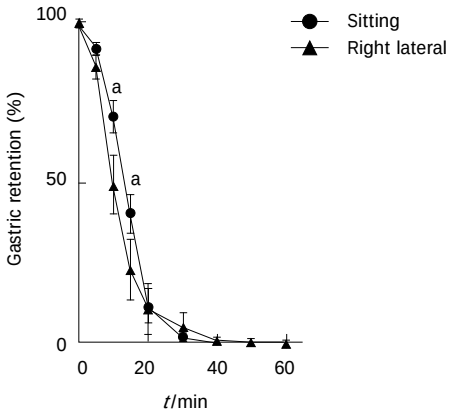


Figure 2 Percent gastric retention-time curves created by Wagner-Nelson analysis. Error bars indicate SE. The asterisk indicates a significant difference ($^aP < 0.05$ *vs* right lateral group).

followed by *post hoc* comparisons at each measurement time with Student's paired *t* test with Bonferroni adjustment. The t_{max} and $t_{1/2}$ values are presented as mean ± SD. Differences in t_{max} and $t_{1/2}$ between the two body positions were also assessed with Student's paired *t* test. The level of significance was set at $P = 0.05$ (two-sided probability).

RESULTS

No significant posture effect was found in pulmonary $^{13}CO_2$ output curves ($P = 0.2150$; Figure 1), whereas a significant effect was found in gastric retention curves ($P = 0.0315$; Figure 2). The *post hoc* comparisons revealed that the percent retention values were significantly smaller in the right recumbent position at 10 min ($P < 0.01$) and at 15 min ($P < 0.05$). On the other hand, neither Tmax nor $t_{1/2}$ was significantly different between the two positions (Tmax: 25.0 ± 5.35 min in the sitting position and 23.1 ± 9.23 min in the right recumbent position; $P = 0.644$, $t_{1/2}$: 13.7 ± 2.79 min in the sitting position and 11.6 ± 5.92 min in the right recumbent position; $P = 0.378$).

DISCUSSION

It seems natural that non-caloric fluids in the stomach should gravitate toward the duodenum in the right recumbent position. It is, therefore, reasonable to assume that the right recumbent position would hasten gastric emptying of fluids compared to a sitting position. However, this assumption has not been verified. The present results provided clear evidence that water is emptied significantly faster when a subject is in the right recumbent position than when seated.

Burn-Murdoch *et al*[2] showed that emptying of saline was significantly faster in the right recumbent position than in the left recumbent position whereas it was not significantly different between the right recumbent position and the seated position. We think that the lack of significant difference between the right recumbent position and the seated position in that study might be due to the suboptimal protocol used to measure gastric emptying: the emptying was quantified as the volume aspirated *via* a nasogastric tube at 10 min after ingestion of 750-mL saline. The aspiration technique appears unreliable to measure residual gastric volume[9].

In the present study, the gastric retention at 10 and 15 min indicated a significant positional effect (Figure 2), whereas the pulmonary $^{13}CO_2$ output curve did not (Figure 1). This is possibly because the Wagner-Nelson modification improved the accuracy of breath testing. In contrast to gastric scintigraphy, which allows direct visualization of duodenal transit of gastric contents[10], the conventional ^{13}C-acetate breath test indirectly reflects gastric emptying and thus is less sensitive[6]. The Wagner-Nelson analytical strategy has been introduced into the breath test system to overcome its indirect nature, making the ^{13}C-breath test as accurate as the "gold standard" of scintigraphy[7]. Gastric emptying of non-nutrient fluids in the sitting position per se is so rapid that conventional breath testing would have overlooked a subtle, albeit real acceleration of emptying in the right recumbent position. This subtle difference would be detected only by the gastric retention curve created by Wagner-Nelson analysis. The $t_{1/2}$ values were not significantly different between the two body positions even though values were derived from the "accurate" Wagner-Nelson analysis. This is probably because the $t_{1/2}$ value itself would be a less accurate parameter than the percent gastric retention at a fixed time point, as shown in the expert recommended guidelines for scintigraphic gastric emptying studies[10].

There are some limitations to the present study. First, we used only a small sample size. Second, only a small volume of tap water was used. A larger volume may influence the results.

In conclusion, our results verified the accelerating effect of the right recumbent position on gastric emptying of non-nutritive solutions. Concerning clinical implications, this study suggests that placing patients with acute pain on their right side after oral administration of analgesic drugs in solution is justified as an effective practice

for rapid pain relief. For patients with gastrointestinal reflux symptoms, sleeping in the right recumbent position may reduce nocturnal symptoms, as delayed gastric emptying can cause reflux symptoms.

COMMENTS

Background

Conventionally, gastric emptying of non-caloric fluids is thought to be accelerated when a person is in the right recumbent position. However, evidence supporting this hypothesis is weak. The present study compared the impact of the right recumbent position with the sitting position on gastric emptying of water.

Research frontiers

For calorie-containing meals, the force-resistance balance is primarily regulated by the nutrient-evoked feedback loop that strictly controls duodenal entry of meals so as to optimize intestinal absorption of nutrients. On the other hand, for non-caloric fluids, the feedback system is barely activated and consequently, the stomach behaves like "a motionless bag" in which the fluids passively flow back and forth depending on gravity.

Innovations and breakthroughs

The present study was conducted to elucidate whether gastric emptying of water is faster when lying on the right side than when seated. The sitting position is regarded as "neutral" if the effect of the gravity over intragastric fluids is accounted for, and was thereby set as the reference posture. The ^{13}C-acetate breath test with Wagner-Nelson analysis was used to measure gastric emptying accurately without any invasive procedures.

Applications

Their results verified the accelerating effect of the right recumbent position on gastric emptying of non-nutritive solutions. Concerning clinical implications, this study suggests that placing patients with acute pain on their right side after oral administration of analgesic drugs in solution is justified as an effective practice for rapid pain relief. For patients with gastrointestinal reflux symptoms, sleeping in the right recumbent position may reduce nocturnal symptoms, as delayed gastric emptying can cause reflux symptoms.

Peer review

This is a prospective study to evaluate the gastric emptying of non-caloric liquids in a seated position versus right lateral recumbence. A total of 8 healthy male volunteers were given 100 mL of tap water labeled with ^{13}C-acetate and evaluated in a seated position on one occasion and, on a separate day, evaluated in a right lateral recumbence position. Breath samples were collected at given intervals and tested for the pulmonary recovery of $^{13}CO_2$. The values were plotted against time to create a time-breath $^{13}CO_2$ excretion curve, which is an indirect measurement of the gastric emptying. The Wagner-Nelson procedure was then used to further analyze the data and extrapolate percent gastric retention-time curve.

REFERENCES

1 **Schulze K**. Imaging and modelling of digestion in the stomach and the duodenum. *Neurogastroenterol Motil* 2006; **18**: 172-183 [PMID: 16487408 DOI: 10.1111/j.1365-2982.2006.00759.x]

2 **Burn-Murdoch R**, Fisher MA, Hunt JN. Does lying on the right side increase the rate of gastric emptying? *J Physiol* 1980; **302**: 395-398 [PMID: 7411459]

3 **Vance MV**, Selden BS, Clark RF. Optimal patient position for transport and initial management of toxic ingestions. *Ann Emerg Med* 1992; **21**: 243-246 [PMID: 1536482 DOI: 10.1016/S0196-0644(05)80882-0]

4 **Queckenberg C**, Fuhr U. Influence of posture on pharmacokinetics. *Eur J Clin Pharmacol* 2009; **65**: 109-119 [PMID: 18936930 DOI: 10.1007/s00228-008-0579-2]

5 **Braden B**, Adams S, Duan LP, Orth KH, Maul FD, Lembcke B, Hör G, Caspary WF. The [13C]acetate breath test accurately reflects gastric emptying of liquids in both liquid and semisolid test meals. *Gastroenterology* 1995; **108**: 1048-1055 [PMID: 7698571 DOI: 10.1016/0016-5085(95)90202-3]

6 **Sanaka M**, Nakada K. Stable isotope breath tests for assessing gastric emptying: A comprehensive review. *J Smooth Muscle Res* 2010; **46**: 267-280 [PMID: 21441715 DOI: 10.1540/jsmr.46.267]

7 **Sanaka M**, Nakada K, Nosaka C, Kuyama Y. The Wagner-Nelson method makes the [13C]-breath test comparable to radioscintigraphy in measuring gastric emptying of a solid/liquid mixed meal in humans. *Clin Exp Pharmacol Physiol* 2007; **34**: 641-644 [PMID: 17581222 DOI: 10.1111/j.1440-1681.2007.04624.x]

8 **Sanaka M**, Yamamoto T, Ishii T, Kuyama Y. The Wagner-Nelson method can generate an accurate gastric emptying flow curve from CO2 data obtained by a 13C-labeled substrate breath test. *Digestion* 2004; **69**: 71-78 [PMID: 15031624 DOI: 10.1159/000077391]

9 **Parrish CR,** McClave SA. Checking gastric residual volumes-A practice in search of science? *Prac Gastroenterol* 2008; **32:** 33-47

10 **Abell TL**, Camilleri M, Donohoe K, Hasler WL, Lin HC, Maurer AH, McCallum RW, Nowak T, Nusynowitz ML, Parkman HP, Shreve P, Szarka LA, Snape WJ, Ziessman HA. Consensus recommendations for gastric emptying scintigraphy: a joint report of the American Neurogastroenterology and Motility Society and the Society of Nuclear Medicine. *Am J Gastroenterol* 2008; **103**: 753-763 [PMID: 18028513 DOI: 10.1111/j.1572-0241.2007.01636.x]

P- Reviewers Koch TR, Ghoshal UC **S- Editor** Gou SX
L- Editor A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i3.366

World J Gastroenterol 2013 January 21; 19(3): 366-374
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Role of surgical resection for multiple hepatocellular carcinomas

Sung Hoon Choi, Gi Hong Choi, Seung Up Kim, Jun Yong Park, Dong Jin Joo, Man Ki Ju, Myoung Soo Kim, Jin Sub Choi, Kwang Hyub Han, Soon Il Kim

Sung Hoon Choi, Gi Hong Choi, Dong Jin Joo, Man Ki Ju, Myoung Soo Kim, Jin Sub Choi, Soon Il Kim, Department of Surgery, Yonsei University College of Medicine, Seoul 120-752, South Korea
Seung Up Kim, Jun Yong Park, Kwang Hyub Han, Department of Internal Medicine, Yonsei University College of Medicine, Seoul 120-752, South Korea
Author contributions: Choi SH, Choi GH and Choi JS designed the research, analyzed the data, performed the study and wrote the paper; Choi JS reviewed the study population data; Kim SU, Park JY, Joo DJ, Ju MK, Kim MS, Han KH and Kim SI collected the data.
Correspondence to: Jin Sub Choi, MD, PhD, Department of Surgery, Yonsei University Health System, 50 Yonsei-ro, Seodaemoon-gu, Seoul 120-752, South Korea. choi5491@yuhs.ac
Telephone: +82-2-22282122 Fax: +82-2-3138289
Received: May 13, 2012 Revised: December 3, 2012
Accepted: December 15, 2012
Published online: January 21, 2013

Abstract

AIM: To clarify the role of surgical resection for multiple hepatocellular carcinomas (HCCs) compared to transarterial chemoembolization (TACE) and liver transplantation (LT).

METHODS: Among the HCC patients who were managed at Yonsei University Health System between January 2003 and December 2008, 160 patients who met the following criteria were retrospectively enrolled: (1) two or three radiologically diagnosed HCCs; (2) no radiologic vascular invasion; (3) Child-Pugh class A; (4) main tumor smaller than 5 cm in diameter; and (5) platelet count greater than 50 000/mm^3. Long-term outcomes were compared among the following three treatment modalities: surgical resection or combined radiofrequency ablation (RFA) (n = 36), TACE (n = 107), and LT (n = 17). The survival curves were computed using the Kaplan-Meier method and compared with a log-rank test. To identify the patients who gained a survival benefit from surgical resection, we also investigated prognostic factors for survival following surgical resection. Multivariate analyses of the prognostic factors for survival were performed using the Cox proportional hazard model.

RESULTS: The overall survival (OS) rate was significantly higher in the surgical resection group than in the TACE group (48.1% *vs* 28.9% at 5 years, $P < 0.005$). LT had the best OS rate, which was better than that of the surgical resection group, although the difference was not statistically significant (80.2% *vs* 48.1% at 5 years, $P = 0.447$). The disease-free survival rates were also significantly higher in the LT group than in the surgical resection group (88.2% *vs* 11.2% at 5 years, $P < 0.001$). Liver cirrhosis was the only significant prognostic factor for poor OS after surgical resection. Clinical liver cirrhosis rates were 55.6% (20/36) in the resection group and 93.5% (100/107) in the TACE group. There were 19 major and 17 minor resections. *En bloc* resection was performed in 23 patients, multisite resection was performed in 5 patients, and combined resection with RFA was performed in 8 patients. In the TACE group, only 34 patients (31.8%) were recorded as having complete remission after primary TACE. Seventy-two patients (67.3%) were retreated with repeated TACE combined with other therapies. In patients who underwent surgical resection, the 16 patients who did not have cirrhosis had higher 5-year OS and disease-free survival rates than the 20 patients who had cirrhosis (80.8% *vs* 25.5% 5-year OS rate, $P = 0.006$; 22.2% *vs* 0% 5-year disease-free survival rate, $P = 0.048$). Surgical resection in the 20 patients who had cirrhosis did not provide any survival benefit when compared with TACE (25.5% *vs* 24.7% 5-year OS rate, $P = 0.225$). Twenty-nine of the 36 patients who underwent surgical resection experienced recurrence. Of the patients with cirrhosis, 80% (16/20) were within the Milan criteria at the time of recurrence

after resection.

CONCLUSION: Among patients with two or three HCCs, no radiologic vascular invasion, and tumor diameters ≤ 5 cm, surgical resection is recommended only in those without cirrhosis.

© 2013 Baishideng. All rights reserved.

Key words: Hepatocellular carcinoma; Hepatectomy; Liver transplantation; Chemoembolization; Cirrhosis

Choi SH, Choi GH, Kim SU, Park JY, Joo DJ, Ju MK, Kim MS, Choi JS, Han KH, Kim SI. Role of surgical resection for multiple hepatocellular carcinomas. *World J Gastroenterol* 2013; 19(3): 366-374 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i3/366.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i3.366

INTRODUCTION

Surgical resection is the established treatment modality for hepatocellular carcinoma (HCC) in patients with preserved liver function, and surgical outcomes have been greatly improved with mortality rates of 0%-6.4% and excellent 5-year survival rates of more than 50%[1-3]. However, for patients with multiple HCCs, unfavorable disease-free and overall survival (OS) rates following surgical resection have led to the contraindication of surgical treatment. This contraindication is reflected in the guidelines suggested by the American Association for the Study of Liver Disease[4] and the European Association for the Study of the Liver[5] based on the Barcelona Clinic Liver Cancer (BCLC) staging system. Thus, liver transplantation is recommended as the best option for patients with multiple HCCs, but a worldwide shortage of donor organs greatly limits the application of this recommendation. Therefore, although multidisciplinary strategies are used in the treatment of multiple HCCs, multiple HCCs still pose a therapeutic challenge and are a matter of debate.

Currently, the main treatment modality for multiple HCCs is transarterial chemoembolization (TACE). Although several expert centers have recently reported encouraging durable long-term outcomes of surgical resection for multiple HCCs (50%-60% at 5 years) in patients with well-preserved liver function[6,7], few comparative studies of surgical resection and liver transplantation (LT).

ACE have been reported[8,9]. Therefore, we designed this study to clarify the role of surgery for multiple HCCs by comparing the long-term outcomes following surgical resection, TACE, and LT and investigating prognostic factors in patients who underwent surgical resection.

MATERIALS AND METHODS

Patient evaluation and follow-up

We analyzed a single-institution database of 3928 patients who received their initial treatments for HCCs at Yonsei University Health System, Seoul, South Korea between January 2003 and December 2008. The Institutional Review Board of Yonsei University Health System approved this study. Of the patients who underwent surgical resection (n = 304), TACE (n = 854), and LT (n = 45), the patients who met the following criteria were enrolled in this retrospective, single-cohort study: (1) two or three radiologically diagnosed HCCs; (2) no radiologic vascular invasion; (3) Child-Pugh class A; (4) main tumor smaller than 5 cm in diameter; and (5) a platelet count greater than 50 000/mm^3. Small satellite nodules found in the resected specimen were not included in this study.

The cutoff value of tumor size for therapeutic decision making is a debated issue. The 7th edition of cancer staging of the American Joint Committee on Cancer describes main tumor size greater than 5 cm in multiple tumors as an independent prognostic factor for survival[10]. Additionally, a study comparing the pathologically proven necrosis rate following TACE reported the following frequencies of complete necrosis according to tumor size: 66.7%, 30% and 0% for ≤ 3.0 cm, 3.1-5.0 cm, and > 5.0 cm, respectively[11]. Although TACE has been performed as palliative care for larger HCCs, the OS rates of patients with tumors larger than 5 cm in diameter are very poor[12,13]. In addition to tumor size, the presence of vascular involvement of the tumor is a significant prognostic factor for poor outcome. The 5-year survival rates among patients with tumors with and without macroscopic vascular invasion are significantly different (48% ± 3% *vs* 14% ± 5%, $P < 0.001$)[14]. Therefore, patients with a tumor ≥ 5 cm in diameter or with macroscopic vascular involvement were excluded from this study because those patients were unsuitable for curative therapy.

Ultimately, we included 36 patients who underwent surgical resection or combined resection with radiofrequency ablation (RFA), 107 patients who received TACE, and 17 patients who underwent LT, and we compared long-term outcomes following the respective treatments.

Patients were evaluated preoperatively by abdominal ultrasonography (US), computed tomography (CT), magnetic resonance imaging (MRI), and hepatic angiography, if indicated. All patients were assessed using alpha-fetoprotein (AFP), protein induced by vitamin K absence or antagonist Ⅱ (PIVKA Ⅱ), hepatitis B surface antigen, anti-hepatitis C viral antibody, liver biochemistry, coagulation test, and indocyanine green retention rate at 15 min (ICG R15).

The selection of therapeutic options was determined by the anatomical locations of the tumors, liver functional reserve, and patient preference. TACE was generally considered when the tumor was ineligible for complete surgical removal, low remnant liver volume was expected after resection, or the patients declined operative inter-

vention. The patients who had an available liver donor underwent LT.

Eradicating all of the multifocal tumors required a multimodality approach using not only *en-bloc* resection but also separate multi-site resection or resection combined with RFA. All of the patients who underwent surgical resection were routinely assessed by intra-operative US. The effectiveness and safety of combined hepatectomy with RFA for multi-site HCCs were reported by Choi *et al*[15], and our previous work has also shown comparable results between en-bloc resection and multi-site resection or combination hepatectomy with RFA[16]. Separate multi-site resection or resection plus RFA was performed for the multifocal tumors ineligible for en-bloc resection because of bilobar involvement or when there was not enough hepatic function reserve after *en-bloc* resection. Wedge resection was considered for superficial tumors, and RFA was performed for tumors less than 3 cm in diameter that were located deep in the liver.

The median follow-up period for the patients who underwent surgical resection was 38.6 mo (range: 1-94 mo). Surveillance after treatment was conducted with regular monitoring of AFP, PIVKA Ⅱ, and US or CT every three to six months. Suspicious intrahepatic recurrence was confirmed by MRI, hepatic angiography, or image-guided fine-needle biopsy, if needed.

To identify the patients who gained a survival benefit from surgical resection, we also investigated prognostic factors for survival following surgical resection. Twelve clinical variables recorded at the time of diagnosis were analyzed. The variables included age at diagnosis; sex; serum albumin; alanine aminotransferase (ALT) and aspartate aminotransferase levels; ICG R15; serum AFP level; clinical liver cirrhosis; main tumor size and number of tumors on preoperative image studies; lobar distribution of the tumor; and type of operation.

Clinically diagnosed liver cirrhosis was defined as follows: (1) history of overt complications of liver cirrhosis, such as ascites, variceal bleeding, and hepatic encephalopathy; (2) evidence of clinical portal hypertension, including esophageal or gastric varices, or splenomegaly (maximal diameter > 12 cm) with platelet count < 100 000 mm^3; and (3) liver morphology suggesting the presence of cirrhosis on preoperative image studies, including hypertrophy of the left lobe and/or caudate lobe, relative volume reduction of the right lobe, nodularity of the liver surface, presence of regenerative or dysplastic nodules, or the presence of a portosystemic shunt[17-19]. All of the analysis in the current study were performed using a clinical diagnosis of liver cirrhosis to evaluate its clinical usefulness. Minor resection was defined as hepatectomy of two or fewer liver segments.

Statistical analysis

Continuous variables are presented as the mean ± SD and were compared by Student's *t* test. Categorical variables are expressed as frequencies with percentages and were compared by the χ^2 test. Cumulative overall and disease-free survival rates were computed by the Kaplan-Meier method, and differences between the survival curves were compared using a log-rank test. Multivariate analyses of the prognostic factors for survival were performed using the Cox proportional hazard model and included the factors that had *P* values less than 0.1 upon univariate analysis. Statistical analyses were performed using SPSS 15 for Windows (SPSS Inc., Chicago, IL, United States). Statistical significance was set at a *P* value less than 0.05.

RESULTS

Clinical characteristics of the surgical resection group and TACE group

The patients who underwent TACE were older and had lower platelet counts, higher ALT levels, and a higher rate of clinical liver cirrhosis than those in the resection group. Clinical liver cirrhosis rates were 55.6% (20/36) in the resection group and 93.5% (100/107) in the TACE group. Surgical resection was performed more frequently in patients with larger diameter tumors (Table 1).

Operative procedures in the surgical resection group and additional treatments in the TACE group

Table 2 lists the operative procedures and combined treatments with RFA in the resection group. There were 19 and 17 major and minor resections, respectively. *En bloc* resection was performed in 23 patients, multi-site resection was performed in 5 patients, and combined resection with RFA was performed in 8 patients. In the TACE group, only 34 patients (31.8%) were recorded as having complete remission after primary TACE. Seventy-two patients (67.3%) were retreated with repeated TACE, one patient was retreated with repeated TACE and intra-arterial chemotherapy, two patients were retreated with RFA, two patients were retreated with radiation therapy, one patient was retreated with percutaneous ethanol injection, two patients were retreated with intra-arterial chemotherapy and systemic chemotherapy, two patients were retreated with intra-arterial chemotherapy and radiation therapy, and five patients were retreated with holmium therapy.

Long-term outcomes according to treatment modality

The OS rate was significantly higher in the surgical resection group than in the TACE group (48.1% *vs* 28.9% at 5 years, $P < 0.005$) (Figure 1A). LT had the best OS rate, which was better than that of the surgical resection group, although the difference was not statistically significant (80.2% *vs* 48.1% at 5 years, $P = 0.447$) (Figure 1A). The disease-free survival rates were also significantly higher in the LT group than in the surgical resection group (88.2% *vs* 11.2% at 5 years, $P < 0.001$) (Figure 1B).

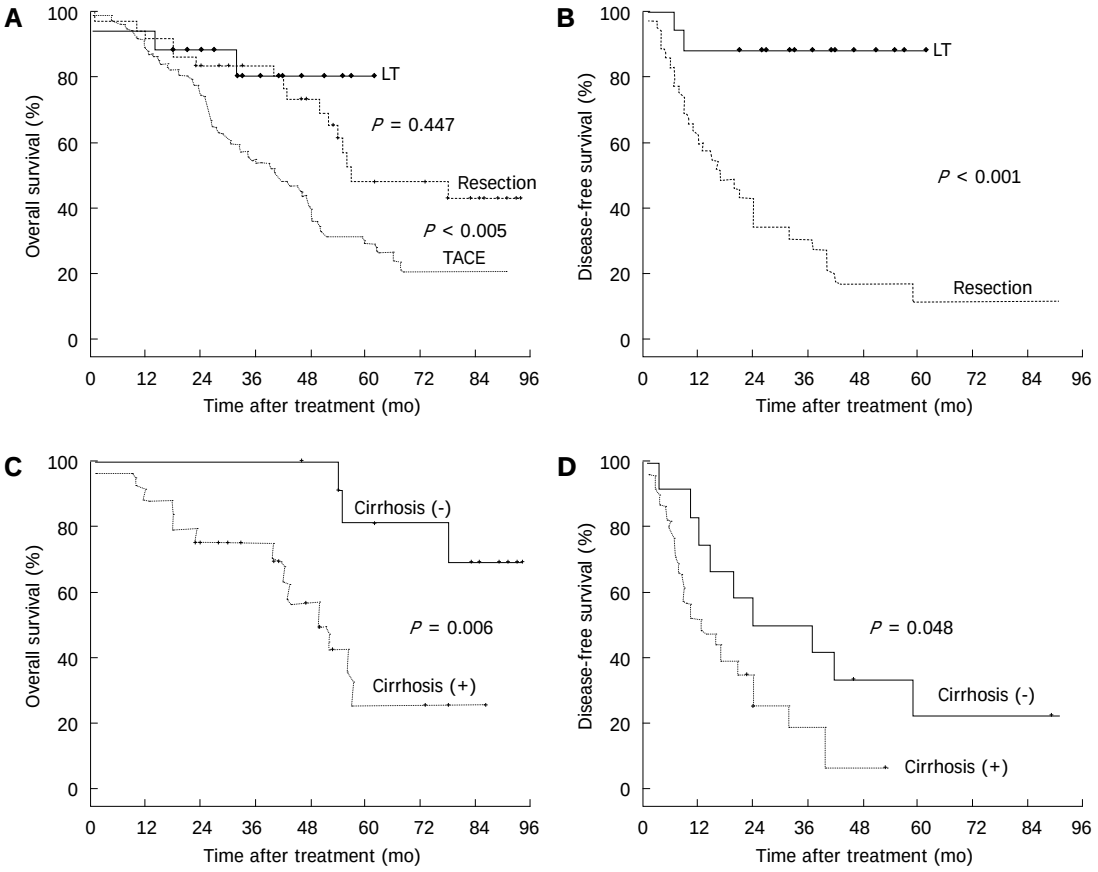


Figure 1 The overall and disease-free survival curves according to treatment modality and presence of liver cirrhosis in surgical resection patients. A: The 1-, 3- and 5-year overall survival (OS) rates were 94.1%, 80.2% and 80.2%, respectively, in the liver transplantation (LT) group; 91.7%, 83.3% and 48.1%, respectively, in the resection group; and 88.7%, 55.6% and 28.9%, respectively, in the transarterial chemoembolization (TACE) group. The OS rate was significantly higher in the surgical resection group than in the TACE group ($P < 0.005$). LT showed the best OS rate (better than the surgical resection group, but not statistically significant, $P = 0.447$); B: The 1-, 3- and 5-year disease-free survival rates were 88.2%, 88.2% and 88.2% in the LT group and 60%, 30.3% and 11.2% in the resection group, respectively. The disease-free survival rates were also significantly higher in the transplantation group than in the surgical resection group ($P < 0.001$); C: The 1-, 3- and 5-year OS rates were 100%, 100% and 80.8% in patients without cirrhosis (-) and 87.5%, 75% and 25.5% in patients with cirrhosis (+), respectively ($P = 0.006$); D: The 1-, 3- and 5-year disease-free survival rates were 75.0%, 50.0% and 22.2% in patients without cirrhosis (-) and 52.3%, 18.7% and 0% in patients with cirrhosis (+), respectively ($P = 0.048$).

Table 1 Clinical characteristics of the surgical resection patients *vs* the transarterial chemoembolization patients

Variable	Surgical resection (n = 36)	TACE (n = 107)	P value
Age (yr)	54.3 ± 8.6	61.2 ± 9.3	< 0.001
Gender (male:female)	34:2	86:21	0.047
Platelet (k/mm^3)	153.4 ± 53.9	121.0 ± 51.8	0.002
Albumin (g/dL)	4.05 ± 0.50	3.93 ± 0.48	0.203
ALT (IU/L)	36.4 ± 15.8	59.0 ± 40.4	0.001
AST (IU/L)	41.2 ± 25.8	53.2 ± 44.7	0.130
HBsAg	28 (77.8)	67 (62.6)	0.099
Clinical liver cirrhosis	20 (55.6)	100 (93.5)	< 0.001
Tumor number			0.549
2	30 (83.3)	90 (84.1)	
3	6 (16.7)	17 (15.9)	
Main tumor size (cm)			0.005
< 3	19 (52.8)	17 (47.2)	
⩾ 3	29 (27.1)	78 (72.9)	
AFP > 1000 IU/mL	2 (5.6)	8 (7.5)	0.696

Data are expressed as absolute n (%) or mean ± SD. M: Male; F: Female; TACE: Transarterial chemoembolization; ALT: Alanine aminotransferase (reference range, 5-46 IU/L); AST: Aspartate aminotransferase (reference range, 13-34 IU/L); HBsAg: Hepatitis B surface antigen; AFP: α-fetoprotein.

Table 2 Operative procedures in the resection group

Degree of resection	Operative procedure	Number of patients (n = 36)
Major resection (n = 19)	Extended right hepatectomy	1
	Right hepatectomy only	12
	+ wedge resection	1
	+ RFA	1
	Left hepatectomy only	1
	+ wedge resection	1
	Central bisectionectomy only	2
Minor resection (n = 17)	Left lateral sectionectomy only	3
	+ RFA	1
	Sectionectomy only	2
	+ wedge resection	2
	+ RFA	2
	Bisegmentectomy only	1
	+ wedge resection	1
	+ RFA	2
	Segmentectomy only	1
	+ RFA	1
	Wedge resection + RFA	1

En bloc resection (n = 23); multi-site resection (n = 5); resection plus radiofrequency ablation (RFA) (n = 8).

Table 3 Prognostic factors for overall survival in surgical resection patients

Variable	Patients (n = 36)	1-yr OS	3-yr OS	5-yr OS	P value
Age (yr)					0.146
≤ 60	27	88.9%	81.5%	42.3%	
> 60	9	100%	88.9%	66.7%	
Gender					0.245
Male	34	91.2%	82.4%	45.1%	
Female	2	100%	100%	100%	
Serum albumin (g/dL)					0.642
≤ 3.5	10	90.0%	60.0%	48.0%	
> 3.5	26	92.3%	87.7%	46.1%	
ALT (IU/L)					0.593
≤ 50	27	92.6%	81.5%	53.7%	
> 50	9	88.9%	71.1%	23.7%	
AST (IU/L)					0.87
≤ 50	31	90.3%	80.6%	52.4%	
> 50	5	100%	100%	50.0%	
ICG R 15 (%)					0.992
≤ 14	25	92.0%	84.0%	47.1%	
> 14	9	88.9%	77.8%	58.3%	
α-fetoprotein					0.471
≤ 1000 IU/mL	32	90.6%	81.3%	47.3%	
> 1000 IU/mL	4	100%	100%	50.0%	
Cirrhosis					0.023
No	16	100%	93.8%	69.9%	
Yes	20	85.0%	75.0%	26.5%	
Main tumor size (cm)					0.629
< 3.0	17	94.1%	86.3%	43.1%	
≥ 3.0	19	89.5%	73.7%	52.6%	
Number of tumors					0.061
2	31	90.3%	80.6%	40.7%	
3	5	100%	100%	100%	
Lobar distribution of tumors					0.892
One lobes	24	91.7%	83.3%	49.4%	
Two lobe	12	91.7%	83.3%	48.6%	
Operation type					0.568
En bloc resection	23	91.3%	82.6%	43.4%	
Multiple resection or combined with RFA	13	92.3%	84.6%	57.1%	

OS: Overall survival; RFA: Radiofrequency ablation; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ICG R 15: Indocyanine green retention rate at 15.

Prognostic factors for OS in patients who underwent surgical resection

Cirrhosis was the only significant prognostic factor for poor OS after resection in both the univariate (P = 0.023) and multivariate analyses (P = 0.034, odds ratio = 0.552, 95%CI: 0.105-0.915) (Table 3). Clinically diagnosed liver cirrhosis was correlated with pathological cirrhosis with a positive predictive value of 100%, a negative predictive value of 75%, a sensitivity of 83.3%, and a specificity of 100%.

Long-term outcomes of patients who underwent surgical resection according to the presence of cirrhosis

The 1-, 3- and 5-year OS rates were 100%, 100% and 80.8%, respectively, in 16 patients without cirrhosis, and 87.5%, 75% and 25.5%, respectively, in 20 patients with cirrhosis (P = 0.006) (Figure 1C). The disease-free 1-, 3- and 5-year survival rates were 75.0%, 50% and 22.2%, respectively, in patients without cirrhosis and 52.3%, 24.9% and 0%, respectively, in patients with cirrhosis (P = 0.048) (Figure 1D). The OS rates were not different between the surgical resection group and the TACE group among the patients with cirrhosis (87.5%, 75.0% and 25.5% *vs* 91.8%, 61.7% and 24.7% at 1-, 3- and 5-year, respectively, P = 0.225) (Figure 2).

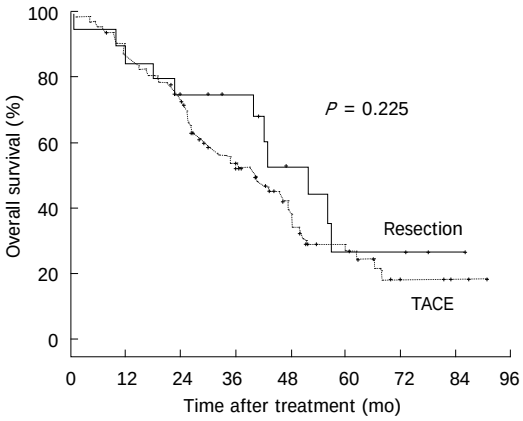


Figure 2 Overall survival curves of surgical resection and transarterial chemoembolization in patients with liver cirrhosis. Among patients with cirrhosis, the overall survival (OS) rates were not different between the surgical resection group and the transarterial chemoembolization (TACE) group (25.5% *vs* 24.7% 5-year OS rate, P = 0.225).

Recurrence pattern after surgical resection

Twenty-nine of the 36 patients who underwent surgical resection experienced recurrence. Of the 29 patients with recurrence, 1 (4%) had a marginal recurrence on the resection margin, 21 (84%) had intra-hepatic recurrences, and 3 (12%) had extra-hepatic recurrences with one in the lung and two in bone. All of the patients with marginal and intra-hepatic recurrence were retreated by TACE. Chemotherapy, radiation therapy, and a clinical trial were used to treat extra-hepatic recurrences. Notably, of the patients with cirrhosis, 80% (16/20) were within the Milan criteria at the time of recurrence after resection.

DISCUSSION

Our study demonstrates that surgery for patients with multiple HCCs is recommended in patients without cirrhosis. Our data revealed that the survival of patients who underwent surgical resection was better than that of patients who received TACE. However, the survival of patients with HCCs was affected not only by HCC itself, but also by underlying liver disease. The majority of patients (93.5%, 100/107) who received TACE had liver cirrhosis. Therefore, when the survival of the patients in the surgical resection group was compared with the TACE group according to the presence of cirrhosis, surgical resection showed no survival benefit in cirrhotic patients.

To confirm the efficacy of surgical resection for mul-

tiple HCCs, its superiority over non-surgical treatment (TACE) should be proven. A recent retrospective cohort study by Ho *et al*[8] reported that hepatectomy yields better survival than TACE even in patients with multiple HCCs in various stages. The authors compared the prognosis of the patients according to stages in different staging systems; however, as the authors note, their study had several weaknesses. Although each staging system represents the prognosis of the patients who have HCC, and in particular, the BCLC system accounts for portal hypertension and the bilirubin level in addition to the Child-Pugh classification in staging, the liver function status in each stage encompasses a wide range and the prognosis in the subgroups of each stage differs significantly[20]. Additionally, the patients in each stage of their study were heterogeneous. Therefore, it is unclear whether the degree of underlying liver disease was evenly distributed between the compared groups. In our study, we limited the inclusion criteria to patients with platelet counts greater than 50 000/mm^3 in addition to a Child-Pugh A classification to exclude patients who had severely advanced liver cirrhosis, for whom surgery is contraindicated due to likely postoperative deterioration of liver function and poor prognosis[17,21].

Liver cirrhosis is a well-known potent predictive factor for OS in patients with HCC[14,22,23], as shown in our patient cohort. According to our analysis, the survival rates of cirrhotic patients with multiple HCCs who underwent surgical resection were extremely disappointing. The 5-year OS rate of the patients with cirrhosis was 25.5%, which was significantly worse than that of the patients without cirrhosis (69.3%, $P = 0.006$) (Figure 1C and D). Furthermore, the outcomes of surgical resection and TACE in the patients with cirrhosis were not different (Figure 2). Therefore, surgical resection for multiple HCCs would be beneficial in patients without cirrhosis, but it remains debatable in patients with cirrhosis.

Despite the fact that surgical resection for multiple HCCs showed acceptable OS rates in our study as well as in several other publications[6-8,22,24], a high recurrence rate is a major drawback of surgical resection as a curative therapy. According to our data, the 5-year disease-free survival rates were 0% in patients with cirrhosis and 22.2% in non-cirrhotic patients. Thus, our single cohort study demonstrated that LT might be the preferred treatment option to offer the chance of a cure for multiple HCCs; the OS and disease-free survival rates were 80.2% and 88.2%, respectively (Figure 1A and B). However, in addition to inevitable immuneesuppressive therapy, which has an adverse oncologic effect, the scarcity of liver donors is another great obstacle to the wide application of LT.

Which loco-regional therapy is superior as a bridge to LT is an issue because of the long waiting time on the transplant list, which results in patients progressing and falling outside the transplant criteria. According to Llovet *et al*[25], approximately 10% of patients are dropped from the transplant list during the waiting period because of tumor progression or liver failure. TACE and RFA have been studied in detail and widely used as a bridge therapy in several transplant centers, but the efficacy of these modalities has not yet been established[26-28].

Recently, Belghiti *et al*[29] proposed three different roles of resection for HCC prior to LT: (1) As a primary therapy, resection can delay or avoid transplantation and can be followed by salvage transplantation for recurrence and deteriorated liver function; (2) As an initial therapy, resection can provide pathologic information about the whole specimen, which enables selection of the best candidates for transplantation; and (3) As a bridge therapy, resection can offer the best control of HCC in patients listed for LT through the possibility of downstaging and providing detailed pathologic information. In addition to these benefits, liver resection can provide superior control and a good survival rate[29,30]. Furthermore, several studies report that 60%-80% of patients who recur after resection for HCC are still amenable to transplantation, and these results are not different between patients with solitary and oligonodular primary HCCs[22,31,32]. Our results also showed that 80% of the recurred patients with cirrhosis following resection were within the Milan criteria. Thus, surgical resection for cirrhotic patients with multiple HCCs might be performed as a bridge to LT. Surgical resection as a bridge is justified by the improved safety of liver surgery and no survival impairment in the event of subsequent LT[31].

Recent studies demonstrated that salvage LT does not compromise the operative morbidity and mortality compared with primary LT[31,33,34]. In contrast, Adam *et al*[35] reported that secondary LT is associated with a higher operative morbidity and mortality; they also argued that patients treated by resection when they were initially transplantable had a higher recurrence rate with more frequent extrahepatic metastasis and vascular invasion, which impair the transplantability and long-term survival of the patients. Therefore, it is too early to conclude whether resection can be performed as bridge therapy. Nevertheless, these efforts to use the limited number of donor organs effectively are necessary, as is further investigation of this issue.

We did not analyze the outcomes according to the types of multiple HCCs in the present study because our data included patients who received resection combined with RFA, which did not allow for pathologic analysis. Discrimination of intrahepatic metastasis (IM) and *de novo* multicentric (MC) HCCs may be important because generally, IMs that have acquired metastatic ability exhibit more aggressive biologic behavior[36,37] and thus influence therapeutic strategy decisions. According to the guidelines of the Liver Cancer Study Group of Japan[38], IM is diagnosed if the tumors definitely originated from portal vein tumor thrombi, if the tumors arose in multiple satellite nodules surrounding a main tumor, or if a satellite tumor near the main tumor shows similar or

poorer histological differentiation than the main tumor. Otherwise, multiple HCCs that do not meet these conditions are deemed *de novo* MC tumors. Although these conventional pathological criteria are convenient, they are relatively subjective. Currently, the most precise method to determine the origin of HCC is DNA clonal analysis[39,40], and clinical differentiation between IM and MC is not possible preoperatively. If a credible diagnosis could be possible for preoperative distinction of the origin of multiple nodules, it might be helpful for the selection of therapy.

In conclusion, surgical resection for HCCs with two or three radiologically identified tumors, no radiologic vascular invasion, and diameters less than 5 cm is recommended for patients without cirrhosis but debatable for patients with cirrhosis. LT might be the best treatment option for patients with multiple HCCs. The retrospective design and the small number of cases are limitations of this-single cohort study. Therefore, further multi-center trials and randomized, controlled, prospective studies are needed, especially to examine the role of surgical resection in cirrhotic patients with multiple HCCs.

COMMENTS

Background

Although surgical resection is the established treatment modality for hepatocellular carcinoma (HCC) in patients with preserved liver function, however, for patients with multiple HCCs, unfavorable disease-free and overall survival (OS) rates following surgical resection have led to the contraindication of surgical treatment. Therefore, although multidisciplinary strategies are used in the treatment of multiple HCCs, multiple HCCs still pose a therapeutic challenge and are a matter of debate. There has been a lack of studies for the efficacy of surgical resection in patients with multiple HCCs.

Research frontiers

The survival of patients with HCCs was affected not only by HCC itself but also by underlying liver disease. Some studies have reported the superiority of surgical resection for multiple HCCs over non-surgical treatment. However, long-term outcomes of the patients according to not only stage but also the degree of underlying liver disease may help to select a proper treatment modality.

Innovations and breakthroughs

Their study demonstrates that surgery for patients with multiple HCCs is recommended in patients without clinical cirrhosis. When the survival of the patients in the surgical resection group was compared with the transarterial chemoembolization group according to the presence of cirrhosis, surgical resection showed no survival benefit in cirrhotic patients. This study is worthy because the surgical outcomes of the patients with multiple HCCs were compared according to the degree of underlying liver disease.

Applications

Liver cirrhosis is a well-known potent predictive factor for OS in patients with HCC. According to the analysis, the survival rates of cirrhotic patients with multiple HCCs who underwent surgical resection were extremely disappointing. Therefore, surgical resection for multiple HCCs is recommended for patients without cirrhosis but still debatable for patients with cirrhosis. Further multi-center trials and randomized, controlled, prospective studies are needed.

Peer review

This study provides clinical outcomes of surgical resection for multiple HCCs compared with the transarterial chemoembolization and liver transplantation. This issue is still debatable in patients with cirrhosis. The retrospective design and the small number of cases are limitations of this study.

REFERENCES

1 **Wu CC**, Cheng SB, Ho WM, Chen JT, Liu TJ, P'eng FK. Liver resection for hepatocellular carcinoma in patients with cirrhosis. *Br J Surg* 2005; **92**: 348-355 [PMID: 15672423 DOI: 10.1002/bjs.4838]

2 **Esnaola NF**, Mirza N, Lauwers GY, Ikai I, Regimbeau JM, Belghiti J, Yamaoka Y, Curley SA, Ellis LM, Nagorney DM, Vauthey JN. Comparison of clinicopathologic characteristics and outcomes after resection in patients with hepatocellular carcinoma treated in the United States, France, and Japan. *Ann Surg* 2003; **238**: 711-719 [PMID: 14578734 DOI: 10.1097/01.sla.0000094436.34556.ac]

3 **Fan ST**, Lo CM, Liu CL, Lam CM, Yuen WK, Yeung C, Wong J. Hepatectomy for hepatocellular carcinoma: toward zero hospital deaths. *Ann Surg* 1999; **229**: 322-330 [PMID: 10077043]

4 **Bruix J**, Sherman M. Management of hepatocellular carcinoma. *Hepatology* 2005; **42**: 1208-1236 [PMID: 16250051 DOI: 10.1002/hep.20933]

5 **Bruix J**, Sherman M, Llovet JM, Beaugrand M, Lencioni R, Burroughs AK, Christensen E, Pagliaro L, Colombo M, Rodés J. Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J Hepatol* 2001; **35**: 421-430 [PMID: 11592607]

6 **Ishizawa T**, Hasegawa K, Aoki T, Takahashi M, Inoue Y, Sano K, Imamura H, Sugawara Y, Kokudo N, Makuuchi M. Neither multiple tumors nor portal hypertension are surgical contraindications for hepatocellular carcinoma. *Gastroenterology* 2008; **134**: 1908-1916 [PMID: 18549877 DOI: 10.1053/j.gastro.2008.02.091]

7 **Ng KK**, Vauthey JN, Pawlik TM, Lauwers GY, Regimbeau JM, Belghiti J, Ikai I, Yamaoka Y, Curley SA, Nagorney DM, Ng IO, Fan ST, Poon RT. Is hepatic resection for large or multinodular hepatocellular carcinoma justified? Results from a multi-institutional database. *Ann Surg Oncol* 2005; **12**: 364-373 [PMID: 15915370 DOI: 10.1245/ASO.2005.06.004]

8 **Ho MC**, Huang GT, Tsang YM, Lee PH, Chen DS, Sheu JC, Chen CH. Liver resection improves the survival of patients with multiple hepatocellular carcinomas. *Ann Surg Oncol* 2009; **16**: 848-855 [PMID: 19159983 DOI: 10.1245/s10434-008-0282-7]

9 **Schumacher PA**, Powell JJ, MacNeill AJ, Buczkowski AK, Erb SR, Ho SG, Scudamore CH, Steinbrecher UP, Weiss A, Yoshida E, Chung SW. Multimodal therapy for hepatocellular carcinoma: a complementary approach to liver transplantation. *Ann Hepatol* 2010; **9**: 23-32 [PMID: 20308719]

10 **Edge SB**, Compton CC. The American Joint Committee on Cancer: the 7th edition of the AJCC cancer staging manual and the future of TNM. *Ann Surg Oncol* 2010; **17**: 1471-1474 [PMID: 20180029 DOI: 10.1245/s10434-010-0985-4]

11 **Takayasu K**. Chemoembolization for unresectable hepatocellular carcinoma in Japan. *Oncology* 2010; **78** Suppl 1: 135-141 [PMID: 20616596 DOI: 10.1159/000315242]

12 **Takayasu K**, Arii S, Ikai I, Omata M, Okita K, Ichida T, Matsuyama Y, Nakanuma Y, Kojiro M, Makuuchi M, Yamaoka Y. Prospective cohort study of transarterial chemoembolization for unresectable hepatocellular carcinoma in 8510 patients. *Gastroenterology* 2006; **131**: 461-469 [PMID: 16890600 DOI: 10.1053/j.gastro.2006.05.021]

13 **Herber SC**, Otto G, Schneider J, Schuchmann M, Düber C, Pitton MB, Kummer I, Manzl N. Transarterial chemoembolization in patients not eligible for liver transplantation: single-center results. *AJR Am J Roentgenol* 2008; **190**: 1035-1042 [PMID: 18356452 DOI: 10.2214/AJR.07.2755]

14 **Vauthey JN**, Lauwers GY, Esnaola NF, Do KA, Belghiti J,

Mirza N, Curley SA, Ellis LM, Regimbeau JM, Rashid A, Cleary KR, Nagorney DM. Simplified staging for hepatocellular carcinoma. *J Clin Oncol* 2002; **20**: 1527-1536 [PMID: 11896101 DOI: 10.1200/JCO.20.6.1527]

15 **Choi D**, Lim HK, Joh JW, Kim SJ, Kim MJ, Rhim H, Kim YS, Yoo BC, Paik SW, Park CK. Combined hepatectomy and radiofrequency ablation for multifocal hepatocellular carcinomas: long-term follow-up results and prognostic factors. *Ann Surg Oncol* 2007; **14**: 3510-3518 [PMID: 17653800 DOI: 10.1245/s10434-007-9492-7]

16 **Cho MS**, Choi GH, Kim DH, Kang CM, Choi JS, Park YN, Lee WJ. Long-term outcomes after multiple-site resection or combined resection and radiofrequency ablation in patients with multiple hepatocellular carcinoma. *Korean J HBP Surg* 2009; **13**: 227-234

17 **Choi GH**, Park JY, Hwang HK, Kim DH, Kang CM, Choi JS, Park YN, Kim do Y, Ahn SH, Han KH, Chon CY, Lee WJ. Predictive factors for long-term survival in patients with clinically significant portal hypertension following resection of hepatocellular carcinoma. *Liver Int* 2011; **31**: 485-493 [PMID: 21382158 DOI: 10.1111/j.1478-3231.2010.02436.x]

18 **Kim do Y**, Kim SU, Ahn SH, Park JY, Lee JM, Park YN, Yoon KT, Paik YH, Lee KS, Chon CY, Han KH. Usefulness of FibroScan for detection of early compensated liver cirrhosis in chronic hepatitis B. *Dig Dis Sci* 2009; **54**: 1758-1763 [PMID: 19005758 DOI: 10.1007/s10620-008-0541-2]

19 **Brancatelli G**, Federle MP, Ambrosini R, Lagalla R, Carriero A, Midiri M, Vilgrain V. Cirrhosis: CT and MR imaging evaluation. *Eur J Radiol* 2007; **61**: 57-69 [PMID: 17145154 DOI: 10.1016/j.ejrad.2006.11.003]

20 **Grieco A**, Pompili M, Caminiti G, Miele L, Covino M, Alfei B, Rapaccini GL, Gasbarrini G. Prognostic factors for survival in patients with early-intermediate hepatocellular carcinoma undergoing non-surgical therapy: comparison of Okuda, CLIP, and BCLC staging systems in a single Italian centre. *Gut* 2005; **54**: 411-418 [PMID: 15710992 DOI: 10.1136/gut.2004.048124]

21 **Ratti L**, Pozzi M, Bosch J. Pathophysiology of portal hypertension in HCV-related cirrhosis. Putative role of assessment of portal pressure gradient in Peginterferon-treated patients. *Dig Liver Dis* 2005; **37**: 886-893 [PMID: 16172033 DOI: 10.1016/j.dld.2005.04.029]

22 **Poon RT**, Fan ST, Lo CM, Liu CL, Wong J. Long-term survival and pattern of recurrence after resection of small hepatocellular carcinoma in patients with preserved liver function: implications for a strategy of salvage transplantation. *Ann Surg* 2002; **235**: 373-382 [PMID: 11882759]

23 **Dalla Valle R**, Borie D, Hannoun L, Botta GC. Surgical treatment of hepatocellular carcinoma in cirrhosis. *Dig Liver Dis* 2000; **32**: 346-356 [PMID: 11515634]

24 **Wang BW**, Mok KT, Liu SI, Chou NH, Tsai CC, Chen IS, Yeh MH, Chen YC. Is hepatectomy beneficial in the treatment of multinodular hepatocellular carcinoma? *J Formos Med Assoc* 2008; **107**: 616-626 [PMID: 18678545 DOI: 10.1016/S0929-6646(08)60179-5]

25 **Llovet JM**, Fuster J, Bruix J. Intention-to-treat analysis of surgical treatment for early hepatocellular carcinoma: resection versus transplantation. *Hepatology* 1999; **30**: 1434-1440 [PMID: 10573522 DOI: 10.1002/hep.510300629]

26 **Decaens T**, Roudot-Thoraval F, Bresson-Hadni S, Meyer C, Gugenheim J, Durand F, Bernard PH, Boillot O, Boudjema K, Calmus Y, Hardwigsen J, Ducerf C, Pageaux GP, Dharancy S, Chazouilleres O, Dhumeaux D, Cherqui D, Duvoux C. Impact of pretransplantation transarterial chemoembolization on survival and recurrence after liver transplantation for hepatocellular carcinoma. *Liver Transpl* 2005; **11**: 767-775 [PMID: 15973710 DOI: 10.1002/lt.20418]

27 **Graziadei IW**, Sandmueller H, Waldenberger P, Koenigsrainer A, Nachbaur K, Jaschke W, Margreiter R, Vogel W. Chemoembolization followed by liver transplantation for hepatocellular carcinoma impedes tumor progression while on the waiting list and leads to excellent outcome. *Liver Transpl* 2003; **9**: 557-563 [PMID: 12783395 DOI: 10.1053/jlts.2003.50106]

28 **Lu DS**, Yu NC, Raman SS, Lassman C, Tong MJ, Britten C, Durazo F, Saab S, Han S, Finn R, Hiatt JR, Busuttil RW. Percutaneous radiofrequency ablation of hepatocellular carcinoma as a bridge to liver transplantation. *Hepatology* 2005; **41**: 1130-1137 [PMID: 15841454 DOI: 10.1002/hep.20688]

29 **Belghiti J**. Resection and liver transplantation for HCC. *J Gastroenterol* 2009; **44** Suppl 19: 132-135 [PMID: 19148807 DOI: 10.1007/s00535-008-2250-1]

30 **Arii S**, Yamaoka Y, Futagawa S, Inoue K, Kobayashi K, Kojiro M, Makuuchi M, Nakamura Y, Okita K, Yamada R. Results of surgical and nonsurgical treatment for small-sized hepatocellular carcinomas: a retrospective and nationwide survey in Japan. The Liver Cancer Study Group of Japan. *Hepatology* 2000; **32**: 1224-1229 [PMID: 11093728 DOI: 10.1053/jhep.2000.20456]

31 **Belghiti J**, Cortes A, Abdalla EK, Régimbeau JM, Prakash K, Durand F, Sommacale D, Dondero F, Lesurtel M, Sauvanet A, Farges O, Kianmanesh R. Resection prior to liver transplantation for hepatocellular carcinoma. *Ann Surg* 2003; **238**: 885-92; discussion 892-3 [PMID: 14631225 DOI: 10.1097/01.sla.0000098621.74851.65]

32 **Cha CH**, Ruo L, Fong Y, Jarnagin WR, Shia J, Blumgart LH, DeMatteo RP. Resection of hepatocellular carcinoma in patients otherwise eligible for transplantation. *Ann Surg* 2003; **238**: 315-321; discussion 321-323 [PMID: 14501497 DOI: 10.1097/01.sla.0000086548.84705.ef]

33 **Del Gaudio M**, Ercolani G, Ravaioli M, Cescon M, Lauro A, Vivarelli M, Zanello M, Cucchetti A, Vetrone G, Tuci F, Ramacciato G, Grazi GL, Pinna AD. Liver transplantation for recurrent hepatocellular carcinoma on cirrhosis after liver resection: University of Bologna experience. *Am J Transplant* 2008; **8**: 1177-1185 [PMID: 18444925 DOI: 10.1111/j.1600-6143.2008.02229.x]

34 **Cherqui D**, Laurent A, Mocellin N, Tayar C, Luciani A, Van Nhieu JT, Decaens T, Hurtova M, Memeo R, Mallat A, Duvoux C. Liver resection for transplantable hepatocellular carcinoma: long-term survival and role of secondary liver transplantation. *Ann Surg* 2009; **250**: 738-746 [PMID: 19801927 DOI: 10.1097/SLA.0b013e3181bd582b]

35 **Adam R**, Azoulay D, Castaing D, Eshkenazy R, Pascal G, Hashizume K, Samuel D, Bismuth H. Liver resection as a bridge to transplantation for hepatocellular carcinoma on cirrhosis: a reasonable strategy? *Ann Surg* 2003; **238**: 508-518; discussion 518-519 [PMID: 14530722 DOI: 10.1097/01.sla.0000090449.87109.44]

36 **Okusaka T**, Okada S, Nose H, Ishii H, Nakasuka H, Nakayama H, Nagahama H, Yoshimori M, Shimada K, Yamamoto J, Takayama T, Kosuge T, Yamasaki S, Sakamoto M, Hirohashi S. The prognosis of patients with hepatocellular carcinoma of multicentric origin. *Hepatogastroenterology* 1996; **43**: 919-925 [PMID: 8884314]

37 **Wang J**, Li Q, Sun Y, Zheng H, Cui Y, Li H, Zhou H, Hao X. Clinicopathologic features between multicentric occurence and intrahepatic metastasis of multiple hepatocellular carcinomas related to HBV. *Surg Oncol* 2009; **18**: 25-30 [PMID: 18640032 DOI: 10.1016/j.suronc.2008.05.009]

38 **Ueno S**, Sakoda M, Kubo F, Hiwatashi K, Tateno T, Baba Y, Hasegawa S, Tsubouchi H. Surgical resection versus radiofrequency ablation for small hepatocellular carcinomas within the Milan criteria. *J Hepatobiliary Pancreat*

Surg 2009; **16**: 359-366 [PMID: 19300896 DOI: 10.1007/s00534-009-0069-7]

39 **Hodges KB**, Cummings OW, Saxena R, Wang M, Zhang S, Lopez-Beltran A, Montironi R, Nour H, Cheng L. Clonal origin of multifocal hepatocellular carcinoma. *Cancer* 2010; **116**: 4078-4085 [PMID: 20564142 DOI: 10.1002/cncr.25258]

40 **Nakata T**, Seki N, Miwa S, Kobayashi A, Soeda J, Nimura Y, Kawasaki S, Miyagawa S. Identification of genes associated with multiple nodules in hepatocellular carcinoma using cDNA microarray: multicentric occurrence or intrahepatic metastasis? *Hepatogastroenterology* 2008; **55**: 865-872 [PMID: 18705285]

P- Reviewer Eghtesad B **S- Editor** Gou SX
L- Editor A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i3.375

World J Gastroenterol 2013 January 21; 19(3): 375-380
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Comparison of concomitant and subsequent cholangiocarcinomas associated with hepatolithiasis: Clinical implications

Chia-Cheng Lin, Ping-Yi Lin, Yao-Li Chen

Chia-Cheng Lin, Yao-Li Chen, Department of Surgery, Changhua Christian Hospital, Changhua 50006, Taiwan
Ping-Yi Lin, Transplant Medicine and Surgery Research Centre, Changhua Christian Hospital, Changhua 50006, Taiwan
Yao-Li Chen, School of Medicine, Chung Shan Medical University, Taichung 40201, Taiwan
Author contributions: Lin CC and Chen YL designed and performed the research; Lin PY contributed mainly to the data analysis; and Lin CC wrote the manuscript.
Correspondence to: Yao-Li Chen, MD, Department of Surgery, Changhua Christian Hospital, Changhua 50006, Taiwan. 31560@cch.org.tw
Telephone: +886-4-7238595 Fax: +886-4-7228289
Received: March 13, 2012 Revised: September 24, 2012
Accepted: September 29, 2012
Published online: January 21, 2013

Abstract

AIM: To compare the outcomes of concomitant cholangiocarcinoma (C-CCA) and subsequent cholangiocarcinoma (S-CCA) associated with hepatolithiasis.

METHODS: From December 1987 to December 2007, 276 patients underwent hepatic resection for hepatolithiasis in Changhua Christian Hospital. Sixty-five patients were excluded due to incomplete medical records and the remaining 211 patients constituted our study population base. Ten patients were diagnosed with C-CCA based on the preoperative biopsy or postoperative pathology. During the follow-up period, 12 patients developed S-CCA. The diagnosis of S-CCA was made by image-guided biopsy or by pathology if surgical intervention was carried out. Patient charts were reviewed to collect clinical information. Parameters such as CCA incidence, interval from operation to CCA diagnosis, interval from CCA diagnosis to disease-related death, follow-up time, and mortality rate were calculated for both the C-CCA and S-CCA groups. The outcomes of the C-CCA and S-CCA groups were mathematically compared and analysed.

RESULTS: Our study demonstrates the clinical implications and the survival outcomes of C-CCA and S-CCA. Among the patients with unilateral hepatolithiasis, the incidence rates of C-CCA and S-CCA were fairly similar (4.8% *vs* 4.5%, respectively, P = 0.906). However, for the patients with bilateral hepatolithiasis, the incidence rate of S-CCA (12.2%) was higher than that of C-CCA (4.7%), although the sample size was limited and the difference between two groups was not statistically significant (P = 0.211). The average follow-up time was 56 mo for the C-CCA group and 71 mo for the S-CCA group. Regard to the average time intervals from operation to CCA diagnosis, S-CCA was diagnosed after 67 mo from the initial hepatectomy. The average time intervals from the diagnoses of CCA to disease-related death was 41 mo for the C-CCA group and 4 mo for the S-CCA group, this difference approached statistical significance (P = 0.075). Regarding the rates of overall and disease-related mortality, the C-CCA group had significantly lower overall mortality (70% *vs* 100%, P = 0.041) and disease-related mortality (60% *vs* 100%, P = 0.015) than the S-CCA group. For the survival outcomes of two groups, the Kaplan-Meier curves corresponding to each group also demonstrated better survival outcomes for the C-CCA group (log rank P = 0.005). In the C-CCA group, three patients were still alive at the time of data analysis, all of them had free surgical margins and did not have pathologically proven lymph node metastasis at the time of the initial hepatectomy. In the S-CCA group, only one patient had chance to undergo a second hepatectomy, and all 12 S-CCA patients had died at the time of data analysis.

CONCLUSION: C-CCA has better outcomes than S-CCA.

The first hepatectomy is crucial because most patients with recurrent CCA or S-CCA are not eligible for repeated surgical intervention.

© 2013 Baishideng. All rights reserved.

Key words: Hepatolithiasis; Intrahepatic duct stones; Recurrent pyogenic cholangitis; Cholangiocarcinoma; Concomitant cholangiocarcinoma; Subsequent cholangiocarcinoma

Lin CC, Lin PY, Chen YL. Comparison of concomitant and subsequent cholangiocarcinomas associated with hepatolithiasis: Clinical implications. *World J Gastroenterol* 2013; 19(3): 375-380 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i3/375.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i3.375

INTRODUCTION

Hepatolithiasis is the presence of stones in the intrahepatic duct (IHD) proximal to the confluence of the right and left main hepatic ducts. This disease is endemic in Southeast Asia and is also referred to as "IHD stones", "recurrent pyogenic cholangitis", "oriental cholangiohepatitis" and "Hong Kong disease" in the literature[1-3]. Since Sanes and MacCallum pointed out the possible association between hepatolithiasis and cholangiocarcinoma (CCA) in 1942, numerous studies have reported similar observations and formulated theories about CCA pathogenesis[4-7].

Currently, hepatic resection is frequently used for the definitive treatment of hepatolithiasis. Among patients who undergo hepatectomy for hepatolithiasis, approximately 5%-10% are incidentally found to have concomitant cholangiocarcinoma (C-CCA)[8-10]. On the other hand, subsequent cholangiocarcinoma (S-CCA) may appear mo to years after the initial hepatectomy. To the best of our knowledge, no previous study has reported on the clinical course and outcomes of S-CCA. During our daily practice, we observed that many patients with S-CCA progressed to death rather quickly. We wondered if patients with S-CCA really have worse outcomes than those with C-CCA. If this premise is true, it would be reasonable to treat hepatolithiasis more aggressively to minimize the deleterious consequences of S-CCA.

MATERIALS AND METHODS

Study design

From December 1987 to December 2007, a total of 276 patients underwent hepatic resection for hepatolithiasis in Changhua Christian Hospital. After excluding 65 patients with incomplete medical records, the remaining 211 patients constituted the base of our study. For each case, the diagnosis of hepatolithiasis was made by the means of image studies preoperatively (liver ultra-sonography, computed tomography, endoscopic retrograde cholangiopancreatography, or magnetic resonance cholangiopancreatography), which was then confirmed by postoperative pathologic examination. The C-CCA diagnosis was based on the preoperative biopsy or the postoperative pathologic examination. The S-CCA diagnosis was made by image-guided aspiration cytology, or by pathology if surgical intervention was carried out. By this assessment, 10 patients were diagnosed with C-CCA. During the follow-up period, 12 patients developed S-CCA (Figure 1).

Patient parameters evaluated

Patient charts were reviewed to collect clinical information. Parameters such as CCA incidence, interval from operation to CCA diagnosis, interval from CCA diagnosis to disease-related death, follow-up time, and mortality were calculated for both the C-CCA group and the S-CCA group. Finally, a mathematical comparison between the two groups was performed.

Statistical analysis

All data were recorded in a computerized database. Continuous variables are expressed as mean and range. A non-parametric test was used to examine the differences between the two groups. The Mann-Whitney *U* test was used for comparing continuous variables. Categorical variables were tested by the Person χ^2 test. Kaplan-Meier analysis with log rank test was used to compare survival between the two groups. *P* values less than 0.05 were considered statistically significant. The statistical analysis was performed with SPSS version 16.0 (Statistical Package for the Social Sciences; SPSS, Inc., Chicago, IL).

RESULTS

Patient clinical characteristics details are shown in Table 1. Among the patients with unilateral hepatolithiasis, the incidences of C-CCA and S-CCA were similar (4.8% *vs* 4.5%, respectively; $P = 0.906$). However, when we looked at bilateral hepatolithiasis, the C-CCA incidence (4.7%) was comparable with that for unilateral hepatolithiasis, but 12.2% of patients with bilateral hepatolithiasis developed S-CCA. However, the case number was too small to allow determination of a statistically significant difference ($P = 0.211$).

The average ages for the C-CCA group and the S-CCA group were 61 and 59 years old, respectively ($P = 0.742$). In both groups, the gender distribution were similar, and only approximately 10% of the patients were male ($P = 0.892$). After the patients were diagnosed with C-CCA, the average life span until disease-related death was 41 mo. On average, S-CCA is diagnosed after 67 mo from the initial hepatectomy. However, after the diagnosis is established, the average life span in these patients was only 4 mo. This difference between intervals from CCA to disease-related death approached statistical significance ($P = 0.075$).

The clinical outcomes of the C-CCA patients are summarized in Table 2. At the time of data analysis, 70% (7/10) of the C-CCA patients had died. Two of them

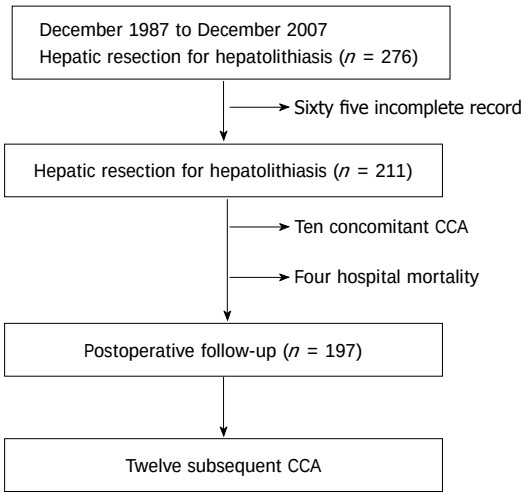


Figure 1 Schematic of the patient selection process. CCA: Cholangiocarcinoma.

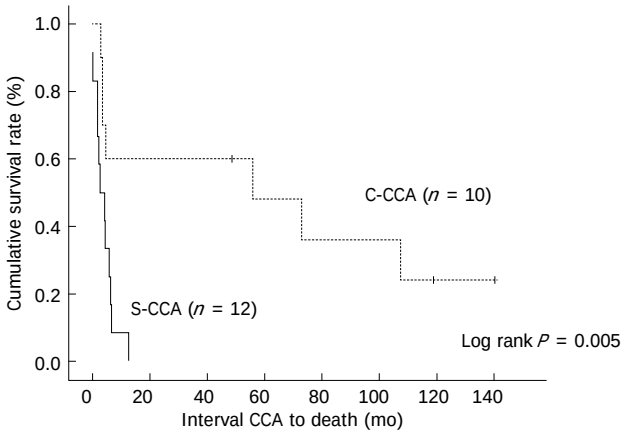


Figure 2 Comparison of survival rates between the concomitant cholangiocarcinoma group and the subsequent cholangiocarcinoma group. C-CCA: Concomitant cholangiocarcinoma; S-CCA: Subsequent cholangiocarcinoma; CCA: Cholangiocarcinoma.

Table 1 Clinical characteristics of patients

	C-CCA group	S-CCA group	*P* value
Incidence (%)	10/211 (4.7)	12/197 (6.1)	0.546
Unilateral stones	8/168 (4.8)	7/156 (4.5)	0.906
Bilateral stones	2/43 (4.7)	5/41 (12.2)	0.211
Age, yr (range)	61 (39-82)	59 (46-69)	0.742
Gender (%)			
Male	1 (10)	1 (8.3)	0.892
Female	9 (90)	11 (91.7)	
Mean interval from operation to CCA, mo (range)	-	67 (7-138)	-
Mean interval from CCA to disease-related death, mo (range)	41 (3-107)	4 (0-13)	0.075
Follow-up time, mo (range)	56 (2-140)	71 (7-144)	0.291
Mortality, *n* (%)			
Overall	7 (70)	12 (100)	0.041
Disease-related	6 (60)	12 (100)	0.015

C-CCA: Concomitant cholangiocarcinoma; S-CCA: Subsequent cholangiocarcinoma; CCA: Cholangiocarcinoma.

(cases 89 and 142) had regional lymph node metastasis at the time of hepatectomy. Three C-CCA patients (cases 119, 212 and 217) had positive surgical margins reported by pathology. One patient (case 227) had a free surgical margin and negative regional lymph nodes; however, she suffered from CCA recurrence at the common bile duct 4 years following the initial hepatectomy. After excision of the common bile duct (free surgical margin), she lived for 5 more years. One patient (case 128) had a free surgical margin and negative regional lymph nodes. However, a hypopharyngeal tumor was diagnosed 3 mo after the initial hepatectomy, and the patient finally expired due to acute respiratory failure. After excluding this last patient, the disease-related mortality was only 60% (6/10). The three patients (cases 90, 253 and 143) who were still alive share some common characteristics. They all had free surgical margins and did not have pathologically-proven lymph node metastasis at the time of the initial hepatectomy. Patients 90 and 253 had been completely disease-free for 140 and 49 mo, respectively. Patient 143 was a victim of bilateral hepatolithiasis, and recurrent IHD stones were detected during the follow-up period. However, she refused treatment until recurrent CCA at the common bile duct was found 5 years following the initial hepatectomy. After excising the common bile duct (free surgical margin), she was disease-free for more than 43 mo. The C-CCA diagnosis was made pre-operatively in one patient (case 142), intra-operatively in four patients (cases 89, 128, 217 and 253), and post-operatively in five patients (cases 90, 119, 143, 212 and 227).

The clinical outcomes of S-CCA patients are summarized in Table 3. All 12 of the patients with S-CCA had died at the time of data analysis, resulting in a 100% overall, and disease-related, mortality. Among them, only one patient (case 11) had undergone a second hepatectomy. However, the surgical margin was positive for cancer and the patient expired 4 mo later.

Figure 2 shows the Kaplan-Meier curves corresponding to each group. The C-CCA group demonstrated clearly better survival outcomes than the S-CCA group ($P = 0.005$, log rank).

DISCUSSION

The S-CCA incidence in unilateral hepatolithiasis is 4.5%, which is comparable to that of C-CCA (4.8%). However, the S-CCA incidence in bilateral hepatolithiasis (12.2%) is more than double that of C-CCA incidence (4.7%). Our case number was too small for this difference to be reflected in statistical significance ($P = 0.211$). We believe, however, that this is quite possibly a true biological difference. This difference can be explained by recent studies that showed that hepatectomy for unilateral hepatolithiasis is associated with high stone clearance rates and low stone recurrence rates[8,11-13]. However, with bilateral hepatolithiasis, there are still very few reports in the literature discussing the best management and long-term outcomes[14]. According to a recent report and our unpublished data, treating bilateral hepatolithiasis with hepatic resection did

Table 2 Summary of patients with concomitant cholangiocarcinoma

Case	Age/sex	Stone location	Procedure	Residual/recurrent stones	Margin	pTNM	Interval from CCA to death (mo)
89	50/F	R	S5/6/7	-/-	Free	T1 N1 M0	3
90	68/F	L	LH	-/-	Free	T1 Nx M0	Alive (140)
119	82/F	L	LL	-/-	Atypical mucinous epithelium	T1 Nx M0	73
128	60/M	L	LH	-/-	Free	T1 N0 M0	3 (hypopharyngeal tumor)
142	39/F	L	LL	-/-	Free	T4 N1 M0	3
212	76/F	L	S2	-/-	Inadequate	T1 Nx M0	56
227	61/F	L	LH + RA	-/+	Free	T1 N0 M0	107 (re-operated 4 years later due to CCA recurrence at CBD)
253	72/F	L	LH	-/-	Free	T1 N0 M0	Alive (49)
143	50/F	BI	LH + S5	-/+	Free	T1 Nx M0	Alive (119) (re-operated 5 years later due to CCA recurrence at CBD)
217	56/F	BI	RH	+/-	Inadequate	T2a Nx M0	5

L: Left; R: Right; BI: Bilateral; LH: Left hemihepatectomy; LL: Left lateral sectionectomy; RH: Right hemihepatectomy; RA: Right anterior sectionectomy; S5: Segment 5; S2: Segment 2; CCA: Cholangiocarcinoma; CBD: Common bile duct; pTNM: Pathologic TNM stage for intrahepatic bile duct according to AJCC 7th edition; F: Female; M: Male.

Table 3 Summary of patients with subsequent cholangiocarcinoma

Case	Age/sex	Stone location	Procedure	Residual/recurrent stones	Interval from hepatectomy to S-CCA (mo)	Second hepatectomy for S-CCA	Interval S-CCA to death (mo)
1	56/F	L	LH	- / -	87	No	< 1
11	59/F	R	S5/6	- / -	91	Yes (with involved surgical margin)	4
66	65/F	R	RH	- / +	114	No	6
69	61/F	R	RP	- / +	67	No	3
137	69/F	L	LL	- / -	95	No	2
203	60/F	L	LH	- / -	47	No	13
236	63/F	L	LL	- / -	7	No	< 1
37	46/F	BI	LL	- / -	138	No	6
96	65/F	BI	LL + S6	- / -	115	No	2
157	53/F	BI	LL + S6	- / -	19	No	4
159	63/F	BI	LL	+ / -	19	No	6
163	50/M	BI	LH	+ / -	10	No	2

L: Left; R: Right; BI: Bilateral; S6: Segment 6; LH: Left hemihepatectomy; LL: Left lateral sectionectomy; RH: Right hemihepatectomy; RP: Right posterior sectionectomy; S-CCA: Subsequent cholangiocarcinoma; F: Female; M: Male.

not show encouraging results as did resection in unilateral disease[15]. We speculate that hepatic resection seems to be less effective in stopping the disease progression toward S-CCA in patients with bilateral hepatolithiasis.

This study showed clearly that the C-CCA group had lower overall mortality (P = 0.041) and disease-related mortality (P = 0.015) than the S-CCA group. This better survival outcome is demonstrated by the Kaplan-Meier curves (Figure 2, P = 0.005, log rank). Considering that the complete removal of the malignancy with a free surgical margin is essential for achieving good survival outcomes[16-18], the role of the first hepatectomy is definitely crucial. Paradoxically, preoperative C-CCA diagnosis is sometimes difficult due to the presence of IHD stones[19-21]. Among our 10 C-CCA patients, only one patient was diagnosed pre-operatively. In order to solve this problem, making good use of intraoperatively-collected frozen sections is recommended. In the case of a strongly suspected C-CCA in the absence of conclusive tissue proof, carrying out the hepatectomy aggressively can increase the likelihood of creating a safe surgical margin.

For the five patients (cases 90, 119, 143, 212 and 227) whose C-CCA diagnoses were made incidentally by post-operative pathology, the survival outcomes were good if they had safe surgical margins (cases 90, 143 and 227). The lymph node status is also an important prognostic factor for these patients; however, these data were incomplete because regional lymph node dissection was not routinely performed on these patients.

All 12 S-CCA patients died shortly after definitive S-CCA diagnosis. A possible explanation is that most patients at the time of S-CCA diagnosis are not eligible for a second hepatectomy due to distant metastasis, locally advanced disease, peritoneal seeding, or insufficient remaining liver volume. Only one S-CCA patient in our series (case 11) had the opportunity to undergo a second hepatectomy. However, the tumor could not be removed completely, and the patient died 4 mo after the operation.

The interval from the initial hepatectomy to S-CCA development ranged from 7 to 138 mo (average 67 mo). After hepatic resection for hepatolithiasis, we observed that in patients who presented residual or recurrent IHD

stones, the S-CCA incidence (11.8%, 4/34) was higher than in S-CCA patients who had achieved complete stone clearance and disease-free status after hepatectomy (4.9%, 8/163). However, 67% of S-CCA patients (8/12) had neither residual nor recurrent IHD stones. We can presume that undiagnosed CCA might be left in the liver remnant, resulting in S-CCA, especially for patients who developed S-CCA relatively soon after the initial hepatectomy (e.g., cases 157, 159, 163 and 236). Interestingly, however, one patient (case 37) had neither residual nor recurrent IHD stones but ultimately developed S-CCA after a follow-up period of 138 mo. Beyond the presence of residual and recurrent IHD stones, we should also consider other predisposing factors for the development of S-CCA such as cancer-related genes, female gender, choledochoenterostomy, liver atrophy, smoking, family history, and viral hepatitis infection[22-26].

This retrospective study is the first published investigation that subdivided and compared hepatolithiasis-related cholangiocarcinoma in concomitant and subsequent groups. The study design included patients who underwent hepatic resection for hepatolithiasis during the past two decades (1987 to 2007) in Changhua Christian Hospital. Unexpectedly, incomplete information in 65 patient medical records was noticed during data collection, forcing us to exclude these patients. The majority of the excluded patients were treated in the first decade (1987 to 1997). However, all CCA patients in our study were operated on after 1997. We believe that these missing records should cause minimal effect on our results, although the data collected in our study were skewed towards the second decade (1997 to 2007). Another limitation of this study could be the relatively small number of enrolled CCA patients. However, considering that the C-CCA incidence is only 5%-10% in our patient population, the difficulty in obtaining very large patient numbers is appreciable.

We want to reemphasize the importance of a successful first hepatectomy, because most patients with recurrent CCA or that develop S-CCA are not candidates for repeated surgical intervention.

COMMENTS

Background

Hepatolithiasis is the presence of stones in the intrahepatic duct (IHD) proximal to the confluence of the right and left main hepatic ducts. This disease is endemic in Southeast Asia and is also referred to as "IHD stones", "recurrent pyogenic cholangitis", "oriental cholangiohepatitis" and "Hong Kong disease" in the literature.

Research frontiers

Concomitant cholangiocarcinoma (C-CCA) has better outcomes than subsequent cholangiocarcinoma (S-CCA). The first hepatectomy is crucial because most patients with recurrent CCA or S-CCA are not eligible for repeated surgical intervention.

Innovations and breakthroughs

Parameters such as cholangiocarcinoma (CCA) incidence, interval from operation to CCA diagnosis, interval from CCA diagnosis to disease-related death, follow-up time, and mortality rate were calculated for both the C-CCA and S-CCA groups. The outcomes of the C-CCA and S-CCA groups were mathematically compared and analysed.

Peer review

C-CCAs associated to hepatolithiasis is a crucial problem in hepatobiliary medicine. The authors can heal well unilateral hepatolithiasis today, but there are not effective methods in a treatment of bilateral hepatolithiasis. S-CCAs associated to hepatolithiasis is a larger problem than unilateral hepatolithiasis. The long-term outcomes are bed. The authors show the average life span only 4 mo. Literature's data are similar. The subject is very interesting, rarely discussed in the literature, and the period of observation and follow-up are commendable. Manuscript is well written. Data table are well-documented. Refferences is appropriately selected.

REFERENCES

1 **Cook J**, Hou PC, Ho HC, Mcfadzean AJ. Recurrent pyogenic cholangeitis. *Br J Surg* 1954; **42**: 188-203 [PMID: 13209043]
2 **Stock FE**, Fung JH. Oriental cholangiohepatitis. *Arch Surg* 1962; **84**: 409-412 [PMID: 14039526]
3 **Mage S**, Morel AS. Surgical experience with cholangiohepatitis (hong kong disease) in canton chinese. *Ann Surg* 1965; **162**: 187-190 [PMID: 14326998]
4 **Sanes S**, Maccallum JD. Primary Carcinoma of the Liver: Cholangioma in Hepatolithiasis. *Am J Pathol* 1942; **18**: 675-687 [PMID: 19970650]
5 **Koga A**, Ichimiya H, Yamaguchi K, Miyazaki K, Nakayama F. Hepatolithiasis associated with cholangiocarcinoma. Possible etiologic significance. *Cancer* 1985; **55**: 2826-2829 [PMID: 2986827]
6 **Nakanuma Y**, Terada T, Tanaka Y, Ohta G. Are hepatolithiasis and cholangiocarcinoma aetiologically related? A morphological study of 12 cases of hepatolithiasis associated with cholangiocarcinoma. *Virchows Arch A Pathol Anat Histopathol* 1985; **406**: 45-58 [PMID: 2986349]
7 **Falchuk KR**, Lesser PB, Galdabini JJ, Isselbacher KJ. Cholangiocarcinoma as related to chronic intrahepatic cholangitis and hepatolithiasis. Case report and review of the literature. *Am J Gastroenterol* 1976; **66**: 57-61 [PMID: 184709]
8 **Chen DW**, Tung-Ping Poon R, Liu CL, Fan ST, Wong J. Immediate and long-term outcomes of hepatectomy for hepatolithiasis. *Surgery* 2004; **135**: 386-393 [PMID: 15041962 DOI: 10.1016/j.surg.2003.09.007]
9 **Chen MF**, Jan YY, Wang CS, Hwang TL, Jeng LB, Chen SC, Chen TJ. A reappraisal of cholangiocarcinoma in patient with hepatolithiasis. *Cancer* 1993; **71**: 2461-2465 [PMID: 8384069]
10 **Ohta G**, Nakanuma Y, Terada T. Pathology of hepatolithiasis: cholangitis and cholangiocarcinoma. *Prog Clin Biol Res* 1984; **152**: 91-113 [PMID: 6089228]
11 **Jan YY**, Chen MF, Wang CS, Jeng LB, Hwang TL, Chen SC. Surgical treatment of hepatolithiasis: long-term results. *Surgery* 1996; **120**: 509-514 [PMID: 8784405]
12 **Uchiyama K**, Kawai M, Ueno M, Ozawa S, Tani M, Yamaue H. Reducing residual and recurrent stones by hepatectomy for hepatolithiasis. *J Gastrointest Surg* 2007; **11**: 626-630 [PMID: 17468921 DOI: 10.1007/s11605-006-0024-8]
13 **Lee KF**, Chong CN, Ng D, Cheung YS, Ng W, Wong J, Lai P. Outcome of surgical treatment for recurrent pyogenic cholangitis: a single-centre study. *HPB* (Oxford) 2009; **11**: 75-80 [PMID: 19590627 DOI: 10.1111/j.1477-2574.2008.00018.x]
14 **Yang T**, Lau WY, Lai EC, Yang LQ, Zhang J, Yang GS, Lu JH, Wu MC. Hepatectomy for bilateral primary hepatolithiasis: a cohort study. *Ann Surg* 2010; **251**: 84-90 [PMID: 20032719 DOI: 10.1097/SLA.0b013e3181b2f374]
15 **Li SQ**, Liang LJ, Peng BG, Hua YP, Lv MD, Fu SJ, Chen D. Outcomes of liver resection for intrahepatic stones: a comparative study of unilateral versus bilateral disease. *Ann Surg* 2012; **255**: 946-953 [PMID: 22504194 DOI: 10.1097/SLA.0b013e31824dedc2]
16 **Lee CC**, Wu CY, Chen GH. What is the impact of coexistence of hepatolithiasis on cholangiocarcinoma? *J Gastroenterol Hepatol* 2002; **17**: 1015-1020 [PMID: 12167124]

17 **Li HY**, Zhou SJ, Li M, Xiong D, Singh A, Guo QX, Liu CA, Gong JP. Diagnosis and cure experience of hepatolithiasis-associated intrahepatic cholangiocarcinoma in 66 patients. *Asian Pac J Cancer Prev* 2012; **13**: 725-729 [PMID: 22524851]
18 **Boland B**, Kim A, Nissen N, Colquhoun S. Cholangiocarcinoma: aggressive surgical intervention remains justified. *Am Surg* 2012; **78**: 157-160 [PMID: 22369822]
19 **Chen MF**, Jan YY, Hwang TL, Jeng LB, Yeh TS. Impact of concomitant hepatolithiasis on patients with peripheral cholangiocarcinoma. *Dig Dis Sci* 2000; **45**: 312-316 [PMID: 10711444]
20 **Menias CO**, Surabhi VR, Prasad SR, Wang HL, Narra VR, Chintapalli KN. Mimics of cholangiocarcinoma: spectrum of disease. *Radiographics* 2008; **28**: 1115-1129 [PMID: 18635632]
21 **Hur H**, Park IY, Sung GY, Lee DS, Kim W, Won JM. Intrahepatic cholangiocarcinoma associated with intrahepatic duct stones. *Asian J Surg* 2009; **32**: 7-12 [PMID: 19321396]
22 **Kuroki T**, Tajima Y, Kanematsu T. Hepatolithiasis and intrahepatic cholangiocarcinoma: carcinogenesis based on molecular mechanisms. *J Hepatobiliary Pancreat Surg* 2005; **12**: 463-466 [PMID: 16365819 DOI: 10.1007/s00534-005-1004-1]
23 **Isse K**, Specht SM, Lunz JG, Kang LI, Mizuguchi Y, Demetris AJ. Estrogen stimulates female biliary epithelial cell interleukin-6 expression in mice and humans. *Hepatology* 2010; **51**: 869-880 [PMID: 20043322 DOI: 10.1002/hep.23386]
24 **Liu ZY**, Zhou YM, Shi LH, Yin ZF. Risk factors of intrahepatic cholangiocarcinoma in patients with hepatolithiasis: a case-control study. *Hepatobiliary Pancreat Dis Int* 2011; **10**: 626-631 [PMID: 22146627]
25 **Suzuki Y**, Mori T, Abe N, Sugiyama M, Atomi Y. Predictive factors for cholangiocarcinoma associated with hepatolithiasis determined on the basis of Japanese Multicenter study. *Hepatol Res* 2012; **42**: 166-170 [PMID: 22151748 DOI: 10.1111/j.1872-034X.2011.00908.x]
26 **Zhou Y**, Zhao Y, Li B, Huang J, Wu L, Xu D, Yang J, He J. Hepatitis viruses infection and risk of intrahepatic cholangiocarcinoma: evidence from a meta-analysis. *BMC Cancer* 2012; **12**: 289 [PMID: 22799744]

P- Reviewers O'Dwyer P, Smigielski JA **S- Editor** Gou SX
L- Editor A **E- Editor** Lu YJ

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i3.381

World J Gastroenterol 2013 January 21; 19(3): 381-388
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Acupuncture transcutaneous electrical nerve stimulation reduces discomfort associated with barostat-induced rectal distension: A randomized-controlled study

Wing-Wa Leung, Alice YM Jones, Simon SM Ng, Cherry YN Wong, Janet FY Lee

Wing-Wa Leung, Alice YM Jones, Department of Rehabilitation Sciences, The Hong Kong Polytechnic University, Hung Hom, Hong Kong 999077, China
Alice YM Jones, Faculty of Health Sciences, The University of Sydney, Sydney, NSW 2006, Australia
Simon SM Ng, Cherry YN Wong, Janet FY Lee, Division of Colorectal Surgery, Department of Surgery, The Chinese University of Hong Kong, Prince of Wales Hospital, Shatin, Hong Kong 999077, China
Author contributions: Leung WW, Jones AYM and Ng SSM designed research; Leung WW contributed to sample recruitment and explanation of procedures to subjects; Wong CYN contributed to data collection and analytic procedure in laboratory; Lee JFY analyzed data; and Leung WW, Jones AYM and Ng SSM wrote the paper.
Correspondence to: Wing-Wa Leung, MSc, PT, Department of Rehabilitation Sciences, The Hong Kong Polytechnic University, Hung Hom, Hong Kong 999077, China. leungww@surgery.cuhk.edu.hk
Telephone: +852-26321495 Fax: +852-26377974
Received: May 29, 2012 Revised: September 5, 2012
Accepted: September 22, 2012
Published online: January 21, 2013

Abstract

AIM: To explore the effectiveness of acupuncture transcutaneous electrical nerve stimulation (Acu-TENS), a non-invasive modality in reduction of rectal discomfort during barostat-induced rectal distension.

METHODS: Forty healthy subjects were randomized to receive 45 min of either Acu-TENS or placebo-TENS (no electrical output) over acupuncture points *Hegu* (large-intestine 4), *Neiguan* (pericardium 6) and *Zusanli* (stomach 36). A balloon catheter attached to a dual-drive barostat machine was then inserted into the subjects' rectum. A step-wise (4 mmHg) increase in balloon pressure was induced until maximal tolerable or 48 mmHg. Visual analogue scale and a 5-point subjective discomfort scale (no perception, first perception of distension, urge to defecate, discomfort/pain and extreme pain) were used to assess rectal discomfort at each distension pressure. Blood beta-endorphin levels were measured before, immediately after intervention, at 24 mmHg and at maximal tolerable distension pressure.

RESULTS: There was no difference in the demographic data and baseline plasma beta-endorphin levels between the two groups. Perception threshold levels were higher in the Acu-TENS group when compared to the placebo group, but the difference reached statistical significance only at the sensations "urge to defecate" and "pain". The distension pressures recorded at the "urge to defecate" sensation for the Acu-TENS and placebo-TENS groups were 28.0 ± 4.5 mmHg and 24.6 ± 5.7 mmHg, respectively (P = 0.043); and the pressures recorded for the "pain" sensation for these two groups were 36.0 ± 4.2 mmHg and 30.5 ± 4.3 mmHg respectively (P = 0.002). Compared to the placebo group, a higher number of participants in the Acu-TENS group tolerated higher distension pressures (> 40 mmHg) (65% in Acu-TENS *vs* 25% in placebo, P = 0.02). The plasma beta-endorphin levels of the Acu-TENS group were significantly higher than that of the placebo group at barostat inflation pressure of 24 mmHg (1.31 ± 0.40 ng/mL *vs* 1.04 ± 0.43 ng/mL, P = 0.044) and at maximal inflation pressure (1.46 ± 0.53 ng/mL *vs* 0.95 ± 0.38 ng/mL, P = 0.003).

CONCLUSION: Acu-TENS reduced rectal discomfort during barostat-induced rectal distension and concurrently associated with a rise in beta-endorphin level.

© 2013 Baishideng. All rights reserved.

Key words: Colonoscopy; Rectal discomfort; Transcutaneous electrical nerve stimulation; Acupuncture; Visceral pain

Leung WW, Jones AYM, Ng SSM, Wong CYN, Lee JFY. Acupuncture transcutaneous electrical nerve stimulation reduces discomfort associated with barostat-induced rectal distension: A randomized-controlled study. *World J Gastroenterol* 2012; 19(3): 381-388 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i3/381.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i3.381

INTRODUCTION

Early colonoscopy screening is encouraged in prevention of development of colorectal cancer[1]. However, the procedure is often associated with abdominal pain and discomfort[2]. The unpleasant feeling during examination may affect the patient's overall tolerance and thereby jeopardizing the accuracy of outcome findings. A combination of narcotic analgesia and benzodiazepines is often used to decrease the discomfort during colonoscopy[3,4]. However these medications are also associated with gastrointestinal upset and although uncommon, respiratory and cardiac arrests during colonoscopy were reported[5,6]. Report of a European colorectal cancer screening project revealed that more than 50% of the subjects developed hypoxaemia and 6% had severe bradycardia after colonoscopy with sedation[7].

Electroacupuncture (EA) is widely accepted in China and is considered a possible treatment option for acute and chronic pain of various origins[8-10]. The role of acupuncture in managing pain and anxiety during colonoscopy however is unclear. A randomized sham-controlled study[11] suggested that patients receiving EA to acupoints *Zusanli* (stomach meridian ST-36) and *Hegu* (large intestine meridian LI-4) experienced lower pain level during colonoscopy than those receiving sham acupuncture (SA), but the difference was non-significant statistically. Recently we have shown that application of EA to *Hegu* (large-intestine 4), *Neiguan* (pericardium 6) and *Zusanli* (stomach 36) was able to effectively reduce rectal discomfort during Barostat-induced rectal distension[12]. Acupuncture however is invasive, and its application requires an experienced acupuncturist. Application of transcutaneous electrical nerve stimulation (TENS) over acupuncture points (Acu-TENS) is a non-invasive modality and a novel analgesic therapy that combines the advantages of acupuncture and TENS in management of painful conditions[13,14]. Acu-TENS has been shown to be effective in reducing postoperative analgesic requirement[14]. This pilot study aims to explore the potential of Acu-TENS in reduction of visceral pain. Prior to investigation of its role at the actual colonoscopy procedure, where the intraluminal pressures often varied in different subjects, it is necessary to first establish its role using a model which standardizes the intensity and duration of the stimulation. A dual drive barostat device was therefore used to mimic the discomfort caused by gaseous distension during colonoscopy, such model allows the nature of discomfort, distension pressure and duration of stimulation to be standardized.

MATERIALS AND METHODS

Subjects

Hong Kong Chinese subjects with age between 18 to 65 years registered for a screening colonoscopy examination (a free health care service provided by the Hong Kong Hospital Authority for all local permanent residents with family history of colorectal cancer) were invited to participate in the study prior to the actual colonoscopy screening procedure. Exclusion criteria included known cardiovascular dysfunction (American Society of Anaesthesiologists grade Ⅲ or above), irritable bowel syndrome (Rome Ⅱ classification[15]), renal impairment, previous abdominal surgery or experience of colonoscopy, gastrointestinal complaints, pregnancy or allergy to acupuncture needles. Subjects who met the inclusion criteria were randomized to either the Acu-TENS or placebo Acu-TENS (PA) group. The respective intervention code was generated randomly by a computer program (Random Allocation Software, Version 1.0, Isfahan University of Medical Sciences, Iran). The group allocation numbers were then placed in sealed opaque envelopes. An envelope was drawn and allocated to each subject by a secretary blinded to the groupings. The envelope was opened by the investigator who then applied the identified intervention accordingly. The subjects, surgeons and all other parties, including an independent outcome assessor, were blinded to the implementation of Acu-TENS or PA.

Bowel preparation

All subjects were instructed to have low fiber diet three days prior to the study. On the day before examination, all subjects underwent standard mechanical bowel preparation with 4 liters of polyethylene glycol (Klean-Prep, Norgine Ltd., Middlesex, United Kingdom) to induce bowel movement and to clear the bowel in preparation for the colonoscopy procedure. Participants were asked to empty their bowel at home before coming to the hospital for examination.

Equipment

A dual-lumen polyethylene catheter (Model CR3-0005, Mui Scientific, Mississauga, Ontario, Canada) was attached to a 10 cm long and 600 mL capacity polyethylene bag (Model CT-BP600R, Mui Scientific) using a sterile surgical silk suture (Ethicon, Mersilk Soie, Somerville, New Jersey, United States), with the surgical knots sealed by latex glue. One lumen of the catheter allowed passage of compressed air for bag inflation and the other was attached to a transducer for monitoring of the pressure inside the bag. The catheter was attached to the dual drive barostat (Distender Series Ⅱ; G and J Electronics, Inc., Toronto, Canada) which controlled the rate of inflation and deflation of the bag electronically. To ensure

the bag attachment was airtight, the bag was inflated to 60 mmHg for 5 min and placed in sterile warm water to ensure there was no leakage of air from the system.

Intervention protocols

Acupuncture points *Hegu* (large-intestine 4), *Neiguan* (pericardium 6) and *Zusanli* (stomach 36) were first identified by the investigator before the procedure. LI 4 is located on the dorsum of the hand, between the 1st and 2nd metacarpal bones; Pericardium 6 is located on the palmar aspect of the forearm, 2 "cuns" above the transverse crease of the wrist between the flexor carpi radialis and palmaris longus tendons; ST 36 is located on the anterior aspect of the leg, at 3 cuns below the knee cap and one finger-breadth from the anterior crest of the tibia. One "cun" is the distance between the interphalangeal creases of the subject's middle finger[16]. These points were chosen because large-intestine 4 and pericardium 6 are reportedly used to reduce abdominal pain[17,18] and ST36 was shown to regulate colorectal muscle contractility in conscious rats[19,20]. All of the selected acupuncture points were swabbed with alcohol following a standard antiseptic process before the TENS electrodes were applied. Six gel pads were cut into size of 5 mm × 5 mm and placed over the subject's acupuncture points (bilaterally). Each cleaned rubber electrode was placed over the small gel pad (HD-001, ITO Company Limited, Tokyo, Japan), this allowed electrical stimulation to be focused mainly over the acupuncture points. The electrodes were then attached to a dual channel TENS machine (Model 120Z, ITO Company Limited, Tokyo, Japan). Subjects in the Acu-TENS group received a constant mode of electrical stimulation at 2 Hz and pulse width at 200 μs for 45 min. Intensity was set to just initiate muscle contraction. This stimulation protocol has been shown to be effective in inducing endorphin production[21,22]. Electrode placements were similar for subjects in the PA group, except that the electrical output from the TENS unit was disconnected. The output light however remained active and subjects were told that they may or may not feel the current.

Experimental procedures

All subjects arrived at the laboratory at 9 am on the assessment day. They were instructed to rest in the supine position for 15 min and randomized to receive 45 min of either Acu-TENS or PA in the supine position. The subject then adopted a left side-lying position with both knees flexed to 90º. The polyethylene bag which attached to the barostat was slowly inserted into subject's rectum to a distance 10 cm from the anal verge. The tube was taped to the buttock with micropores and the subject then resumed the supine position. After rested in this position for 15 min the bag was inflated for 60 s and then completely deflated in 60 s, a resting period of 1 minute then followed, this constituted one complete cycle of inflation. An incremental stepwise increase in rectal pressure at 4 mmHg was instilled at subsequent cycles until a pressure of 48 mmHg or when the subject could not tolerate further discomfort. Acu-TENS or PA continued during the whole barostat procedure. When the maximal 48 mmHg or an intolerable pressure was reached, the stimulation was discontinued and all electrodes and gel pads removed.

Outcome measures

Subjective discomfort scale: During each 60-s phase of sustained incremental pressure, the subject was asked to rate their rectal sensation using an electronic panel attached to a computer. Ratings were "no perception", "first perception of distension", "urge to defecate", "discomfort or pain" and "extreme pain". Concurrently, each subject was also asked to rate the degree of rectal discomfort using a visual analogue scale (VAS)-a 10 cm ungraduated line, with words "no discomfort at all" anchored to "0" end and "discomfort cannot be tolerated" anchored to the number "10". The VAS and subjective discomfort scales were recorded by an independent research assistant who was blinded to the intervention allocation.

Beta-endorphin measurement: Venous blood (3 mL) was drawn (from a cannula inserted into the cubital vein of each subject under aseptic technique) before the randomization process, immediately after the 45 min intervention, at a distension pressure of 24 mmHg and at highest tolerable pressure. The blood samples were transferred to the biochemistry laboratory of the involved hospital in EDTA tubes stored in a 4 ºC ice box. The blood sample was then centrifuged and frozen until further assayed. Batch analyzed for beta-endorphin (Human) were analyzed using EIA kit (Phoenix Pharmaceuticals Inc, 330 Beach Road, Burlingame, CA94010, United States). All subjects then undertook the colonoscopy screening procedure in the same afternoon following the barostat study.

Sample size estimation

Sample size estimation was based on the assumption that Acu-TENS could increase the mean pain threshold by 8 mmHg (this was based on our previous work which explored the effect of acupuncture in relief of discomfort induced by the barostat[12]. To yield a power of 80% with a significant level of 0.05, a sample size of at least 17 subjects in each group was required (sample size estimation was determined by SPSS Version 15.0 for Windows, SPSS Inc., Chicago, IL, United States).

Ethical approval

This study was conducted at a local district hospital from September 2009 to May 2010. The protocol was approved by the Ethics Review Committees of the involved hospital and ClinicalTrials.gov prior to data collection (Approval numbers: NCT01551654 and CRE-2008.546-T). Written informed consent was obtained from each subject prior to commencement of the study.

Table 1 Demographic data of subjects in the transcutaneous electrical nerve stimulation over acupuncture points and placebo transcutaneous electrical nerve stimulation over acupuncture points groups (mean ± SD)

Item	Acu-TENS group (n = 20)	PA group (n = 20)	P value
Age (yr)	53.4 ± 3.8	53.9 ± 3.5	0.669
Gender (male/female)	8/12	8/12	1.000
Body weight (kg)	63.9 ± 14.4	63.0 ± 9.35	0.825
Body height (m)	1.62 ± 0.07	1.61 ± 0.07	0.692
BMI(kg/m^2)	23.9 ± 3.61	24.0 ± 2.53	0.899

Acu-TENS: Transcutaneous electrical nerve stimulation over acupuncture points; PA: Placebo transcutaneous electrical nerve stimulation over acupuncture points; BMI: Body mass index.

Table 2 Number of subjects in the transcutaneous electrical nerve stimulation over acupuncture points and placebo transcutaneous electrical nerve stimulation over acupuncture points groups identified with colonic polyps during the colonoscopy screening procedure

Location of polyps	Acu-TENS group (n = 20)	PA group (n = 20)
Right side (caecum, ascending colon, hepatic flexure, transverse colon)	4	3
Left side (splenic flexure, descending colon, sigmoid)	1	1
Rectosigmoid junction	0	0
Rectum	0	0
No abnormality	15	16

Acu-TENS: Transcutaneous electrical nerve stimulation over acupuncture points; PA: Placebo transcutaneous electrical nerve stimulation over acupuncture points.

Statistical analysis

All data were analyzed using the Statistical Package for the Social Sciences (SPSS Version 15.0 for Windows, SPSS Inc., Chicago, IL, United States). Repeated measures ANOVA were used to determine the change in sensation at different bag inflation pressures. Between-group sensation at each pressure was compared using independent sample t test for parametric data, and Pearson χ^2 test or Mann-Whitney U test for nonparametric data. A statistical significant level was set at $P < 0.05$.

RESULTS

A total of 40 healthy subjects (16 male, 24 female; mean age 53.7 ± 3.62 years) were recruited to the study with 20 subjects randomized to either the Acu-TENS or PA group (Figure 1). The demographic data of all subjects were similar between the two groups (Table 1). All subjects completed the procedure and no adverse effects were observed during the intervention. Results of the colonoscopy screening procedure for this subject cohort are displayed in Table 2. While colonic polyps were found in some subjects, none were identified with rectal polyps or lesions. The pressures at which the subjects reported "urge to defecate" and "discomfort or pain" sensations were significantly higher in the Acu-TENS group compared to the PA group (Table 3). Furthermore, there were more subjects in the Acu-TENS group who tolerated a higher rectal distension pressures (at 40 mmHg, 44 mmHg and 48 mmHg) ($P < 0.05$) (Table 3). At each recorded pressure, the VAS score reported by the Acu-TENS group was lower (Figure 2A) although the differences did not reach a statistical significant level. The mean maximal tolerable pressure recorded during Acu-TENS (45.6 ± 5.09 mmHg) was higher than that during PA (39.8 ± 7.62 mmHg) ($P = 0.007$). The beta-endorphin level raised after 45 min Acu-TENS and continued to rise with increased inflation pressure (Table 3). Changes in beta-endorphin level in the PA group were not apparent. At 24 mmHg and maximal tolerable pressure, the beta-endorphin levels measured were significantly higher in the Acu-TENS when compared with the PA group (Figure 2B and Table 3).

Table 3 Perception threshold, distension pressures, levels of blood beta-endorphin in transcutaneous electrical nerve stimulation over acupuncture points and placebo transcutaneous electrical nerve stimulation over acupuncture points groups (mean ± SD)

	Acu-TENS	PA	P value
Perception threshold (mmHg)			
First perception of distension	18.4 ± 4.2	16.8 ± 6.4	0.358
Urge to defecate	28.0 ± 4.5	24.6 ± 5.7	0.043
Discomfort or pain	36.0 ± 4.2	30.5 ± 4.3	0.002
Extreme pain	40.0 ± 6.7	35.4 ± 5.6	0.179
Pressure (n = 20)			
40 mmHg	17 (85)	10 (50)	0.020
44 mmHg	13 (65)	3 (15)	0.001
48 mmHg	11 (55)	2 (10)	0.022
Levels of blood beta-endorphin (ng/mL)			
Baseline	1.04 ± 0.35	1.22 ± 0.52	0.216
After 45 min of intervention	1.14 ± 0.41	1.13 ± 0.48	0.980
At 24 mmHg pressure	1.31 ± 0.40	1.04 ± 0.43	0.044
At maximal tolerable pressure or 48 mmHg	1.46 ± 0.53	0.95 ± 0.38	0.003

Acu-TENS: Transcutaneous electrical nerve stimulation over acupuncture points; PA: Placebo transcutaneous electrical nerve stimulation over acupuncture points.

DISCUSSION

To the best of the authors' knowledge, this is the first report on the effect of Acu-TENS in reduction of rectal discomfort in human subjects. This study demonstrated Acu-TENS to large-intestine 4, pericardium 6 and stomach 36 reduced discomfort associated with rectal distension induced by a barostat. TENS is a non-invasive physical therapeutic modality commonly used for pain relief in conditions such as osteoarthritis and low back pain[23]. Although widely used in clinical situations, the working mechanism of TENS remains unclear. The pain-gate hypothesis proposed by Melzack and Wall[24] is considered a possible mechanism of control of stimulation

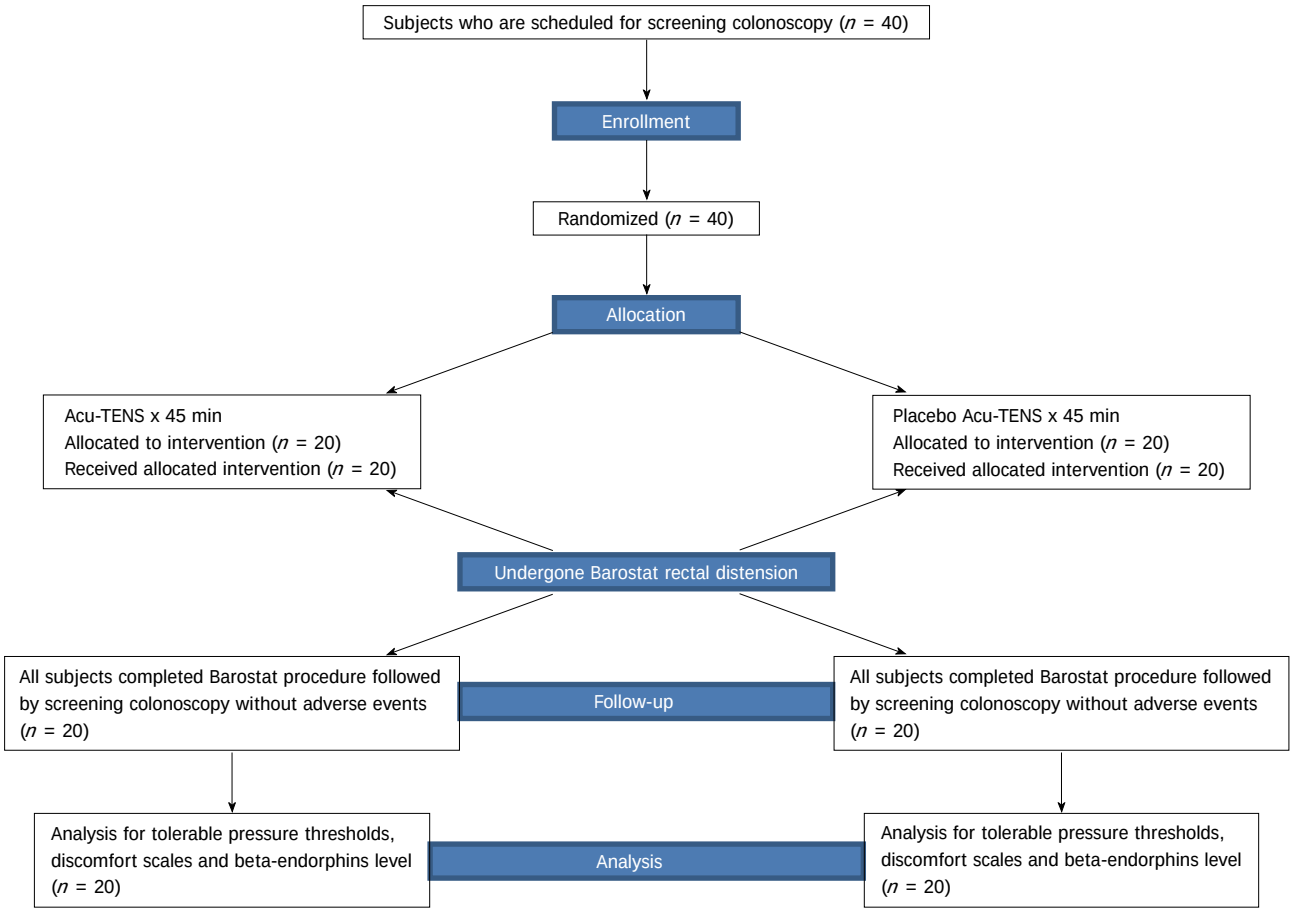


Figure 1 Flow diagram of the study. Acu-TENS: Transcutaneous electrical nerve stimulation over acupuncture points.

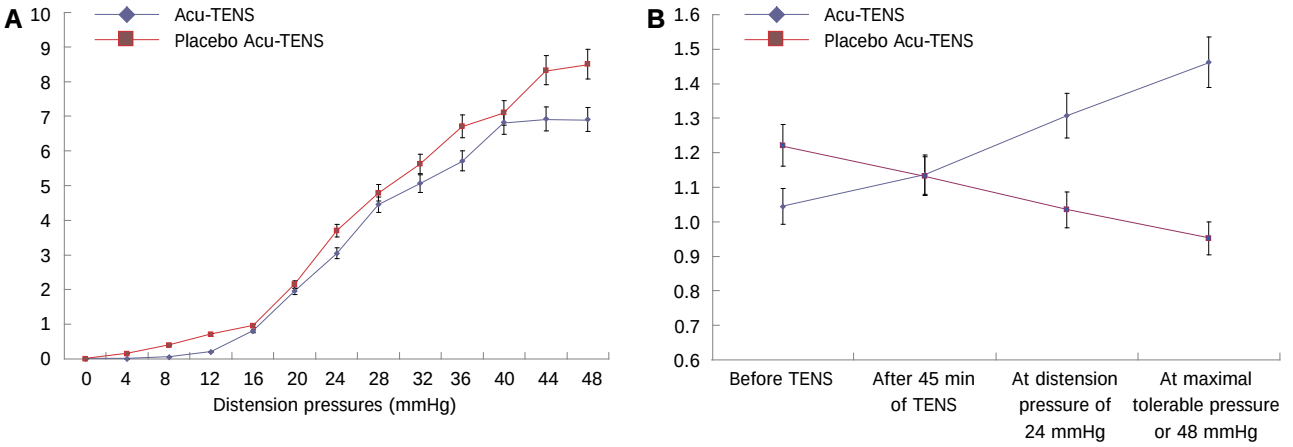


Figure 2 Visual analog discomfort score recorded at different distension pressures (A) and mean beta-endorphin levels recorded at different time-points (B). Acu-TENS: Transcutaneous electrical nerve stimulation over acupuncture points.

pathways during TENS. It was also believed that TENS inhibits pain signals via the descending pathway[25,26], the dorsal horn cell, and the spinothalamic tract[27]. Another prevalent theory discussed in literature is the release of endogenous opioids such as beta-endorphins, enkephalins and dynorphin after TENS[28-30]. Acu-TENS was previously shown to increase beta-endorphin levels in subjects with chronic respiratory disease[31]; finding of this current study further confirms that application of 45 min Acu-TENS induced the release of beta-endorphins in subjects with rectal discomfort. Our Acu-TENS protocol adopts a low stimulation frequency at 2 Hz. It was believed that low frequency TENS was able to trigger μ-and delta opioid receptors, the correspondents of beta-endorphin[29,31]. On the other hand, high frequency (100 Hz) stimulation was believed to trigger κ-opioid receptor and results in the release of dynorphin[29,31]. Irrespective of the working mechanisms, this study has shown that Acu-TENS

appears to be able to reduce the rectal distension pain induced by the barostat model and concurrently associated with an increase in beta-endorphin level. It was hypothesized that beta-endorphin possibly suppresses the action potential of neurons during neural transmission through the release of excess sodium molecules, consequently leading to inhibition of transmission of pain signals[32]. This is the first study which reports the effect of Acu-TENS on visceral pain in humans. We have previously demonstrated that acupuncture was an effective modality in reducing rectal discomfort associated with barostat induced rectal distension[12]. This study has shown that similar effect can be achieved non-invasively using Acu-TENS. The main difference in findings between our previous acupuncture study and this current one is that while the level of beta-endorphin increased immediately after 45 min of Acu-TENS, the increase reached a statistical significant level at 90 min after stimulation. However, in our previous study, the difference in change of beta-endorphin level between acupuncture and placebo acuptuncture was significant at immediately after 45 min of acupuncture stimulation. This probably is associated with the fact that one of the acupoints adopted in our studies, ST36, is situated in a much deeper anatomical position[33] and because Acu-TENS works in a "transcutaneous" manner, more time is therefore required for Acu-TENS to achieve the expected effect. Interestingly the level of beta-endorphin remained high for a longer period of time after Acu-TENS when compared with acupuncture stimulation. Acupuncture points large-intestine 4, pericardium 6 and stomach 36 were selected in this study because stimulation of *Hegu* (large-intestine 4) was associated with excitation of sensory nerve fibers in the pelvic complex and induced regulation of pelvic floor muscle contraction[34]. Stimulation of large-intestine 4 was also shown to be effective in management of labor pain and plantar fasciitis[34,35]. Stimulation of *Neiguan* (pericardium 6) and *Zusanli* (stomach 36) were reported to have significant clinical results in management of gastrointestinal symptoms[36,37]. Apart from being a non-invasive modality, another advantage of employing Acu-TENS over acupuncture is the low costing involved in Acu-TENS. The TENS machine is inexpensive, the Acu-TENS procedure is simple and experienced acupuncturists are not required for its application. Furthermore, apart from allergy to the gel used in a very minority of subjects, no side effects were reported with the use of Acu-TENS.

This study showed that while a higher distention pressure was tolerated during Acu-TENS, the difference at the VAS scores at any pressure level however did not reach a statistical difference. This could possibly be a consequence of both the slow release of endorphin during Acu-TENS, as well as the small sample size of this study. However convincing subjects who signed up for colonoscopy screening to pay an extra visit to the theatre for experimental recording of discomfort induced by a barostat machine was deemed demanding by many subjects. Another limitation of this study is that only Acu-TENS and placebo groups were included in the study, the strength of the placebo effect cannot be accurately evaluated without inclusion of a control group of no intervention. Inclusion of a control group however requires increasing the sample size, and as previously explained, this was difficult to comply. This study used the barostat model to mimic rectal discomfort. Previous reports on the use of the barostat model were mainly on patients with irritable bowel syndrome[38]. The barostat-induced rectal discomfort cannot accurately reproduce the same painful sensation as the stretching of mesenteric tissues during actual colonoscopy procedure. However, gaseous distension during colonoscopy also creates visceral pain and discomfort[39]. Our previous experience suggests that using the barostat model to mimic sensation of gaseous distension during colonoscopy appears to be a good model for evaluation of pain and distension discomfort under a standardized, controllable environment.

In conclusion, this study showed that 45 min of Acu-TENS over large-intestine 4, pericardium 6 and stomach 36, compared to placebo Acu-TENS, appeared to reduce the level of rectal discomfort induced by barostat distension and allowed the subjects to withstand a higher distension pressure. Subjective toleration of a higher inflation pressure was shown to be accompanied with a significant increase in beta-endorphin level. The role of Acu-TENS in application during clinical colonoscopy warrants further investigation.

COMMENTS

Background

Colonoscopy procedure is often associated with abdominal pain and discomfort. Application of transcutaneous electrical nerve stimulation over acupoints (Acu-TENS) is a non-invasive treatment modality for management of musculoskeletal pain, but its role for pain relief in endoscopic procedure has not been investigated.

Research frontiers

TENS has extensively been used as a physical modality for pain relief for musculoskeletal condition, however, the mechanism of action remains unclear. Beta-endorphin is a natural painkiller and largely found in the hypothalamus and pituitary gland. In this study, employing the barostat, an electronic device as an experimental pain model to standardize and mimic visceral pain caused by gaseous distension, the authors demonstrated that application of Acu-TENS is associated with the release of beta-endorphin effecting pain relief.

Innovations and breakthroughs

Endoscopic procedure is often associated with abdominal pain and discomfort, especially when there is increased gaseous distension. The unpleasant feeling during examination may affect the patient's overall tolerance and thereby jeopardizing the accuracy of outcome findings. Electroacupuncture is considered worldwide as a possible treatment option for acute and chronic pain of various origins, however, acupuncture is invasive and require an experienced acupuncturist. Acu-TENS, on the other hand, is a novel non-invasive modality that combines the advantage of TENS and acupuncture in the management of acute painful conditions.

Applications

Findings of this study suggest that Acu-TENS is potentially a therapeutic, non-invasive physical modality that could reduce rectal discomfort during an endoscopic procedure. The role of Acu-TENS during clinical colonoscopy warrants further investigation.

Terminology

Acu-TENS is the application of transcutaneous electrical nerve stimulation over acupuncture points. Visceral pain is an unpleasant sensation as a result of activation of nociceptors of the abdominal organs. Such type of pain is associated

with distension of the visceral tissue and often described as diffuse and difficult to localize. The electronic barostat served as an experimental pain model in this study to standardize and mimic visceral pain caused by gaseous distension.

Peer review

This is an interesting and well-designed study showing that Acu-TENS, a non-invasive analgesic therapy, reduced rectal discomfort during barostat-induced distensions. Moreover, reduction of pain was accompanied with a significant increase in beta-endorphin level, implying a key-role of endorphins in the mechanism of TENS.

REFERENCES

1 **Baxter NN**, Goldwasser MA, Paszat LF, Saskin R, Urbach DR, Rabeneck L. Association of colonoscopy and death from colorectal cancer. *Ann Intern Med* 2009; **150**: 1-8 [PMID: 19075198]

2 **Lee DW**, Li AC, Ko CW, Chu DW, Chan KC, Poon CM, Sin KS, Leung KF, Sze TS, Chan AC, Chung SC. Use of a variable-stiffness colonoscope decreases the dose of patient-controlled sedation during colonoscopy: a randomized comparison of 3 colonoscopes. *Gastrointest Endosc* 2007; **65**: 424-429 [PMID: 17321243 DOI: 10.1016/j.gie.2006.08.027]

3 **Keeffe EB**, O'Connor KW. 1989 A/S/G/E survey of endoscopic sedation and monitoring practices. *Gastrointest Endosc* 1990; **36**: S13-S18 [PMID: 2351253]

4 **Froehlich F**, Gonvers JJ, Fried M. Conscious sedation, clinically relevant complications and monitoring of endoscopy: results of a nationwide survey in Switzerland. *Endoscopy* 1994; **26**: 231-234 [PMID: 8026371 DOI: 10.1055/s2007-1008949]

5 **Iber FL**, Sutberry M, Gupta R, Kruss D. Evaluation of complications during and after conscious sedation for endoscopy using pulse oximetry. *Gastrointest Endosc* 1993; **39**: 620-625 [PMID: 8224681 DOI: 10.1016/S0016-5107(93)70211-4]

6 **Ristikankare M**, Julkunen R, Mattila M, Laitinen T, Wang SX, Heikkinen M, Janatuinen E, Hartikainen J. Conscious sedation and cardiorespiratory safety during colonoscopy. *Gastrointest Endosc* 2000; **52**: 48-54 [PMID: 10882962 DOI: 10.1067/mge.2000.105982]

7 **Khalid-de Bakker CA**, Jonkers DM, Hameeteman W, de Ridder RJ, Masclee AA, Stockbrügger RW. Cardiopulmonary events during primary colonoscopy screening in an average risk population. *Neth J Med* 2011; **69**: 186-191 [PMID: 21527807]

8 **Wayne PM**, Krebs DE, Macklin EA, Schnyer R, Kaptchuk TJ, Parker SW, Scarborough DM, McGibbon CA, Schaechter JD, Stein J, Stason WB. Acupuncture for upper-extremity rehabilitation in chronic stroke: a randomized sham-controlled study. *Arch Phys Med Rehabil* 2005; **86**: 2248-2255 [PMID: 16344019 DOI: 10.1016/j.apmr.2005.07.287]

9 **Witt CM**, Jena S, Brinkhaus B, Liecker B, Wegscheider K, Willich SN. Acupuncture in patients with osteoarthritis of the knee or hip: a randomized, controlled trial with an additional nonrandomized arm. *Arthritis Rheum* 2006; **54**: 3485-3493 [PMID: 17075849 DOI: 10.1002/art.22154]

10 **Carlsson CP**, Sjölund BH. Acupuncture for chronic low back pain: a randomized placebo-controlled study with long-term follow-up. *Clin J Pain* 2001; **17**: 296-305 [PMID: 11783809 DOI: 10.1097/00002508-200112000-00003]

11 **Wang HH**, Chang YH, Liu DM, Ho YJ. A clinical study on physiological response in electroacupuncture analgesia and meperidine analgesia for colonoscopy. *Am J Chin Med* 1997; **25**: 13-20 [PMID: 9166993 DOI: 10.1142/S0192415X97000408]

12 **Leung WW**, Jones AY, Ng SS, Wong CY, Lee JF. Electroacupuncture in reduction of discomfort associated with barostat-induced rectal distension--a randomized controlled study. *J Gastrointest Surg* 2011; **15**: 660-666 [PMID: 21327534 DOI: 10.1007/s11605-011-1446-5]

13 **Chao AS**, Chao A, Wang TH, Chang YC, Peng HH, Chang SD, Chao A, Chang CJ, Lai CH, Wong AM. Pain relief by applying transcutaneous electrical nerve stimulation (TENS) on acupuncture points during the first stage of labor: a randomized double-blind placebo-controlled trial. *Pain* 2007; **127**: 214-220 [PMID: 17030438 DOI: 10.1016/j.pain.2006.08.016]

14 **Chen L**, Tang J, White PF, Sloninsky A, Wender RH, Naruse R, Kariger R. The effect of location of transcutaneous electrical nerve stimulation on postoperative opioid analgesic requirement: acupoint versus nonacupoint stimulation. *Anesth Analg* 1998; **87:** 1129-1134 [DOI: 10.1097/00000539-199811000-00028]

15 **Thompson WG**, Longstreth GF, Drossman DA, Heaton KW, Irvine EJ, Müller-Lissner SA. Functional bowel disorders and functional abdominal pain. *Gut* 1999; **45** Suppl 2: II43-II47 [PMID: 10457044 DOI: 10.1136/gut.45.2008.ii43]

16 **Roboubi B**. What is cun? *Reg Anesth Pain Med* 2006; **31**: 91-92 [PMID: 16418038 DOI: 10.1016/j.rapm.2005.09.006]

17 **Chen LL**, Hsu SF, Wang MH, Chen CL, Lin YD, Lai JS. Use of acupressure to improve gastrointestinal motility in women after trans-abdominal hysterectomy. *Am J Chin Med* 2003; **31**: 781-790 [PMID: 14696681 DOI: 10.1142/S0192415X03001466]

18 **Xu HY**, Yang F, Zhu J, He ZP, Yan C. [Effect of electroacupuncture at Hegu (LI 4) and Sanyinjiao (SP 6) on short-term adverse effects of drug-induced abortion]. *Zhongguo Zhenjiu* 2007; **27**: 103-105 [PMID: 17370490]

19 **Petti F**, Bangrazi A, Liguori A, Reale G, Ippoliti F. Effects of acupuncture on immune response related to opioid-like peptides. *J Tradit Chin Med* 1998; **18**: 55-63 [PMID: 10437265]

20 **Luo D**, Liu S, Xie X, Hou X. Electroacupuncture at acupoint ST-36 promotes contractility of distal colon via a cholinergic pathway in conscious rats. *Dig Dis Sci* 2008; **53**: 689-693 [PMID: 17768682 DOI: 10.1007/s10620-007-9929-7]

21 **Sjölund BH**, Eriksson MB. The influence of naloxone on analgesia produced by peripheral conditioning stimulation. *Brain Res* 1979; **173**: 295-301 [PMID: 487091 DOI: 10.1016/0006-8993(79)90629-2]

22 **Lau KS**, Jones AY. A single session of Acu-TENS increases FEV1 and reduces dyspnoea in patients with chronic obstructive pulmonary disease: a randomised, placebo-controlled trial. *Aust J Physiother* 2008; **54**: 179-184 [PMID: 18721121 DOI: 10.1016/S00049514(08)70024-2]

23 **Berman BM**, Bausell RB. The use of non-pharmacological therapies by pain specialists. *Pain* 2000; **85**: 313-315 [PMID: 10781905 DOI: 10.1016/S0304-3959(00)00258-X]

24 **Melzack R**, Wall PD. Pain mechanisms: a new theory. *Science* 1965; **150**: 971-979 [PMID: 5320816 DOI: 10.1126/science.150.3699.971]

25 **Wall PD**, Sweet WH. Temporary abolition of pain in man. *Science* 1967; **155**: 108-109 [PMID: 6015561 DOI: 10.1126/science.155.3758.108]

26 **Melzack R**. From the gate to the neuromatrix. *Pain* 1999; Suppl 6: S121-S126 [PMID: 10491980 DOI: 10.1016/S0304-3959(99)00145-1]

27 **Garrison DW**, Foreman RD. Decreased activity of spontaneous and noxiously evoked dorsal horn cells during transcutaneous electrical nerve stimulation (TENS). *Pain* 1994; **58**: 309-315 [PMID: 7838579 DOI: 10.1016/0304-3959(94)90124-4]

28 **Salar G**, Job I, Mingrino S, Bosio A, Trabucchi M. Effect of transcutaneous electrotherapy on CSF beta-endorphin content in patients without pain problems. *Pain* 1981; **10**: 169-172 [PMID: 6267542 DOI: 10.1016/0304-3959(81)90192-5]

29 **Hughes GS**, Lichstein PR, Whitlock D, Harker C. Response of plasma beta-endorphins to transcutaneous electrical nerve stimulation in healthy subjects. *Phys Ther* 1984; **64**: 1062-1066 [PMID: 6330773]

30 **Han JS**, Chen XH, Sun SL, Xu XJ, Yuan Y, Yan SC, Hao JX, Terenius L. Effect of low- and high-frequency TENS on Met-

enkephalin-Arg-Phe and dynorphin A immunoreactivity in human lumbar CSF. *Pain* 1991; **47**: 295-298 [PMID: 1686080 DOI: 10.1016/0304-3959(91)90218-M]

31 **Ngai SP**, Jones AY, Hui-Chan CW, Ko FW, Hui DS. Effect of 4 weeks of Acu-TENS on functional capacity and beta-endorphin level in subjects with chronic obstructive pulmonary disease: a randomized controlled trial. *Respir Physiol Neurobiol* 2010; **173**: 29-36 [PMID: 20601209 DOI: 10.1016/j.resp.2010.06.005]

32 **Joshi N**, Araque H. Neurophysiologic basis for the relief of human pain by acupuncture. *Acupunct Electrother Res* 2009; **34**: 165-174 [PMID: 20344884]

33 **Chou PC**, Chu HY, Lin JG. Safe needling depth of acupuncture points. *J Altern Complement Med* 2011; **17**: 199-206 [PMID: 21417806 DOI: 10.1089/acm.2010.0192]

34 **Ternov K**, Nilsson M, Löfberg L, Algotsson L, Akeson J. Acupuncture for pain relief during childbirth. *Acupunct Electrother Res* 1998; **23**: 19-26 [PMID: 9607101]

35 **Zhang SP**, Yip TP, Li QS. Acupuncture treatment for plantar fasciitis: a randomized controlled trial with six months follow-up. *Evid Based Complement Alternat Med* 2011; **2011**: 154108 [PMID: 19933769]

36 **Gu Y**. Treatment of acute abdomen by electro-acupuncture--a report of 245 cases. *J Tradit Chin Med* 1992; **12**: 110-113 [PMID: 1495331]

37 **Diehl DL**. Acupuncture for gastrointestinal and hepatobiliary disorders. *J Altern Complement Med* 1999; **5**: 27-45 [PMID: 10100029 DOI: 10.1089/acm.1999.5.27]

38 **Kanazawa M**, Palsson OS, Thiwan SI, Turner MJ, van Tilburg MA, Gangarosa LM, Chitkara DK, Fukudo S, Drossman DA, Whitehead WE. Contributions of pain sensitivity and colonic motility to IBS symptom severity and predominant bowel habits. *Am J Gastroenterol* 2008; **103**: 2550-2561 [PMID: 18684175 DOI: 10.1111/j.1572-0241.2008.02066.x]

39 **Drewes AM**, Petersen P, Qvist P, Nielsen J, Arendt-Nielsen L. An experimental pain model based on electric stimulations of the colon mucosa. *Scand J Gastroenterol* 1999; **34**: 765-771 [PMID: 10499476 DOI: 10.1080/00365529975002568]

P- Reviewers Ladas S, Annese V **S- Editor** Gou SX
L- Editor A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i3.389

World J Gastroenterol 2013 January 21; 19(3): 389-393
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Preoperative carcinoembryonic antibody is predictive of distant metastasis in pathologically T1 colorectal cancer after radical surgery

Zheng Lou, Rong-Gui Meng, Wei Zhang, En-Da Yu, Chuan-Gang Fu

Zheng Lou, Rong-Gui Meng, Wei Zhang, En-Da Yu, Chuan-Gang Fu, Department of Colorectal Surgery, Changhai Hospital, Shanghai 200433, China

Author contributions: Lou Z, Meng RG, Zhang W and Yu ED performed the majority of patient treatment; Meng RG and Fu CG coordinated and collected all the clinical data in addition to providing financial support for this work; Lou Z and Meng RG designed the study, wrote and revised the manuscript.

Supported by Changhai Hospital 1255 Project Fund, No. CH125542500

Correspondence to: Dr. Rong-Gui Meng, Department of Colorectal Surgery, Changhai Hospital, No. 168, Changhai Road, Shanghai 200433, China. rongguimeng@163.com
Telephone: +86-21-31161608 Fax: +86-21-31161608

Received: September 19, 2012 Revised: December 12, 2012
Accepted: December 22, 2012
Published online: January 21, 2013

Abstract

AIM: To identify the predictors of distant metastasis in pathologically T1 (pT1) colorectal cancer (CRC) after radical resection.

METHODS: Variables including age, gender, preoperative carcinoembryonic antibody (CEA) level, tumor location, tumor size, lymph node status, and histological grade were recorded. Patients with and without metastasis were compared with regard to age, gender, CEA level and pathologic tumor characteristics using the independent *t* test or χ^2 test, as appropriate. Risk factors were determined by logistic regression analysis.

RESULTS: Metastasis occurred in 6 (3.8%) of the 159 patients during a median follow-up of 67.0 (46.5%) mo. The rates of distant metastasis in patients with pT1 cancer of the colon and rectum were 6.7% and 2.9%, respectively ($P < 0.001$). The rates of distant metastasis between male and female patients with T1 CRC were 6.25% and 1.27%, respectively ($P < 0.001$). The most frequent site of distant metastasis was the liver. Age ($P = 0.522$), gender ($P = 0.980$), tumor location ($P = 0.330$), tumor size ($P = 0.786$), histological grade ($P = 0.509$), and high serum CEA level ($P = 0.262$) were not prognostic factors for lymph node metastasis. Univariate analysis revealed that age ($P = 0.231$), gender ($P = 0.137$), tumor location ($P = 0.386$), and tumor size ($P = 0.514$) were not risk factors for distant metastasis after radical resection for T1 colorectal cancer. Postoperative metastasis was only significantly correlated with high preoperative serum CEA level ($P = 0.001$). Using multivariate logistic regression analysis, high preoperative serum CEA level ($P = 0.004$; odds ratio 15.341; 95%CI 2.371-99.275) was an independent predictor for postoperative distant metastasis.

CONCLUSION: The preoperative increased serum CEA level is a predictive risk factor for distant metastasis in CRC patients after radical resection. Adjuvant chemotherapy may be necessary in such patients, even if they have pT1 colorectal cancer.

© 2013 Baishideng. All rights reserved.

Key words: Colorectal cancer; Risk factor; Metastasis; Pathologically T1; Carcinoembryonic antigen

Lou Z, Meng RG, Zhang W, Yu ED, Fu CG. Preoperative carcinoembryonic antibody is predictive of distant metastasis in pathologically T1 colorectal cancer after radical surgery. *World J Gastroenterol* 2013; 19(3): 389-393 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i3/389.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i3.389

INTRODUCTION

Colorectal cancer (CRC) is one of the most common ma-

lignancies and a leading cause of cancer-related deaths in Europe and the United States[1-3]. Similarly, it is the fifth leading cause of cancer deaths in China and the incidence of CRC is rapidly increasing[4,5].

The standard surgical treatment for CRC is radical resection. Recent advances in diagnostic methods have led to an increase in the detection of T1 CRC[6,7]. Local excision has been substituted for radical resection in some patients with early CRC. However, local excision may result in a higher rate of local recurrence than radical resection in patients with T1 CRC. Local excision leaves metastatic lymph nodes in 8%-15% of pathologically T1 (pT1) CRC patients without definitively retrieving regional lymph nodes[8], resulting in a recurrence rate of 4.1%-39%[9]. However, the recurrence rate has been reported to be 1.3%-2.8% after radical resection of early CRC[6,10]. To minimize recurrence, radical surgery has been performed in China for the treatment of most patients with pT1 CRC.

Previous studies have focused on evaluating the risk factors for lymph node metastasis[10-13], and there is little evidence with regard to the risk factors for postoperative distant metastasis in patients with pT1 CRC. The aim of the present study was to identify the predictive risk factors that may suggest postoperative distant metastasis in patients with pT1 CRC after radical resection.

MATERIALS AND METHODS

One hundred fifty-nine patients with pT1 CRC who had undergone radical resection with lymph node dissection between January 2005 and June 2011 were enrolled. All patients were followed up until December 2011. The study was performed after approval by the Ethics Committee at the Second Military Medical University in Shanghai, China. None of these patients had received preoperative radiotherapy or neoadjuvant chemotherapy. Patients with pT1 CRC who were treated by endoscopic mucosal resection or transanal resection were excluded. Other exclusion criteria were recurrent CRC or cancer associated with familial adenomatous polyposis and inflammatory bowel disease.

Preoperative investigations included colonoscopy, chest X-rays, ultrasonography, computed tomography (CT) of the liver, and blood tests for carcinoembryonic antigen (CEA). A 3-mL peripheral blood sample from each patient was obtained. Serum CEA levels were determined using an enzyme immunoassay test kit (Beckman Coulter, Inc., Fullerton, CA, United States) with the upper limit of 5 ng/mL being defined as normal according to the kit manufacturer.

We established a 5- to 10-year follow-up period which included serum CEA measurements every 3 mo for the first 2 years and every 6 mo for the next 3 years, hepatic imaging (ultrasonography or CT) and chest X-rays every 3 mo, pelvic CT for rectal cancer every 6 mo, and colonoscopy every year.

Continuous variables were presented as means (standard deviation) and dichotomous variables were presented as number and percentage values. Patients with and without metastasis were compared with regard to age, gender, and clinicopathologic characteristics using the independent *t* test or χ^2 test, as appropriate. Logistic regression analysis was used to identify risk factors for distant metastasis. Variables significant at $P < 0.10$ by univariate analysis were subjected to stepwise logistic regression analysis to identify independent risk factors ($P < 0.05$) for distant metastasis. All analysis were performed with SPSS version 17 statistical software package (SPSS, Inc., Chicago, IL, United States).

RESULTS

Demographic data of the 159 patients with pT1 CRC are shown in Table 1. Distant metastasis occurred in 6 (3.8%) of the 159 patients during a median follow-up of 67.0 (46.5%) mo. The rates of distant metastasis in patients with pT1 cancer of the colon and rectum were 6.7% and 2.9%, respectively ($P < 0.001$). The recurrence rates among male and female patients with pT1 CRC were 6.25% and 1.27%, respectively ($P < 0.001$). The most frequent site of metastasis was the liver in pT1 CRC. Preoperative serum CEA level was higher in patients with distant metastasis than in patients without distant metastasis (11.35 ng/mL *vs* 3.25 ng/mL). Comparisons of patients with and without distant metastasis are shown in Table 1. The distant metastasis negative and positive groups were similar with regard to patient demographics and clinicopathologic features.

Based on univariate analysis of the correlation between lymph node metastasis (LNM) and clinicopathologic features, we found that age ($P = 0.522$), gender ($P = 0.980$), tumor location ($P = 0.330$), tumor size ($P = 0.786$), histological grade ($P = 0.509$), and high serum CEA level ($P = 0.262$) were not predictive factors for LNM (Table 2).

Univariate analysis revealed that age ($P = 0.231$), gender ($P = 0.137$), tumor location ($P = 0.386$), and tumor size ($P = 0.514$) were not risk factors for distant metastasis after radical resection for pT1 CRC (Table 2). The patients with unfavorable histological grade [odds ratio (OR) 1.365] were more likely to have metastasis, although the difference did not reach statistical significance ($P = 0.086$). Postoperative metastasis was only significantly correlated with a high serum CEA level ($P = 0.001$, Table 2). Using multivariate logistic regression analysis, high serum CEA level (OR 15.341, 95%CI 2.371-99.275, $P = 0.004$) was an independent predictor for postoperative distant metastasis.

Details of patients with distant metastasis are shown in Table 3. All distant metastases were found less than 3 years after surgery. Two of the six patients died due to the metastases, and the remaining patients are still alive after hepatic resection.

Table 1 Characteristics of 159 patients *n* (%)

Characteristics	With T1 colorectal cancer	Without metastasis (*n* = 153)	With metastasis (*n* = 6)	*P* value
Age, yr, mean ± SD	60.7 ± 11.7	60.48 ± 11.83	66.33 ± 7.012	0.231
Gender				0.099
Male	80 (50.3)	75	5	
Female	79 (49.7)	78	1	
Primary site				0.647
Cecum	3 (1.9)	3	0	
Ascending colon	9 (5.7)	8	1	
Transverse colon	6 (3.8)	6	0	
Descending colon	6 (3.8)	5	1	
Sigmoid colon	21 (13.2)	20	1	
Rectosigmoid	8 (5.0)	8	0	
Rectum	104 (65.4)	101	3	
Anus canal	2 (1.3)	2	0	
Pathology				0.919
Well differentiated	11 (6.9)	11	0	
Moderately differentiated	83 (52.2)	79	4	
Poorly differentiated	2 (1.3)	2	0	
Mucinous	4 (2.5)	4	0	
Localized canceration	59 (37.1)	57	2	
Lymphnode metastasis				0.611
N0	146 (91.8)	141	5	
N1	11 (6.9)	10	1	
N2	2 (1.3)	2	0	
CEA, ng/mL, mean ± SD	3.60 ± 4.01	3.29 ± 3.50	11.35 ± 7.87	0.054

CEA: Carcinoembryonic antibody.

Table 2 Risk factors for lymph node metastasis and distant metastasis in T1 colorectal cancer

Parameter	Odds ratio (95%CI)	*P* value
Lymph node metastasis		
Age	1.015 (0.970, 1.062)	0.522
Gender	0.986 (0.329, 2.954)	0.980
Location	0.518 (0.138, 1.943)	0.330
Tumor size	0.859 (0.287, 2.574)	0.786
Histological grade	0.929 (0.748, 1.155)	0.509
CEA	0.445 (0.115, 1.805)	0.262
Distant metastasis		
Age	1.050 (0.969, 1.138)	0.231
Gender	0.192 (0.022, 1.685)	0.137
Location	0.485 (0.095, 2.491)	0.386
Tumor size	0.563 (0.100, 3.163)	0.514
Histological grade	1.365 (0.957, 1.945)	0.086
LNM	2.154 (0.234, 19.850)	0.498
CEA (preoperation)	18.400 (3.106, 109.006)	0.001

CEA: Carcinoembryonic antibody; LNM: Lymph node metastasis.

DISCUSSION

Patients with pT1 CRC have a favorable prognosis, however, some patients develop recurrence including local recurrence and distant metastasis after radical resection[14,15]. Total recurrence rates have been reported to be as low as 0%-4% and as high as 17%-31% in T1 CRC[16]. The rate of distant metastasis in the present study was 3.8% which was consistent with a previous report.

Various factors such as serum CEA level, histological grade, and LNM for distant metastasis in CRC have been identified in previous reports[17-20], but most of these reports included pT2-T4 patients, and there is a paucity of evidence on the risk factors for distant metastasis in pT1 CRC[21-25]. Following univariate analysis of our data, we found that preoperative serum CEA level (OR 18.400, 95%CI 3.106-109.006, $P = 0.001$) and histological grade (OR 1.365, 95%CI 0.957-1.945, $P = 0.086$) were risk factors for predicting postoperative distant metastasis in patients with pT1 CRC.

Previous studies have reported that the LNM rate is up to 21% for T1-T2 CRC[21,22]. LNM is considered a risk factor for distant metastasis after radical resection for CRC[22]. It is noteworthy that LNM did not reach statistical significance in our series, with a higher OR in univariate analysis (OR 2.154, 95%CI 0.234-19.850, $P = 0.498$). Although our results did not show the same conclusion as previous reports, it is difficult to confidently exclude a correlation between LNM and distant metastasis in T1 CRC, because most patients (5/6, 83.3%) had less than 12 lymph nodes investigated (mean 6.2 lymph nodes).

CEA has been proved to be important in the assessment of prognosis of advanced CRC. Koca *et al*[11] conducted a study on 221 individuals, comprised of 69 (31.2%) patients with clinical stage Ⅱ and 152 (68.8%) with clinical stage Ⅲ, to evaluate potential predictors of recurrence and survival. They found that high serum CEA level was one of the risk factors for recurrence. Kim *et al*[26] also found that elevation of serum CEA was an independent factor for pulmonary metastasis after curative resection in 105 patients with CRC. In multivariate analysis of our data, we found that preoperative serum CEA level was an independent risk factor (OR 15.341, 95%CI 2.371-99.275, $P = 0.004$) in the prediction of postoperative distant metastasis in patients with pT1 CRC. Adjuvant chemotherapy might be necessary for such patients. Regular surveillance after radical resection in CRC patients should be performed, even if they have pT1 CRC, especially in patients with an increased serum CEA level. To our knowledge, this is the first study demonstrating a predictive role for serum CEA level in distant metastasis after radical resection in patients with pT1 CRC.

Interestingly, in our study, with a median follow-up period of 67.0 mo, the rates of distant metastasis in patients with T1 cancer of the colon and rectum were 6.7% and 2.9%, respectively ($P < 0.001$). The rates of distant metastasis in male and female patients with T1 CRC were 6.25% and 1.27%, respectively ($P < 0.001$). It is necessary to accumulate evidence in further studies to confirm these differences, because there is a limit to the number of cases seen in a single institution.

On the basis of our study of 159 consecutive patients with pT1 CRC, we propose that the increased preoperative serum CEA level is the independent risk factor for distant metastasis after radical resection. Adjuvant chemotherapy and regular surveillance after radical resection

Table 3 Details of distant metastasis patients

No.	Age (yr)	Gender	Location	CEA (ng/mL)	Histological grade	LNM	DFS	Recurrent site	Prognosis
1	62	Male	Ascending colon	13.66	Moderately differentiated	0/3	8	Liver and spleen	Diseased
2	61	Male	Descending colon	24.00	Localized canceration	0/15	23	Liver	Survived
3	68	Male	Rectum	15.00	Moderately differentiated	1/1	10	Liver	Survived
4	63	Female	Sigmoid colon	7.91	Moderately differentiated	0/10	30	Liver and lung	Diseased
5	80	Male	Rectum	5.20	Localized canceration	0/2	11	Liver	Survived

CEA: Carcinoembryonic antibody; LNM: Lymph node metastasis; DFS: Disease-free survival.

for such patients should be performed.

COMMENTS

Background

Colorectal cancer (CRC) is one of the most common malignancies and a leading cause of cancer-related deaths. Previous studies have focused on evaluating the risk factors for lymph node metastasis, and there is little evidence with regard to the risk factors for postoperative distant metastasis in patients with pathologically T1 (pT1) CRC.

Research frontiers

Patients with pT1 CRC have a favorable prognosis, but some patients develop recurrence including local recurrence and distant metastasis after radical resection. In this study, the authors demonstrate that the preoperative carcinoembryonic antibody (CEA) level could be predictive of distant metastasis in pT1 CRC after radical surgery.

Innovations and breakthroughs

The authors described the relationship between preoperative CEA levels and distant metastasis in pT1 CRC after surgery. This is the first study to demonstrate a predictive role for serum CEA level in distant metastasis after radical resection in patients with pT1 CRC.

Applications

Preoperative CEA level could be predictive of distant metastasis in pT1 CRC patients after radical surgery. This study suggests that adjuvant chemotherapy might be necessary for such patients.

Terminology

Preoperative serum CEA level is a risk factor in the prediction of postoperative distant metastasis in patients with pT1 CRC.

Peer review

The authors present an interesting study on risk factors for the occurrence of distant metastasis in early pT1 colorectal carcinomas. The manuscript is well structured and the cited literature is comprehensive and up-to-date. This is a clinically very interesting topic. Their results are very valuable.

REFERENCES

1 **Landis SH**, Murray T, Bolden S, Wingo PA. Cancer statistics, 1999. *CA Cancer J Clin* 1999; **49**: 8-31 [PMID: 10200775 DOI: 10.3322/canjclin.49.1.8]

2 **Weinberg DS**, Schoen RE. Colorectal cancer screening: America's next top model? *Ann Intern Med* 2012; **157**: 673-674 [PMID: 23128867 DOI: 10.7326/0003-4819-157-9-201211060-00017]

3 **Patel SS**, Floyd A, Doorly MG, Ortega AE, Ault GT, Kaiser AM, Senagore AJ. Current controversies in the management of colon cancer. *Curr Probl Surg* 2012; **49**: 398-460 [PMID: 22682507 DOI: 10.1067/j.cpsurg.2012.03.002]

4 **Zhao P**, Dai M, Chen W, Li N. Cancer trends in China. *Jpn J Clin Oncol* 2010; **40**: 281-285 [PMID: 20085904 DOI: 10.1093/jjco/hyp187]

5 **Wang JH**, King TM, Chang MC, Hsu CW. Oxaliplatin-induced severe anaphylactic reactions in metastatic colorectal cancer: case series analysis. *World J Gastroenterol* 2012; **18**: 5427-5433 [PMID: 23082060 DOI: 10.3748/wjg.v18.i38.5427]

6 **Iida S**, Hasegawa H, Okabayashi K, Moritani K, Mukai M, Kitagawa Y. Risk factors for postoperative recurrence in patients with pathologically T1 colorectal cancer. *World J Surg* 2012; **36**: 424-430 [PMID: 22187130 DOI: 10.1007/s00268-011-1378-y]

7 **Ramos-Esquivel A**. Screening flexible sigmoidoscopy for colon cancer. *N Engl J Med* 2012; **367**: 1064-1065; author reply 1065-1066 [PMID: 22970953 DOI: 10.1056/NEJMc1208513]

8 **Okabe S**, Shia J, Nash G, Wong WD, Guillem JG, Weiser MR, Temple L, Sugihara K, Paty PB. Lymph node metastasis in T1 adenocarcinoma of the colon and rectum. *J Gastrointest Surg* 2004; **8**: 1032-1039; discussion 1039-1040 [PMID: 15585391 DOI: 10.1016/j.gassur.2004.09.038]

9 **Dias AR**, Nahas CS, Marques CF, Nahas SC, Cecconello I. Transanal endoscopic microsurgery: indications, results and controversies. *Tech Coloproctol* 2009; **13**: 105-111 [PMID: 19484350 DOI: 10.1007/s10151-009-0466-6]

10 **Gao JY**, Song BR, Peng JJ, Lu YM. Correlation between mitochondrial TRAP-1 expression and lymph node metastasis in colorectal cancer. *World J Gastroenterol* 2012; **18**: 5965-5971 [PMID: 23139614 DOI: 10.3748/wjg.v18.i41.5965]

11 **Koca D**, Binicier C, Oztop I, Yavuzsen T, Ellidokuz H, Yilmaz U. Prognostic factors affecting recurrence and survival in patients with locally advanced rectal cancer. *J BUON* 2012; **17**: 291-298 [PMID: 22740208]

12 **Suh JH**, Han KS, Kim BC, Hong CW, Sohn DK, Chang HJ, Kim MJ, Park SC, Park JW, Choi HS, Oh JH. Predictors for lymph node metastasis in T1 colorectal cancer. *Endoscopy* 2012; **44**: 590-595 [PMID: 22638780 DOI: 10.1055/s-0031-1291665]

13 **Chang HC**, Huang SC, Chen JS, Tang R, Changchien CR, Chiang JM, Yeh CY, Hsieh PS, Tsai WS, Hung HY, You JF. Risk factors for lymph node metastasis in pT1 and pT2 rectal cancer: a single-institute experience in 943 patients and literature review. *Ann Surg Oncol* 2012; **19**: 2477-2484 [PMID: 22396007 DOI: 10.1245/s10434-012-2303-9]

14 **Mroczkowski P**, Schmidt U, Sahm M, Gastinger I, Lippert H, Kube R. Prognostic factors assessed for 15,096 patients with colon cancer in stages I and II. *World J Surg* 2012; **36**: 1693-1698 [PMID: 22411087 DOI: 10.1007/s00268-012-1531-2]

15 **Stipa F**, Giaccaglia V, Burza A. Management and outcome of local recurrence following transanal endoscopic microsurgery for rectal cancer. *Dis Colon Rectum* 2012; **55**: 262-269 [PMID: 22469792 DOI: 10.1097/DCR.0b013e318241ef22]

16 **Kobayashi H**, Mochizuki H, Sugihara K, Morita T, Kotake K, Teramoto T, Kameoka S, Saito Y, Takahashi K, Hase K, Oya M, Maeda K, Hirai T, Kameyama M, Shirouzu K, Muto T. Characteristics of recurrence and surveillance tools after curative resection for colorectal cancer: a multicenter study. *Surgery* 2007; **141**: 67-75 [PMID: 17188169 DOI: 10.1016/j.surg.2006.07.020]

17 **Shin R**, Jeong SY, Yoo HY, Park KJ, Heo SC, Kang GH, Kim WH, Park JG. Depth of mesorectal extension has prognostic significance in patients with T3 rectal cancer. *Dis Colon Rectum* 2012; **55**: 1220-1228 [PMID: 23135579 DOI: 10.1097/DCR.0b013e31826fea6a]

18 **Lee SD**, Kim TH, Kim DY, Baek JY, Kim SY, Chang HJ, Park SC, Park JW, Oh JH, Jung KH. Lymph node ratio is an independent prognostic factor in patients with rectal

cancer treated with preoperative chemoradiotherapy and curative resection. *Eur J Surg Oncol* 2012; **38**: 478-483 [PMID: 22465588 DOI: 10.1016/j.ejso.2012.03.002]

19 **Storli KE**, Søndenaa K, Bukholm IR, Nesvik I, Bru T, Furnes B, Hjelmeland B, Iversen KB, Eide GE. Overall survival after resection for colon cancer in a national cohort study was adversely affected by TNM stage, lymph node ratio, gender, and old age. *Int J Colorectal Dis* 2011; **26**: 1299-1307 [PMID: 21562744 DOI: 10.1007/s00384-011-1244-2]

20 **Klaver YL**, Lemmens VE, Nienhuijs SW, Luyer MD, de Hingh IH. Peritoneal carcinomatosis of colorectal origin: Incidence, prognosis and treatment options. *World J Gastroenterol* 2012; **18**: 5489-5494 [PMID: 23112540 DOI: 10.3748/wjg.v18.i39.5489]

21 **Nano M**, Ferronato M, Solej M, D'Amico S. T1 adenocarcinoma of the rectum: transanal excision or radical surgery? *Tumori* 2006; **92**: 469-473 [PMID: 17260485]

22 **Mehrkhani F**, Nasiri S, Donboli K, Meysamie A, Hedayat A. Prognostic factors in survival of colorectal cancer patients after surgery. *Colorectal Dis* 2009; **11**: 157-161 [PMID: 18462239 DOI: 10.1111/j.1463-1318.2008.01556.x]

23 **Kobayashi H**, Mochizuki H, Kato T, Mori T, Kameoka S, Shirouzu K, Saito Y, Watanabe M, Morita T, Hida J, Ueno M, Ono M, Yasuno M, Sugihara K. Is total mesorectal excision always necessary for T1-T2 lower rectal cancer? *Ann Surg Oncol* 2010; **17**: 973-980 [PMID: 19953330 DOI: 10.1245/s10434-009-0849-y]

24 **Huh JW**, Kim HR, Kim YJ. Lymphovascular or perineural invasion may predict lymph node metastasis in patients with T1 and T2 colorectal cancer. *J Gastrointest Surg* 2010; **14**: 1074-1080 [PMID: 20431977 DOI: 10.1007/s11605-010-1206-y]

25 **Kobayashi H**, Mochizuki H, Morita T, Kotake K, Teramoto T, Kameoka S, Saito Y, Takahashi K, Hase K, Oya M, Maeda K, Hirai T, Kameyama M, Shirouzu K, Sugihara K. Characteristics of recurrence after curative resection for T1 colorectal cancer: Japanese multicenter study. *J Gastroenterol* 2011; **46**: 203-211 [PMID: 21152938 DOI: 10.1007/s00535-010-0341-2]

26 **Kim CH**, Huh JW, Kim HJ, Lim SW, Song SY, Kim HR, Na KJ, Kim YJ. Factors influencing oncological outcomes in patients who develop pulmonary metastases after curative resection of colorectal cancer. *Dis Colon Rectum* 2012; **55**: 459-464 [PMID: 22426271 DOI: 10.1097/DCR.0b013e318246b08d]

P- Reviewers Linnebacher M, Tsuchida A **S- Editor** Huang XZ
L- Editor A **E- Editor** Li JY



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i3.394

World J Gastroenterol 2013 January 21; 19(3): 394-398
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Single-incision *vs* three-port laparoscopic cholecystectomy: Prospective randomized study

Ming-Xin Pan, Ze-Sheng Jiang, Yuan Cheng, Xiao-Ping Xu, Zhi Zhang, Jia-Sheng Qin, Guo-Lin He, Ting-Cheng Xu, Chen-Jie Zhou, Hai-Yan Liu, Yi Gao

Ming-Xin Pan, Ze-Sheng Jiang, Yuan Cheng, Xiao-Ping Xu, Zhi Zhang, Jia-Sheng Qin, Guo-Lin He, Ting-Cheng Xu, Chen-Jie Zhou, Hai-Yan Liu, Yi Gao, Second Department of Hepatobiliary Surgery, Zhujiang Hospital, Southern Medical University, Guangzhou 510282, Guangdong Province, China

Author contributions: Pan MX, Jiang ZS and Cheng Y contributed equally to this work; Pan MX and Gao Y designed the trial; Pan MX, Jiang ZS, Xu XP, Zhang Z, Qin JS, He GL, Xu TC, Zhou CJ and Liu HY performed the surgery; Pan MX and Cheng Y revised the paper.

Correspondence to: Yi Gao, MD, Second Department of Hepatobiliary Surgery, Zhujiang Hospital, Southern Medical University, No. 253, Gongye Avenue, Haizhu District, Guangzhou 510282, Guangdong Province, China. gaoyi6146@163.com
Telephone: +86-20-61643207 Fax: +86-20-84318988
Received: September 27, 2012 Revised: November 23, 2012
Accepted: December 15, 2012
Published online: January 21, 2013

Abstract

AIM: To compare the clinical outcome of single-incision laparoscopic cholecystectomy (SILC) with three-port laparoscopic cholecystectomy (TPLC).

METHODS: Between 2009 and 2011, one hundred and two patients with symptomatic benign gallbladder diseases were randomized to SILC (n = 49) or TPLC (n = 53). The primary end point was post operative pain score (at 6 h and 7 d). Secondary end points were blood loss, operation duration, overall complications, postoperative analgesic requirements, length of hospital stay, cosmetic result and total cost. Surgical techniques were standardized and all operations were performed by one experienced surgeon, who had performed more than 500 laparoscopic cholecystectomies.

RESULTS: One patient in the SILC group required conversion to two-port LC. There were no open conversions or major complications in either treatment groups. There were no differences in terms of estimated blood loss (mean ± SD, 14 ± 6.0 mL *vs* 15 ± 4.0 mL), operation duration (mean ± SD, 41.8 ± 17.0 min *vs* 38.5 ± 22.0 min), port-site complications (contusion at incision: 5 cases *vs* 4 cases and hematoma at incision: 2 cases *vs* 1 case), total cost (mean ± SD, 12 075 ± 1047 RMB *vs* 11 982 ± 1153 RMB) and hospital stay (mean ± SD, 1.0 ± 0.5 d *vs* 1.0 ± 0.2 d) , respectively. TPLC had a significantly worse visual analogue pain score at 8 h after surgery (mean ± SD, 3.5 ± 1.6 *vs* 2.0 ± 1.5), however, the scores were similar on day 7 (mean ± SD, 2.5 ± 1.4 *vs* 2.0 ± 1.3). Cosmetic satisfaction, as determined by a survey at 2 mo follow-up favored SILC (mean ± SD, 8 ± 0.4 *vs* 6 ± 0.2).

CONCLUSION: SILC is a safe and feasible approach in selected patients. The main advantages are a better cosmetic result and less pain.

© 2013 Baishideng. All rights reserved.

Key words: Cholecystectomy; Laparoscopic; Single-incision; Randomized; Laparoscopic cholecystectomy

Pan MX, Jiang ZS, Cheng Y, Xu XP, Zhang Z, Qin JS, He GL, Xu TC, Zhou CJ, Liu HY, Gao Y. Single-incision *vs* three-port laparoscopic cholecystectomy: Prospective randomized study. *World J Gastroenterol* 2013; 19(3): 394-398 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i3/394.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i3.394

INTRODUCTION

Laparoscopic cholecystectomy (LC) has become one of the most effective procedures for the treatment of benign gallbladder pathology since its introduction in 1985[1]. However, surgical standards of practice continue to evolve toward less invasive approaches, therefore many researchers have

attempted to minimize the invasiveness by reducing the number and size of the ports. In this context, single-port laparoscopic cholecystectomy (SILC) emerged in 1997[2]. The proposed advantages of SILC include fewer port sites with the potential for decreased pain, cosmetic benefit and faster recovery[3-10]. Some investigators have predicted that it may become a standard approach to LC[11,12]. However, it has also been suggested that SILC has many disadvantages, such as the technique is difficult to handle, prolonged operative time, cost of special instruments and increased risk of per-operative complications[9,13]. It is difficult to make an unbiased comparison between SILC and multi-port LC because of lack of prospective, randomized controlled trials (RCT). Therefore, we designed and conducted this RCT to evaluate the advantages and disadvantages of SILC and three-port laparoscopic cholecystectomy (TPLC).

MATERIALS AND METHODS

Inclusion criteria were as follows: (1) patients aged between 18 and 70 years; (2) body mass index less than 40 kg/m^2; (3) preoperative diagnosis of symptomatic gallstones or gallbladder polyps, and (4) willing to comply with the protocol requirements and signed written informed consent.

Exclusion criteria were as follows: (1) American Society of Anesthesiologists class Ⅳ and Ⅴ; (2) Patients with contraindication to laparoscopy; (3) Patients with suspected Mirizzi syndrome, common duct stones or malignancy; (4) Patients with previous upper abdominal surgery; (5) Patients on long-term anticoagulant treatment; (6) Acute cholecystitis or choledocholiathiasis; and (7) Gallstones > 3 cm in diameter.

Eligible patients were randomized into two groups (SILC and TPLC) using sealed opaque envelopes which contained a computer-generated random number. Before the trial, all patients underwent basic investigations such as blood tests, electrocardiogram, ultrasonography of the abdomen and radiologic imaging such as chest radiograph. All operations were performed by one experienced surgeon, who had performed more than 500 laparoscopic cholecystectomies.

Operative techniques

Surgical techniques were standardized. SILC was performed with the help of 2 slings of sutures, which included four steps: (1) Under general anesthesia, a 20 mm (approximately) bracket-shaped skin incision was made through the inner margin of the umbilicus and a pneumoperitoneum was set at 13 mmHg. One 10 mm trocar (Kanger, Tong Lu, China) to allow the insertion of a 30 degree laparoscope (Olympus, Tokyo, Japan) was inserted through the abdomen at the left side of the incision and a 5 mm trocar (Kanger, Tong Lu, China) was inserted at the right side for the harmonic scalpel. The tissues between the trocars were preserved to prevent air leakage; (2) The first suture using a straight needle was inserted through the right 7th intercostal space in the anterior axillary line, and the seromuscular layer of the gallbladder fundus was punctured and retracted toward the anterior abdominal wall. Hartmann's pouch was punctured and retracted using the second suture to expose Calot's triangle (Figure 1); (3) The harmonic scalpel (Ethicon Endo-Surgery, Cincinnati, OH, United States) was used to dissect Calot's triangle. Once the cystic artery and duct have been exposed, the cystic artery was cut using the harmonic scalpel, and the cystic duct was triple clipped and divided; and (4) The harmonic scalpel was used to dissect the gallbladder from the gallbladder fossa. When the gallbladder was free, the 5 mm trocar was exchanged for a 10 mm one, and through the 10 mm port, a specemen bag was inserted and the gallbladder was extracted after removal of the suspending sutures from the abdominal wall. The umbilical incision was closed and restored and no drainage tube was left in place (Figure 2).



Figure 1 A sketch of the suspension procedure.



Figure 2 The incision is reconstructed after single-incision laparoscopic cholecystectomy.

In TPLC, a sub-umbilical incision, sub-xiphoid incision and right sub-costal incision were made. A 10 mm trocar placed in the sub-umbilical incision allowed the introduction of laparoscope and the other two trocars, a 10 mm and a 5 mm, respectively, were placed for the grasp and harmonic scalpel. The operation was performed following the routine TPLC procedure[14], however, the cystic artery was divided and cut using the harmonic scalpel instead of being clipped and divided. The primary end-point of the

Table 1 Patients data and outcome

	SILC (n = 49)	TPLC (n = 53)	P value
Age (yr)	43.8 ± 14.0	45.2 ± 11.0	0.5493
Female	26	31	0.5810
WI (kg/m^2)	24.3 ± 6.0	25.1 ± 5.0	0.4649
ASA	1.5 ± 0.2	1.6 ± 0.3	0.0676
Clinical diagnosis			0.7790
Cholecystolithiasis	32	36	
Cystic polyps	17	17	
Operation duration (min)	41.8 ± 17.0	38.5 ± 22.0	0.4222
EBL (mL)	14 ± 6.0	15 ± 4.0	0.2643
VAS (1-10)			
8 h after surgery	2.0 ± 1.5	3.5 ± 1.6	0.0000
Day 7 after surgery	2.0 ± 1.3	2.5 ± 1.4	0.0651
Complications			
Contusion at incision	5	4	0.7350
Hematoma at incision	2	1	0.6070
Hospital stay (d)	1.0 ± 0.5	1.0 ± 0.2	1.0000
Cosmetic score	8 ± 0.4	6 ± 0.2	0.0000
Total cost (RMB)	12 075 ± 1047	11 982 ± 1153	0.6715

ASA: American Society of Anesthesiology; SILC: Single-incision laparoscopic cholecystectomy; TPLC: Three-port laparoscopic cholecystectomy; WI: Weight index; EBL: Estimated blood loss; VAS: Visual analog score; RMB: Renminbi yuan/Chinese yuan.

study was the postoperative pain score (at 6 h and 7 d). Secondary end-points included blood loss, operation duration, overall complications (intra- and post-operative complications), postoperative analgesic requirements, length of hospital stay, cosmetic result and total cost. A standard visual analog scale [range, 0 (no pain) to 10 (maximum pain)] was used for assessments at 8 h after surgery and on postoperative day 7. Cosmetic satisfaction of the surgical scar was rated on a scale [range, 0 (worst) to 10 (best)], and was evaluated at the 2-mo follow-up visit.

Statistical analysis

Statistical analysis was accomplished using the SPSS program for Windows 12.0 (SPSS, Chicago, IL, United States). The χ^2 test or the t test was used as indicated. All data were presented as mean ± SD. All P values were 2-sided. A P value of 0.05 was considered statistically significant.

RESULTS

From January 2009 to March 2011, 108 eligible patients were randomized to SILC (n = 51) or TPLC (n = 57). Two patients in the TPLC group and four patients in the SILC group refused to participate before surgery. In total, 102 patients (SILC, n = 49; TPLC, n = 53) were analyzed. Demographic variables in the two groups were similar (Table 1). All patients in the TPLC group successfully underwent three-port laparoscopic cholecystectomy without conversion to open surgery. Of 49 patients in the SILC group, one patient was converted to two-port LC due to anatomical difficulties and the operator felt it was unsafe to proceed with SILC. Overall, No major intra- or post-operative complications, such as biliary injury, abscess, bleeding, biliary collection or port-site hernia were observed in the two treatment groups. Several patients experienced port-site complications such as contusion and hematoma, however, all recovered a few days after surgery. There were no significant differences in estimated blood loss (14 ± 6.0 mL *vs* 15 ± 4.0 mL; P = 0.2643), operation duration (41.8 ± 17.0 min *vs* 38.5 ± 22.0 min; P = 0.4222), port-site complications (contusion at incision: 5 cases *vs* 4 cases; P = 0.7350 and hematoma at incision: 2 *vs* 1; P = 0.6070), hospital stay (1.0 ± 0.5 d *vs* 1.0 ± 0.2 d; P = 1.0000) and total cost (12 075 ± 1047 RMB *vs* 11 982 ± 1153 RMB; P = 0.6715) between the SILC and TPLC groups. However, the TPLC group had a significantly worse visual analogue pain score at 8 h after surgery (3.5 ± 1.6 *vs* 2.0 ± 1.5; P = 0.0000), but the score was similar on day 7 (2.5 ± 1.4 *vs* 2.0 ± 1.3; P = 0.0651). Cosmetic satisfaction as shown by the cosmetic score was significantly higher in the SILC group than in the TPLC group (8 ± 0.4 *vs* 6 ± 0.2; P = 0.0000) (Table 1).

DISCUSSION

SILC has attracted wide attention because of its potential cosmetic results. It may even be possible for this approach to become a gold standard for cholecystectomy[11,12]. However, there is still a long way to go before this approach is a gold standard, as standardization, safety, and the cosmetic results of SILC require validation[15]. Although recent reports have focused on comparisons between SILC and multi-port LC, the safety, better cosmetic results and faster recovery following SILC have been agreed, however, SILC has not been standardized and there is much technical variation. On the one hand, different surgeons have attempted SILC in different ways. For example, in exposing Calot's triangle, trials using sutures, Kirschner wires and loop retractors have been reported, in addition to different manipulative instruments, such as straight instruments and reticulating instruments[7]. On the other hand, devices used to prevent air leakage vary from one surgeon to another: some use common trocars[8], some tend to use SILS multi-port[7] and others favor self-designed devices such as sterile gloves[16]. In our center, we have tried many approaches in the initial stage. For example, to prevent air leakage, we have tried tri-port and gel-port, but due to their high cost in special ports and larger lesions of about 3 cm requiring a trans-umbilical incision these devices are no longer used. We decided to select routine trocars in our practice because they were sufficient to prevent air leakage and were more economical. With regard to the selection of surgical instruments, we use the suture suspension method in SILC, which requires only a 30 degree laparoscope and a manipulative instrument, eliminating the need for more instruments intra-operatively.

In practice, we focus on improving the auxiliary procedure with suspension sutures. To achieve ideal exposure of Calot's triangle, we select the puncture spot at the superior chest wall along the costal margin so that the suspension suture can draw the liver up a bit more than in other

places, different to Piskun *et al*[17] that the puncture spot should be selected at the inferior costal margin. In addition, the harmonic scalpel is effective for occluding 3 mm blood vessels and dissecting tissues[18]. In our experience, cystic arteries in the trial were cut using the harmonic scalpel, indicating the safety of this scalpel. Intra-operative management of the gallbladder is critical for the safety in SILC. Initially, we were perplexed as to how to handle intra-operative bile leakage. First, we placed the suspension sutures into the seromuscular layer to avoid perforation of the cyst wall, but this was inevitable sometimes. Subsequently, we found that this technique was useful as long as evacuating and repeated rinsing were conducted.

There have been a few comparisons between SILC and conventional multi-port LC[19-22]. These studies concluded that SILC is superior to multi-port LC in terms of cosmetic outcome, but not in terms of total cost, surgical time and postoperative pain. A similar cosmetic outcome in both treatment groups was observed in our study. However, there was no significant difference in the total cost between SILC and traditional LC when standard materials were selected. In addition, we found that SILC resulted in less postoperative pain than TPLC, and no significant difference in operation duration was observed between the two techniques. The higher pain score may be explained by the fact that there are more incisions in TPLC and the size of the incisions is larger than those in SILC. The different outcome of surgical time could be explained by difference in experience of the surgeon in SILC.

In conclusion, the results of this prospective trial demonstrate that SILC is a safe procedure when conducted by experienced surgeons, with some outcomes similar to that of TPLC, however, SILC was superior in terms of cosmetic outcome and postoperative pain. In the future, more high-powered randomized studies comparing SILC with conventional LC will be needed to validate its objective benefits and clinical role during the follow-up.

COMMENTS

Background

Laparoscopic cholecystectomy (LC) has been regarded as the "gold standard" for the management of benign gallbladder diseases since its introduction in 1985. However, surgical standards of practice continue to evolve toward less invasive approaches, therefore many researchers have attempted to minimize the invasiveness by reducing the number and size of the ports. In this context, single-incision laparoscopic cholecytectomy (SILC) emerged as a complement to standard LC. The proposed advantages of SILC include fewer port sites with the potential for decreased pain, cosmetic benefit and faster recovery. Some investigators have predicted that it may become a standard approach in LC. However, it has also been suggested that SILC has many disadvantages, such as the technique is difficult to handle, prolonged operative time, cost of special instruments and increased risk of per-operative complications. Therefore, it is difficult to make an unbiased comparison between SILC and multi-port LC due to the lack of prospective, randomized controlled trials.

Research frontiers

SILC has attracted wide attention because of its potential cosmetic results. However, it is unknown whether SILC could become a new standard preocedure, and therefore it is necessary to compare the clinical outcome of SILC and multiple-port laparoscopic cholecystectomy.

Innovations and breakthroughs

This prospective randomized study was performed to explore the safety and feasibility of SILC for the treatment of benign gallbladder diseases, and to compare the clinical outcomes with three-port laparoscopic cholecystectomy (TPLC).

Applications

In selected patients with benign gallbladder diseases, single-incision laparoscopic cholecystectomy is safe and feasible. The main advantages are that SILC result in a better cosmetic outcome and less pain compared with TPLC.

Terminology

SILC is a minimally invasive surgical procedure in which cholecystectomy is accomplished exclusively through a single 15-25 mm incision in the patient's navel. It is complementary to standard LC and an alternative to natural orifice transluminal endoscopic surgery. Unlike the traditional multi-port laparoscopic approach, SILC leaves only a single small scar in the navel.

Peer review

The authors have compared the clinical outcomes of SILC with TPLC and have concluded that SILC is a safe and feasible approach in selected patients. The main advantage is its better cosmetic result and less pain. The study is well organized and the content is adequately written, patient number is good and the authors have used good technique (harmonic for cystic artery).

REFERENCES

1 **Solomon D**, Bell RL, Duffy AJ, Roberts KE. Single-port cholecystectomy: small scar, short learning curve. *Surg Endosc* 2010; **24**: 2954-2957 [PMID: 20401494 DOI: 10.1007/s00464-010-1070-6]

2 **Navarra G**, Pozza E, Occhionorelli S, Carcoforo P, Donini I. One-wound laparoscopic cholecystectomy. *Br J Surg* 1997; **84**: 695 [PMID: 9171771 DOI: 10.1046/j.1365-2168.1997.02586.x]

3 **Barbaros U**, Sümer A, Tunca F, Gözkün O, Demirel T, Bilge O, Randazzo V, Dinççağ A, Seven R, Mercan S, Budak D. Our early experiences with single-incision laparoscopic surgery: the first 32 patients. *Surg Laparosc Endosc Percutan Tech* 2010; **20**: 306-311 [PMID: 20975500 DOI: 10.1097/SLE.0b013e3181f20cf6]

4 **Yu WB**, Zhang GY, Li F, Yang QY, Hu SY. Transumbilical single port laparoscopic cholecystectomy with a simple technique: initial experience of 33 cases. *Minim Invasive Ther Allied Technol* 2010; **19**: 340-344 [PMID: 20964560 DOI: 10.3109/13645706.2010.527772]

5 **Gajda Z**. 3 attempts at isolating lectures on dentistry at the Jagellonian University in the 1st half of the 19th century. *Czas Stomatol* 1979; **32**: 199-202 [PMID: 0374010 DOI: 10.1089/lap.2009.0395]

6 **Hirano Y**, Watanabe T, Uchida T, Yoshida S, Tawaraya K, Kato H, Hosokawa O. Single-incision laparoscopic cholecystectomy: single institution experience and literature review. *World J Gastroenterol* 2010; **16**: 270-274 [PMID: 20066749 DOI: 10.3748/wjg.v16.i2.270]

7 **Ponsky TA**. Single port laparoscopic cholecystectomy in adults and children: tools and techniques. *J Am Coll Surg* 2009; **209**: e1-e6 [PMID: 19854392 DOI: 10.1016/j.jamcollsurg]

8 **Kirschniak A**, Bollmann S, Pointner R, Granderath FA. Transumbilical single-incision laparoscopic cholecystectomy: preliminary experiences. *Surg Laparosc Endosc Percutan Tech* 2009; **19**: 436-438 [PMID: 20027084 DOI: 10.1097/SLE.0b013e3181c3f12b]

9 **Edwards C**, Bradshaw A, Ahearne P, Dematos P, Humble T, Johnson R, Mauterer D, Soosaar P. Single-incision laparoscopic cholecystectomy is feasible: initial experience with 80 cases. *Surg Endosc* 2010; **24**: 2241-2247 [PMID: 20198490 DOI: 10.1007/s00464-010-0943-z]

10 **Rivas H**, Varela E, Scott D. Single-incision laparoscopic cholecystectomy: initial evaluation of a large series of patients. *Surg Endosc* 2010; **24**: 1403-1412 [PMID: 20035355 DOI: 10.1007/s00464-009-0786-7]

11 **Emami CN**, Garrett D, Anselmo D, Torres M, Nguyen NX. Single-incision laparoscopic cholecystectomy in children: a feasible alternative to the standard laparoscopic approach. *J Pediatr Surg* 2011; **46**: 1909-1912 [PMID: 22008326 DOI:

10.1016/j.jpedsurg.2011.03.066]
12 **Jacob DA**, Raakow R. Single-port transumbilical endoscopic cholecystectomy: a new standard?. *Dtsch Med Wochenschr* 2010; **135**: 1363-1367 [PMID: 20589582 DOI: 10.1055/s-0030-1262419]
13 **Elsey JK**, Feliciano DV. Initial experience with single-incision laparoscopic cholecystectomy. *J Am Coll Surg* 2010; **210**: 620-624, 624-626 [PMID: 20421017]
14 **Trichak S**. Three-port vs standard four-port laparoscopic cholecystectomy. *Surg Endosc* 2003; **17**: 1434-1436 [PMID: 12799892 DOI: 10.1007/s00464-002-8713-1]
15 **Allemann P**, Schafer M, Demartines N. Critical appraisal of single port access cholecystectomy. *Br J Surg* 2010; **97**: 1476-1480 [PMID: 20641051 DOI: 10.1002/bjs.7189]
16 **Song SC**, Ho CY, Kim MJ, Kim WS, You DD, Choi DW, Choi SH, Heo JS. Clinical Analysis of Single-Port Laparoscopic Cholecystectomies: Early Experience. *J Korean Surg Soc* 2011; **80**: 43- 50 [DOI: 10.4174/jkss.2011.80.1.43]
17 **Piskun G**, Rajpal S. Transumbilical laparoscopic cholecystectomy utilizes no incisions outside the umbilicus. *J Laparoendosc Adv Surg Tech A* 1999; **9**: 361-364 [PMID: 10488834 DOI: 10.1089/lap.1999.9.361]
18 **Bessa SS**, Al-Fayoumi TA, Katri KM, Awad AT. Clipless laparoscopic cholecystectomy by ultrasonic dissection. *J Laparoendosc Adv Surg Tech A* 2008; **18**: 593-598 [PMID: 18721011 DOI: 10.1016/j.colsurfa.2005.04.039]
19 **Leung D**, Yetasook AK, Carbray J, Butt Z, Hoeger Y, Denham W, Barrera E, Ujiki MB. Single-incision surgery has higher cost with equivalent pain and quality-of-life scores compared with multiple-incision laparoscopic cholecystectomy: a prospective randomized blinded comparison. *J Am Coll Surg* 2012; **215**: 702-708 [PMID: 22819642 DOI: 10.1016/j.bbr.2011.03.031]
20 **Lee PC**, Lo C, Lai PS, Chang JJ, Huang SJ, Lin MT, Lee PH. Randomized clinical trial of single-incision laparoscopic cholecystectomy versus minilaparoscopic cholecystectomy. *Br J Surg* 2010; **97**: 1007-1012 [PMID: 20632264 DOI: 10.1002/bjs.7087]
21 **Lai EC**, Yang GP, Tang CN, Yih PC, Chan OC, Li MK. Prospective randomized comparative study of single incision laparoscopic cholecystectomy versus conventional four-port laparoscopic cholecystectomy. *Am J Surg* 2011; **202**: 254-258 [PMID: 21871979]
22 **Gangl O**, Hofer W, Tomaselli F, Sautner T, Függer R. Single incision laparoscopic cholecystectomy (SILC) versus laparoscopic cholecystectomy (LC)-a matched pair analysis. *Langenbecks Arch Surg* 2011; **396**: 819-824 [PMID: 21695591 DOI: 10.1007/s00423-011-0817-4]

P- Reviewers De Nardi P, Shah OJ **S- Editor** Wen LL
L- Editor A **E- Editor** Li JY



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i3.399

World J Gastroenterol 2013 January 21; 19(3): 399-403
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Helicobacter pylori tumor necrosis factor-α inducing protein promotes cytokine expression *via* nuclear factor-κB

Chun-Li Tang, Bo Hao, Guo-Xin Zhang, Rui-Hua Shi, Wen-Fang Cheng

Chun-Li Tang, Bo Hao, Guo-Xin Zhang, Rui-Hua Shi, Wen-Fang Cheng, Department of Gastroenterology, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China

Author contributions: Tang CL and Hao B completed the searches, collected the data and drafted the manuscript; Cheng WF designed the topic, conducted the meta-analysis, reviewed the data and the manuscript; Zhang GX and Shi RH supervised the whole study and edited the manuscript.

Correspondence to: Wen-Fang Cheng, Associate Chief Physician, Department of Gastroenterology, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China. chengwenfang@yahoo.com.cn
Telephone: +86-25-83718836 Fax: +86-25-83718836
Received: September 12, 2012 Revised: December 10, 2012
Accepted: December 20, 2012
Published online: January 21, 2013

Abstract

AIM: To study the effects of *Helicobacter pylori* (*H. pylori*) tumor necrosis factor-α (TNF) inducing protein (Tip-α) on cytokine expression and its mechanism.

METHODS: We cloned Tip-α from the *H. pylori* strain 26695, transformed *Escherichia coli* with an expression plasmid, and then confirmed the expression product by Western blotting. Using different concentrations of Tip-α that affected SGC7901 and GES-1 cells at different times, we assessed cytokine levels using enzyme-linked immunosorbent assay. We blocked SGC7901 cells with pyrrolidine dithiocarbamate (PDTC), a specific inhibitor of nuclear factor κB (NF-κB). We then detected interleukin (IL)-1β and TNF-α levels in SGC7901 cells.

RESULTS: Western blot analysis using an anti-Tip-α antibody revealed a 23-kDa protein, which indicated that recombinant Tip-α protein was recombined successfully. The levels of IL-1β, IL-8 and TNF-α were significantly higher following Tip-α interference, whether GES-1 cells or SGC-7901 cells were used ($P < 0.05$). However, the levels of cytokines (including IL-1β, IL-8 and TNF-α) secreted by SGC-7901 cells were greater than those secreted by GES-1 cells following treatment with Tip-α at the same concentration and for the same duration ($P < 0.05$). After blocking NF-κB with PDTC, the cells (GES-1 cells and SGC-7901 cells) underwent interference with Tip-α. We found that IL-1β and TNF-α levels were significantly decreased compared to cells that only underwent Tip-α interference ($P < 0.05$).

CONCLUSION: Tip-α plays an important role in cytokine expression through NF-κB.

© 2013 Baishideng. All rights reserved.

Key words: *Helicobacter pylori*; Tumor necrosis factor-α inducing protein; Interleukin-1β; Interleukin-8; Tumor necrosis factor-α; Nuclear factor-κB

Tang CL, Hao B, Zhang GX, Shi RH, Cheng WF. *Helicobacter pylori* tumor necrosis factor-α inducing protein promotes cytokine expression *via* nuclear factor-κB. *World J Gastroenterol* 2013; 19(3): 399-403 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i3/399.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i3.399

INTRODUCTION

Infection with *Helicobacter pylori* (*H. pylori*) leads to chronic gastritis, peptic ulcer, and gastric lymphoma[1-3]. *H. pylori* has also been associated with gastric cancer[4], is classified as a class I carcinogen by the International Agency for Research on Cancer[5], and *H. pylori* exerts its pathogenesis by secreting toxins, including hemolysin, lipopolysaccharides, CagA and VacA[6-9]. CagA and VacA are the major virulence factors. Persistent infection by *H. pylori* enables these toxins to stimulate gastric epithelial cells to produce a large number of cytokines such as tumor necrosis factor (TNF-α) and interleukin 1, 6 and 8 (IL-1, IL-6 and

IL-8), thus generating an inflammatory reaction[10-14]. Tumor necrosis factor-α inducing protein (Tip-α) is a new toxin discovered recently, and likely accelerates the inflammation and cancers caused by *H. pylori*[15]. However, its function and the mechanism underlying these effects remain unclear. The present work was conducted to determine the effects of recombinant Tip-α (rTip-α) on human gastric epithelial cells and gastric cancer cytokine expression, as well as explore the mechanisms involved.

MATERIALS AND METHODS

Materials

H. pylori strain 26695 was obtained from the Shanghai Institute of Digestive Disease. The following reagents were used in this study: Dual Promoter TA Cloning® Kit pCR® II and pET28a vectors (Invitrogen); monoclonal rabbit anti-Tip-α antibody (Beijing Aviva Systems Biology); *Bam*H I , *Xho* I and Prestained Protein Molecular Weight Markers (Fermentas); DNA and gel extraction kit from Tiangen Biotech (Beijing) Co. Ltd.; DNA marker (TaKaRa); His Trap™ *H. pylori* affinity chromatography column (GE Healthcare); and enhanced chemiluminescence kit (Pierce Protein Biology Products). The polymerase chain reaction primer sequences were 5'-TTGGATCCATGCTGCAGGCTTG-3', which contained an *Xho* I restriction site, and 5'-GGCTCGAGCTACATGGCTATAG-3', which contained a *Bam*H I restriction site. The primers were synthesized by Invitrogen. The human gastric epithelial cell line GES-1 and gastric cancer SGC7901 cells were purchased from the Shanghai Cancer Institute. Enzyme-linked immunosorbent assay (ELISA) kits were obtained from MultiSciences Biotech (Shanghai) Co., Ltd., while pyrrolidine dithiocarbamate (PDTC) was purchased from Jingmei Biotech Co., Ltd.

Methods

Expression, purification, and identification of Tip-α: We cloned *Tip-α* from the genome of *H. pylori* strain 26695. The *Tip-α* gene and pET28a vector (His tag) were digested with *Bam*H I and *Xho* I , purified, and then ligated together to generate the ET28a-Tip-α plasmid expressing recombinant Tip-α. This plasmid was transformed into *Escherichia coli* and the resultant protein was purified by Ni-NTA affinity chromatography and verified by Western blotting.

Cell recovery, culture, and passage: Cryopreserved GES-1 and SGC-7901 cells were centrifuged at 1000 rpm for 5 min. After removal of the supernatant, these cells were cultured in 60 mm × 60 mm dishes containing Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum.

IL-1β, IL-8 and TNF-α levels at different times following interference by 12.5 μg/mL rTip-α in GES-1 and SGC7901 cells: GES-1 and SGC7901 cells during their logarithmic growth phase underwent interference with 12.5 μg/mL rTip-α after starvation in serum-free medium for 24 h. The levels of IL-1β, IL-8 and TNF-α cytokines were then assessed at 0, 1, 2, 4 and 8 h post-interference using ELISA.

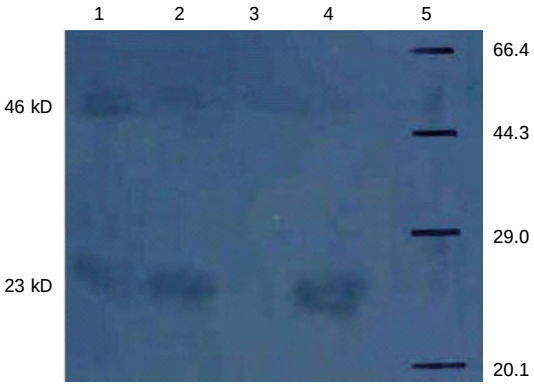


Figure 1 Western blotting identification of recombinant tumor necrosis factor-α inducing protein.

Levels of IL-1β, IL-8 and TNF-α in GES-1 and SGC7901 cells following incubation with different concentrations of rTip-α: We incubated GES-1 and SGC7901 cells with the following concentrations of rTip-α: 0, 12.5, 25 and 50 μg/mL. After 2 h, we examined the levels of IL-1β, IL-8 and TNF-α using ELISA.

Effects of rTip-α on IL-1β and TNF-α expression after PDTC-mediated inhibition of NF-κB: Four groups consisting of the same number of GES-1 and SGC7901 cells were starved in serum-free medium for 24 h before undergoing different treatments. Group A was treated with 12.5 μg/mL rTip-α for 2 h. Group B was treated similarly after PDTC blocking of NF-κB for 4 h. Groups C and D were incubated with serum-free medium and dimethyl sulfoxide (the vehicle with which PDTC was diluted), respectively. ELISA was performed to detect the levels of IL-1β and TNF-α in each group.

Statistical analysis

Data are presented as the mean ± SD and analyzed using SPSS 17.0. The Student's *t* test was used to compare two groups, while one-way analysis of variance was used to compare among several groups. A P value < 0.05 was considered statistically significant.

RESULTS

Identification by Western blotting after rTip-α expression and purification

Western blotting analysis demonstrated that the Tip-α recombinant protein and anti-human Tip-α monoclonal antibody could be specifically bound; specific bands were found (Figure 1). Western blotting analysis by non-denaturing gel electrophoresis showed active dimer bands (46 kDa).

Table 1 Cytokine levels at different times after interference of GES-1 and SGC7901 cells with 12.5 μg/mL recombinant tumor necrosis factor-α inducing protein

Groups	0 h	1 h	2 h	4 h	8 h
GES-1 (IL-1β)	0.34 + 0.04	0.88 + 0.09[a]	2.07 + 0.30[a]	1.35 + 0.20[a]	1.41 + 0.15[a]
SGC-7901 (IL-1β)	0.22 + 0.04	0.35 + 0.05	2.07 + 0.10[a]	1.11 + 0.04[a]	1.14 + 0.04[a]
GES-1 (IL-8)	0.35 + 0.05	0.60 + 0.12[a]	0.84 + 0.11[a]	0.64 + 0.06[a]	0.50 + 0.07[a]
SGC-7901 (IL-8)	0.70 + 0.02	0.78 + 0.19	2.53 + 0.50[a]	2.26 + 0.24[a]	2.14 + 0.68[a]
GES-1 (TNF-α)	0.39 + 0.06	0.39 + 0.07	0.72 + 0.08[a]	0.53 + 0.03[a]	0.51 + 0.14[a]
SGC-7901 (TNF-α)	0.33 + 0.09	1.02 + 0.09[a]	1.41 + 0.10[a]	0.86 + 0.07[a]	0.47 + 0.03[a]

[a]$P < 0.05$ *vs* 0 h. IL-1β: Interleukin-1β; TNF-α: Tumor necrosis factor-α.

Table 2 Cytokine levels in GES-1 and SGC7901 cells after interference with different concentrations of recombinant tumor necrosis factor-α inducing protein for 2 h

Groups	0	12.5 μg/mL	25 μg/mL	50 μg/mL
GES-1 (IL-1β)	0.59 + 0.11	2.07 + 0.30[a]	2.20 + 0.09[a]	1.23 + 0.13[a]
SGC-7901 (IL-1β)	0.36 + 0.01	2.07 + 0.10[a]	1.22 + 0.03[a]	1.02 + 0.04[a]
GES-1 (IL-8)	0.39 + 0.08	0.84 + 0.11[a]	0.75 + 0.09[a]	0.61 + 0.15[a]
SGC-7901 (IL-8)	0.78 + 0.09	2.53 + 0.50[a]	1.50 + 0.16[a]	1.41 + 0.14[a]
GES-1 (TNF-α)	0.30 + 0.06	0.72 + 0.08[a]	0.54 + 0.13[a]	0.63 + 0.10[a]
SGC-7901 (TNF-α)	0.26 + 0.18	1.41 + 0.10[a]	0.62 + 0.07[a]	0.62 + 0.02[a]

[a]$P < 0.05$ *vs* group 0. IL-1β: Interleukin-1β; TNF-α: Tumor necrosis factor-α.

Table 3 Effects of recombinant tumor necrosis factor-α inducing protein on the levels of interleukin-1β and tumor necrosis factor-α in SGC7901 cells under different conditions

Groups	A	B	C	D
SGC-7901 (IL-1β)	2.32 + 0.25	1.05 + 0.75[a]	084 + 0.08	0.57 + 0.09
SGC-7901 (TNF-α)	1.51 + 0.64	0.72 + 0.20[a]	0.43 + 0.07	0.71 + 0.23
GES-1 (IL-1β)	2.07 + 0.30	0.98 + 0.34[a]	0.69 + 0.06	0.63 + 0.06
GES-1 (TNF-α)	0.81 + 0.08	0.36 + 0.03[a]	0.27 + 0.03	0.29 + 0.04

[a]$P < 0.05$ *vs* group A. IL-1β: Interleukin-1β; TNF-α: Tumor necrosis factor-α.

IL-1β, IL-8 and TNF-α levels at different times following interference by 12.5 μg/mL rTip-α in GES-1 and SGC7901 cells

The levels of IL-1β, IL-8 and TNF-α were significantly higher after GES-1 and SGC7901 cells underwent interference by 12.5 μg/mL rTip-α for 1, 2, 4 and 8 h than those at 0 h. Cytokine secretion by GES-1 and SGC7901 cells peaked after rTip-α interference for 2 h, indicating no obvious dependence on time (Tables 1 and 2). However, after interference by rTip-α for 2 h, the levels of IL-8 (2.53 ± 0.50) and TNF-α (1.41 ± 0.10) in SGC7901 cells were significantly higher than those in GES-1 cells (0.84 ± 0.11 for IL-8 and 0.72 ± 0.08 for TNF-α). As shown in Table 1, the levels of IL-1β in GES-1 and SGC7901 cells (2.07 ± 0.10 and 2.07 ± 0.30, respectively) were not statistically different after rTip-α interference for 2 h.

Levels of IL-1β, IL-8 and TNF-α in GES-1 and SGC7901 cells following incubation with different concentrations of rTip-α

The levels of IL-1β, IL-8 and TNF-α were significantly higher than those in the blank control in GES-1 and SGC7901 cells after rTip-α interference for 2 h. Cytokine secretion of GES-1 and SGC7901 cells peaked at 12.5 μg/mL, suggesting that this effect was not concentration-dependent (Table 2).

Effects of rTip-α on IL-1β and TNF-α expression after PDTC-mediated inhibition of NF-κB

The levels of IL-1β and TNF-α in SGC7901 cells in Group B (treated with PDTC + rTip-α) were higher than those in Groups C and D (no rTip-α and PDTC), but markedly lower than those in Group A (only treated with rTip-α). As shown in Table 3, these differences were statistically significant ($P < 0.05$, $F = 40.15$).

DISCUSSION

Tip-α is a novel gene that was discovered recently in *H. pylori*. Located in the *H. pylori* 0596 open reading frame of the *H. pylori* 26695 strain, Tip-α is also called *H. pylori* 0596 protein. Its open reading frame is 519 bp in length and constitutes 173 amino acids. Tip-α has a molecular weight of 19 kDa and can form active homodimers with a molecular weight of 38 kDa through disulfide bonding[15]. Recent studies have found that Tip-α is associated with the adsorption and colonization of *H. pylori* in gastric mucosa[16]. Some studies have shown that the structure of Tip-α is different from penicillin binding proteins. Tip-α is composed of three closely linked domains that interact with other proteins and nucleic acids. In particular, the homodimer formed by cysteine C25 and C27 is essential for Tip-α to serve its role in the gastric mucosal acidic environment[17]. Detected by gene chip technology, expression of the chemokines Cc 12 Cc17, Cc120, Cx11 and Cx15 was enhanced after Tip-α treatment in gastric cancer cells and gastric epithelial cells[18]. These chemokines can induce chemotaxis of immune cells to local sites of infection, resulting in immune regulation and pathology, and ultimately the inflammatory response[19].

Because our pET28a-Tip-α vector also expresses a 3.74 kDa His tag, the recombinant Tip-α protein we produced possesses a molecular weight of about 23 kDa and can form active homodimers with a molecular weight of 46 kDa through disulfide bonding. Our work indicates that after affecting gastric epithelial and cancer

cells, rTip-α can promote the expression of IL-1β, IL-8 and TNF-α. These cytokines are important in promoting the inflammatory response, thus linking Tip-α to inflammation and the occurrence of *H. pylori*-related gastrointestinal diseases. After incubating the cells for 2 h with rTip-α, the levels of cytokine expression peaked at 12.5 μg/mL, which was the best concentration for interference. The levels of cytokine expression induced by rTip-α were not time- or concentration-dependent. These results suggest that Tip-α affects the host by inducing toxin secretion into the exterior environment of the bacteria through the type Ⅱ secretion system[20]. The toxins then enter host cells *via* receptor-mediated endocytosis instead of through injection *via* the Ⅳ secretion system[21-23]. Studies have shown that Tip-α possesses DNA binding activity. In particular, DNA affecting gastric mucosal cells can combine with some transcription factors to promote IL-1β, IL-8 and TNF-α expression, leading to the occurrence and development of *H. pylori*-related gastrointestinal diseases[24].

PDTC is a specific NF-κB inhibitor that works by blocking degradation of the NF-κB p65 subunit or IκB, thereby reducing NF-κB nuclear translocation[25]. Our data demonstrated no significant increase in IL-1β and TNF-α levels after pretreatment of SGC7901 cells with PDTC followed by rTip-α interference. This finding suggests that the promotion of cytokine expression by Tip-α may be regulated by NF-κB. However, further study is required to determine the underlying mechanism.

In addition, we found that when gastric epithelial and cancer cells were treated with the same concentration of Tip-α for the same duration, the level of cytokine expression in gastric cancer SGC7901 cells was significantly higher than that in gastric epithelial GES-1 cells. This difference may be due to differential effects of rTip-α on NF-κB expression in the cell types or may be related to variations in the DNA binding activity of Tip-α in the cells. Some studies suggest that the increased cytokine expression promoted by Tip-α may hasten the invasion and metastasis of gastric cancer[26]. Overall, Tip-α can activate cytokine expression in an NF-κB-dependent manner. Tip-α plays a major role in the pathogenesis of *H. pylori*, however, its mechanism requires further investigation.

COMMENTS

Background

Helicobacter pylori (*H. pylori*) exerts its pathogenesis by secreting toxins. Tumor necrosis factor-α (TNF-α) inducing protein (Tip-α) is a new toxin discovered recently, however, its function and mechanism of pathogenesis remain unclear.

Research frontiers

The pathogenesis of *H. pylori* is partially clear, as *H. pylori* may secrete many types of toxins. With the exception of CagA and VacA, new *H. pylori* toxins have been discovered, such as Tip-α, mammalian, prokaryotic high-temperature requirement A, and the duodenal ulcer-promoting gene. Only the function of the toxins and their mechanism of pathogenesis are clear, as the mechanism of *H. pylori* pathogenesis is known.

Innovations and breakthroughs

Other studies discovered that Tip-α promoted the expression of cytokines, however, this article first showed the difference in the promotion of the expression of cytokines between gastric epithelial cells and cancer cells, and that Tip-α activates cytokine expression in a nuclear factor κB (NF-κB)-dependent manner.

Applications

Tip-α may become a marker of *H. pylori* virulence. The virulence of *H. pylori* may be determined by detecting Tip-α.

Terminology

Tip-α is the abbreviation for tumor necrosis factor-α inducing protein. It was first discovered that this new *H. pylori* toxin can promote the expression of TNF-α, therefore, it was called Tip-α.

Peer review

The authors certified that Tip-α-a new toxin of *H. pylori*, promoted the expression of cytokine, discovered the difference of this function between gastric epithelial cells and cancer cells, and in an NF-κB-dependent manner, further interpreted the mechanism of pathogenesis of *H. pylori*.

REFERENCES

1 **Nomura A**, Stemmermann GN, Chyou PH, Perez-Perez GI, Blaser MJ. Helicobacter pylori infection and the risk for duodenal and gastric ulceration. *Ann Intern Med* 1994; **120**: 977-981 [PMID: 7741826]

2 **Kuipers EJ**. Helicobacter pylori and the risk and management of associated diseases: gastritis, ulcer disease, atrophic gastritis and gastric cancer. *Aliment Pharmacol Ther* 1997; **11** Suppl 1: 71-88 [PMID: 9146793 DOI: 10.1046/j.1365-2036.11.s1.5.x]

3 **Parsonnet J**, Hansen S, Rodriguez L, Gelb AB, Warnke RA, Jellum E, Orentreich N, Vogelman JH, Friedman GD. Helicobacter pylori infection and gastric lymphoma. *N Engl J Med* 1994; **330**: 1267-1271 [PMID: 8145781 DOI: 10.1056/NEJM199405053301803]

4 **Johansson H**. Qualification conditions: invest more firmly in continuing education. *Vardfacket* 1978; **2**: 28-30 [PMID: 249197 DOI: 10.1002/1097-0142(19901215)66]

5 **Møller H**, Heseltine E, Vainio H. Working group report on schistosomes, liver flukes and Helicobacter pylori. *Int J Cancer* 1995; **60**: 587-589 [PMID: 7860130 DOI: 10.1002/ijc.2910600502]

6 **Segal ED**, Tompkins LS. Identification and characterization of a Helicobacter pylori hemolysin. *Infect Agents Dis* 1993; **2**: 178-182 [PMID: 8173790]

7 **Slomiany BL**, Piotrowski J, Sengupta S, Slomiany A. Inhibition of gastric mucosal laminin receptor by Helicobacter pylori lipopolysaccharide. *Biochem Biophys Res Commun* 1991; **175**: 963-970 [PMID: 1827258 DOI: 10.1016/0006-291X(91)91659-Z]

8 **Leunk RD**, Johnson PT, David BC, Kraft WG, Morgan DR. Cytotoxic activity in broth-culture filtrates of Campylobacter pylori. *J Med Microbiol* 1988; **26**: 93-99 [PMID: 3385767 DOI: 10.1099/00222615-26-2-93]

9 **Censini S**, Lange C, Xiang Z, Crabtree JE, Ghiara P, Borodovsky M, Rappuoli R, Covacci A. cag, a pathogenicity island of Helicobacter pylori, encodes type I-specific and disease-associated virulence factors. *Proc Natl Acad Sci USA* 1996; **93**: 14648-14653 [PMID: 8962108 DOI: 10.1073/pnas.93.25.14648]

10 **Crabtree JE**, Court M, Aboshkiwa MA, Jeremy AH, Dixon MF, Robinson PA. Gastric mucosal cytokine and epithelial cell responses to Helicobacter pylori infection in Mongolian gerbils. *J Pathol* 2004; **202**: 197-207 [PMID: 14743502 DOI: 10.1002/path.1498]

11 **Fan XJ**, Chua A, O'Connell MA, Kelleher D, Keeling PW. Interferon-gamma and tumour necrosis factor production in patients with Helicobacter pylori infection. *Ir J Med Sci* 1993; **162**: 408-411 [PMID: 8300378 DOI: 10.1007/BF02996319]

12 **Huang J**, O'Toole PW, Doig P, Trust TJ. Stimulation of interleukin-8 production in epithelial cell lines by Helicobacter pylori. *Infect Immun* 1995; **63**: 1732-1738 [PMID: 7729879]

13 **Noach LA**, Bosma NB, Jansen J, Hoek FJ, van Deventer SJ, Tytgat GN. Mucosal tumor necrosis factor-alpha, interleu-

kin-1 beta, and interleukin-8 production in patients with Helicobacter pylori infection. *Scand J Gastroenterol* 1994; **29**: 425-429 [PMID: 8036458 DOI: 10.3109/00365529409096833]
14 **Gionchetti P**, Vaira D, Campieri M, Holton J, Menegatti M, Belluzzi A, Bertinelli E, Ferretti M, Brignola C, Miglioli M. Enhanced mucosal interleukin-6 and -8 in Helicobacter pylori-positive dyspeptic patients. *Am J Gastroenterol* 1994; **89**: 883-887 [PMID: 8198099]
15 **Suganuma M**, Kurusu M, Suzuki K, Nishizono A, Murakami K, Fujioka T, Fujiki H. New tumor necrosis factor-alpha-inducing protein released from Helicobacter pylori for gastric cancer progression. *J Cancer Res Clin Oncol* 2005; **131**: 305-313 [PMID: 15616827 DOI: 10.1007/s00432-004-0652-x]
16 **Godlewska R**, Pawlowski M, Dzwonek A, Mikula M, Ostrowski J, Drela N, Jagusztyn-Krynicka EK. Tip-alpha (hp0596 gene product) is a highly immunogenic Helicobacter pylori protein involved in colonization of mouse gastric mucosa. *Curr Microbiol* 2008; **56**: 279-286 [PMID: 18172719 DOI: 10.1007/s00284-007-9083-7]
17 **Tosi T**, Cioci G, Jouravleva K, Dian C, Terradot L. Structures of the tumor necrosis factor a inducing protein Tipa: A novel virulence factor from Helicobacter pylori. *FEBS Letters* 2009; **583**: 1581-1585[DOI: 10.1016/j.febslet.2009.04.033]
18 **Kuzuhara T**, Suganuma M, Kurusu M, Fujiki H. Helicobacter pylori-secreting protein Tipalpha is a potent inducer of chemokine gene expressions in stomach cancer cells. *J Cancer Res Clin Oncol* 2007; **133**: 287-296 [PMID: 17393199 DOI: 10.1007/s00432-006-0169-6]
19 **Rossi D**, Zlotnik A. The biology of chemokines and their receptors. *Annu Rev Immunol* 2000; **18**: 217-242 [PMID: 10837058 DOI: 10.1146/annurev.immunol.18.1.217]
20 **Massari P**, Manetti R, Burroni D, Nuti S, Norais N, Rappuoli R, Telford JL. Binding of the Helicobacter pylori vacuolating cytotoxin to target cells. *Infect Immun* 1998; **66**: 3981-3984 [PMID: 9673292]
21 **Odenbreit S**, Püls J, Sedlmaier B, Gerland E, Fischer W, Haas R. Translocation of Helicobacter pylori CagA into gastric epithelial cells by type IV secretion. *Science* 2000; **287**: 1497-1500 [PMID: 10688800 DOI: 10.1126/science.287.5457.1497]
22 **Suganuma M**, Yamaguchi K, Ono Y, Matsumoto H, Hayashi T, Ogawa T, Imai K, Kuzuhara T, Nishizono A, Fujiki H. TNF-alpha-inducing protein, a carcinogenic factor secreted from H. pylori, enters gastric cancer cells. *Int J Cancer* 2008; **123**: 117-122 [PMID: 18412243 DOI: 10.1002/ijc.23484]
23 **Watanabe T**, Tsuge H, Imagawa T, Kise D, Hirano K, Beppu M, Takahashi A, Yamaguchi K, Fujiki H, Suganuma M. Nucleolin as cell surface receptor for tumor necrosis factor-alpha inducing protein: a carcinogenic factor of Helicobacter pylori. *J Cancer Res Clin Oncol* 2010; **136**: 911-921 [PMID: 20049476 DOI: 10.1007/s00432-009-0733-y]
24 **Kuzuhara T**, Suganuma M, Oka K, Fujiki H. DNA-binding activity of TNF-alpha inducing protein from Helicobacter pylori. *Biochem Biophys Res Commun* 2007; **362**: 805-810 [PMID: 17765875 DOI: 10.1084/jem.175.5.1181]
25 **Schreck R**, Meier B, Männel DN, Dröge W, Baeuerle PA. Dithiocarbamates as potent inhibitors of nuclear factor kappa B activation in intact cells. *J Exp Med* 1992; **175**: 1181-1194 [PMID: 1314883]
26 **Suganuma M**, Kuzuhara T, Yamaguchi K, Fujiki H. Carcinogenic role of tumor necrosis factor-alpha inducing protein of Helicobacter pylori in human stomach. *J Biochem Mol Biol* 2006; **39**: 1-8 [PMID: 16466631]

P- Reviewer Fukuhara K **S- Editor** Song XX
L- Editor Webster JR **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i3.404

World J Gastroenterol 2013 January 21; 19(3): 404-410
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Magnifying chromoendoscopy combined with immunohistochemical staining for early diagnosis of gastric cancer

Xian-Mei Meng, Yi Zhou, Tong Dang, Xu-Yang Tian, Jie Kong

Xian-Mei Meng, Yi Zhou, Tong Dang, Department of Gastroenterology, The Second Affiliated Hospital of Baotou Medical College, Baotou 014030, Inner Mongolia Autonomous Region, China
Xian-Mei Meng, Yi Zhou, Tong Dang, Institute of Digestive Diseases of Inner Mongolia Autonomous Region, Baotou 014030, Inner Mongolia Autonomous Region, China
Xu-Yang Tian, Jie Kong, Department of Pathology, The Second Affiliated Hospital of Baotou Medical College, Baotou 014030, Inner Mongolia Autonomous Region, China
Author contributions: Meng XM contributed to conception and design, analysis and interpretation of data; Zhou Y contributed to data gathering, analysis and interpretation of the data, drafting the article; Dang T contributed to conception and design, final approval of the article; Tian XY contributed to immunohistochemistry stain, analysis and interpretation of immunohistochemistry data; Kong J contributed to immunohistochemistry stain.
Supported by Grant from the Medical and Health Research Program of Inner Mongolia Autonomous Region, No. 2010069
Correspondence to: Tong Dang, Professor, Department of Gastroenterology, The Second Affiliated Hospital of Baotou Medical College, Baotou 014030, Inner Mongolia Autonomous Region, China. yizhou1019@163.com
Telephone: +86-472-3169706 Fax: +86-472-3169530
Received: November 7, 2012 Revised: December 7, 2012
Accepted: December 15, 2012
Published online: January 21, 2013

Abstract

AIM: To assess the diagnostic value of using magnifying chromoendoscopy combined with immunohistochemical staining of proliferating cell nuclear antigen (PCNA) and p53 in the detection of gastric precancerous lesions.

METHODS: Ninety-five patients who were treated for abdominal discomfort, abdominal pain, bloating, and acid reflux at our hospital from January 2010 to December 2011 were included in the study. An ordinary gastroscopic procedure was initially performed to select the lesions. All subjects underwent magnifying chromoendoscopy to observe morphological changes of gastric pits. Biopsies were then taken from each area of interest and sent for pathological examination and detection of PCNA and p53 expression by immunohistochemistry. An immunoreactivity score for each lesion was calculated. Based on immunoreactivity scores, immunohistochemical staining was then considered.

RESULTS: Compared to intestinal metaplasia, gastric pits were more diverse in size, more irregular in shape, and more disorderly in arrangement in moderate and severe dysplasia. PCNA and p53 expression was significantly higher in precancerous lesions (intestinal metaplasia and dysplasia) than in chronic gastritis. PCNA expression showed an upward trend in types A-F pits. The number of cases that showed strong PCNA positivity increased significantly with an increase in the severity of lesions. Rank sum test for independent samples showed that p53 expression was significantly higher in types E and F pits than in types A-D pits (H = 33.068, P = 0.000). Rank sum test for independent samples showed that PCNA expression was significantly higher in types E and F pits than in types A-D pits (H = 31.791, P = 0.001).

CONCLUSION: The presence of types E and F pits, in which p53 and PCNA are highly expressed, is highly suggestive of the occurrence of early cancer, and patients developing these changes should be closely followed.

© 2013 Baishideng. All rights reserved.

Key words: Magnifying chromoendoscopy; Gastric precancerous lesions; p53; Proliferating cell nuclear antigen; Early gastric cancer

Meng XM, Zhou Y, Dang T, Tian XY, Kong J. Magnifying chromoendoscopy combined with immunohistochemical stain-

ing for early diagnosis of gastric cancer. *World J Gastroenterol* 2013; 19(3): 404-410 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i3/404.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i3.404

INTRODUCTION

Gastric cancer is one of the most common human malignancies, ranking first in terms of both incidence and mortality among gastrointestinal malignancies in China. Although great technological progress has been made in the diagnosis and treatment of gastric cancer, there is no significant improvement in its prognosis, and the 5-year survival rate of gastric cancer is only about 20%[1]. This is because 67% of patients have already had local spread or distant metastasis at initial diagnosis[1]. Therefore, early diagnosis and treatment are important for improving the prognosis of gastric cancer.

Early gastric cancer only invades the mucosal layer and submucosal layer and has a 5-year survival rate as high as 90% to 95%. Gastroscopy has the advantages of easy operation and low false-negative detection rate and has long been considered the preferred and most reliable diagnostic modality for gastric cancer. It allows detection of minute lesions and flat lesions easily, estimation of the depth of invasion[2], and the performance of histological and molecular biological analysis of diseased tissue; it therefore has a higher detection rate of early cancer and precancerous lesions. Thus, it is important to carry out a regular follow-up of gastric precancerous lesions to increase the detection rate of early gastric cancer. However, the widespread use of gastroscopy does not significantly improve the detection rate of early gastric cancer. A recent study in Japan[3] indicates that endoscopy has a sensitivity of only 81% in detecting early gastric cancer, and this means that the false-negative rate is 19%. Therefore, worldwide efforts are being made to improve the sensitivity of endoscopy in the detection of early cancer and precancerous lesions so that the tumor can be detected at an early treatable stage.

The human genome is composed of about 100 000 genes, and the selective expression of these genes determines the whole human life course. Changes in gene expression play a central role in biological regulatory mechanisms. Tumorigenesis is the result of multiple genetic changes. Activation of oncogenes and inactivation or deletion of tumor suppressor genes are considered closely related to tumorigenesis. The tumor suppressor gene *p53* is well known as the guardian of the cell. To date, extensive and in-depth research on p53 has been conducted. Wild-type p53 is an important negative regulator of cell growth and can antagonize oncogenic functions *in vivo* to maintain the relative stability between positive and negative proliferation signals. Wild-type p53 is involved in many important biological processes such as cell cycle, DNA repair, cell differentiation, and apoptosis.

Proliferating cell nuclear antigen (PCNA) is an acidic nuclear protein that is involved in eukaryotic DNA synthesis and plays an important role in the regulation of the cell cycle. PCNA expression is significantly up-regulated in the process of malignant transformation of normal epithelium and is deemed a marker reflecting cell proliferation activity in most tumor types.

The aim of this study was to assess the diagnostic value of magnifying chromoendoscopy combined with detection of PCNA and p53 in the detection of gastric precancerous lesions.

MATERIALS AND METHODS

Patients

Ninety-five patients who were treated for abdominal discomfort, abdominal pain, bloating, and acid reflux at our hospital from January 2010 to December 2011 were selected. There were 60 males and 35 females. Their ages ranged from 17 to 78 years, with a median age of 52 years.

Methods

Endoscopic examination: The procedure was performed using a Fujinon (EG-590) gastroscope and EPX 4400 processor. An ordinary gastroscopic procedure was initially performed to select the lesions, and magnifying chromoendoscopy was then performed to observe morphological changes of gastric pits. Three to four biopsies were taken from each area of interest and sent for pathological examination and detection of PCNA and p53 expression.

Criteria for morphological classification: Based on morphological classification criteria proposed by Yang *et al*[4], gastric pits were divided into six fundamental types: A (round spot-like pits), B (short rod-like pits, arranged regularly and tightly, with elongations, branches and curvatures), C (linear pits, more sparse and thick compared to type B), D (patchy pits, elongated and tortuous pits connected to form a reticular appearance), E (villous pits, with finger-like tubers similar to enteral villus-like changes) and F (the pits have obscure or disappearing structures and extremely irregular arrangement). The boundaries between bowl-shaped defects on the erosion surface and neighboring structures are unclear. Defect areas may show granular protrusions and irregular thickened capillaries). When two or more types of gastric pits were present in a lesion, the lesion was defined as having the severe type of pits.

Immunohistochemical staining for PCNA and p53: Immunohistochemistry: Immunohistochemical analysis was performed using the streptavidin-peroxidase method to determine the expression of p53 and PCNA in the gastric mucosa. Briefly, tissue samples were fixed in 10% neutral buffered formalin, dehydrated in a graded series of ethanol, cleared, paraffinized, and cut into 4 μm sections. The sections were deparaffinized, hydrated

Table 1 Relation between gastric pit patterns and histopathological phenotypes

Pit type	Case	CSG	CAG	IM	Dysplasia	
					Mild	Severe
A	9	8	1	0	0	0
B	38	32	6	0	0	0
C	42	35	7	0	0	0
D	67	9	56	7	0	0
E	62	2	6	50	3	1
F	30	0	1	3	20	6
Total	248	74	84	60	23	7

CSG: Chronic superficial gastritis; CAG: Chronic atrophic gastritis; IM: Intestinal metaplasia.

Table 2 Expression of p53 and proliferating cell nuclear antigen in various groups *n* (%)

Group	Cases	p53 expression			Positive cases	PCNA expression			Positive cases
		-	+	++		-	+	++	
A	9	7	2	0	2 (22.2)	6	3	0	3 (33.3)
B	38	21	14	3	17 (44.7)	23	12	3	15 (39.7)
C	42	21	18	3	21 (50.0)	16	21	5	26 (61.9)
D	67	28	35	4	39 (58.2)	22	35	10	45 (67.2)
E	62	18	38	6	44 (71.0)	14	35	13	48 (77.1)
F	30	4	19	7	26 (86.7)	2	13	15	28 (93.3)

$P < 0.05$ between-group. PCNA: Proliferating cell nuclear antigen.

in a graded series of ethanol, incubated in a microwave to heat and high pressure cook for 10 min for antigen retrieval, and washed in tris-buffered saline (TBS) (3 × 5 min). After treatment with 3% H_2O_2 for 10 min, slides were washed again in TBS (3 × 5 min). The slides were then incubated with primary anti-PCNA (1:50; Maxim, Fuzhou, China) or anti-p53 (1:100; Maxim) antibody for 30 min at room temperature and washed in TBS (3 × 5 min). Immunolabeling was visualized by reaction with DAB for 10 min at room temperature. The sections were counterstained with hematoxylin, dehydrated and mounted. This experiment was performed by an experienced technician.

Evaluation of immunohistochemical staining: Scoring of immunohistochemical results was performed according to the semi-quantitative method. The percentage of immunopositive cells was scored using a four-point system as follows: 0 point, < 5% of positive cells; 1 point, 5%-25% of positive cells; 2 points, 26%-50% of positive cells; 3 points, 51%-75% of positive cells; 4 points, > 75% of positive cells. The staining intensity was scored similarly, with 0 point for negative staining, 1 point for weak staining (light yellow), 2 points for moderate staining (brown), and 3 points for strong staining (dark brown). Immunoreactivity score for each lesion was calculated as (the score for the percentage of immunopositivity cells + the score for the staining intensity)/2. Based on immunoreactivity scores, immunohistochemical staining was considered negative (< 0.5), weakly positive (0.5-1.5), or strongly positive (> 1.5). Estimation of scores for all lesions was performed by the same pathologist.

Statistical analysis

All statistical analysis were performed using SPSS 11.5 software. P values ⩽ 0.05 were considered statistically significant.

RESULTS

Magnifying chromoendoscopic findings

Relation between gastric pit patterns and histopathological phenotypes: A total of 248 biopsies were taken under a magnifying chromoendoscope. Of these biopsies, 9 had type A gastric pits, 38 had type B, 42 had type C, 67 had type D, 62 had type E, and 30 had type F (Table 1).

Under a magnifying chromoendoscope, intestinal metaplasia was characterized by the presence of type E gastric pits (villous pits). Dysplasia also showed changes similar to those of intestinal metaplasia (e.g., the presence of villous, linear or patchy pits); however, some characteristic changes were observed in some cases of dysplasia. Compared to intestinal metaplasia, gastric pits were more diverse in size, more irregular in shape, and more disorderly in arrangement in moderate and severe dysplasia. Type F gastric pits in the 30 cases of dysplasia showed varying degrees of these changes (Figure 1).

Immunohistochemical results

p53 expression: p53 expression was lowest in type A gastric pits and highest in type F pits, showing an upward trend from types A to F pits. p53 expression intensity also showed an upward trend (Figure 2A and B). Rank sum test for independent samples showed that p53 expression was significantly higher in types E and F pits than in types A-D pits ($H = 33.068$, $P = 0.000$). Between-group comparisons showed that p53 expression was significantly higher in types E and F pits than in type A pits (Table 2).

PCNA expression: PCNA expression was lowest in type A gastric pits and highest in type F pits, showing an upward trend from types A to F pits (Figure 2C and D). The number of cases that showed strong PCNA positivity increased significantly with an increase in the severity of lesions. Rank sum test for independent samples showed that PCNA expression was significantly higher in types E and F pits than in types A-D pits ($H = 31.791$, $P = 0.001$). Between-group comparisons showed that PCNA expression was significantly higher in types E and F pits than in type A pits (Table 2).

DISCUSSION

The normal gastric mucosa surface is divided by criss-cross small grooves into many lesser gastric areas, in

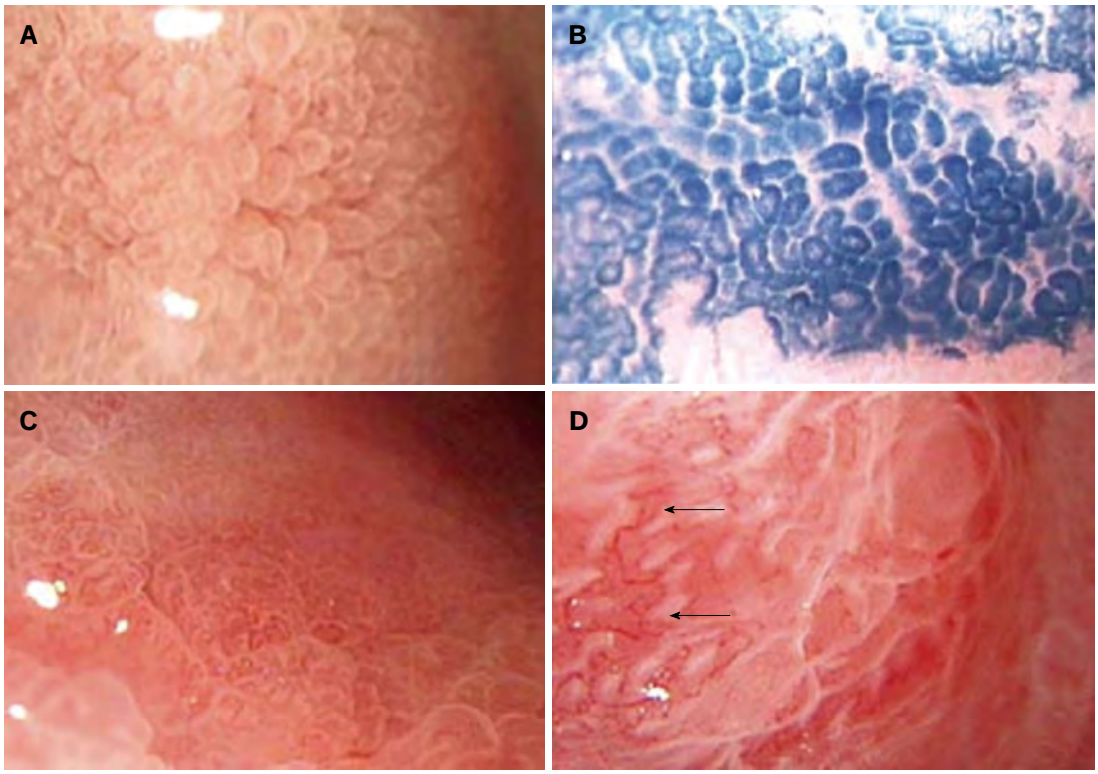


Figure 1 Magnifying chromoendoscopic findings. A: Type E pits; B: Type E pits after dye staining; C: Type F pits; D: Type F pits after dye staining, arrows show disorderly arranged thickened capillaries. Magnification, ×100. Type E: Villous pits, with finger-like tubers similar to enteral villus-like changes; Type F: The pits have obscure or disappearing structures and extremely irregular arrangement.

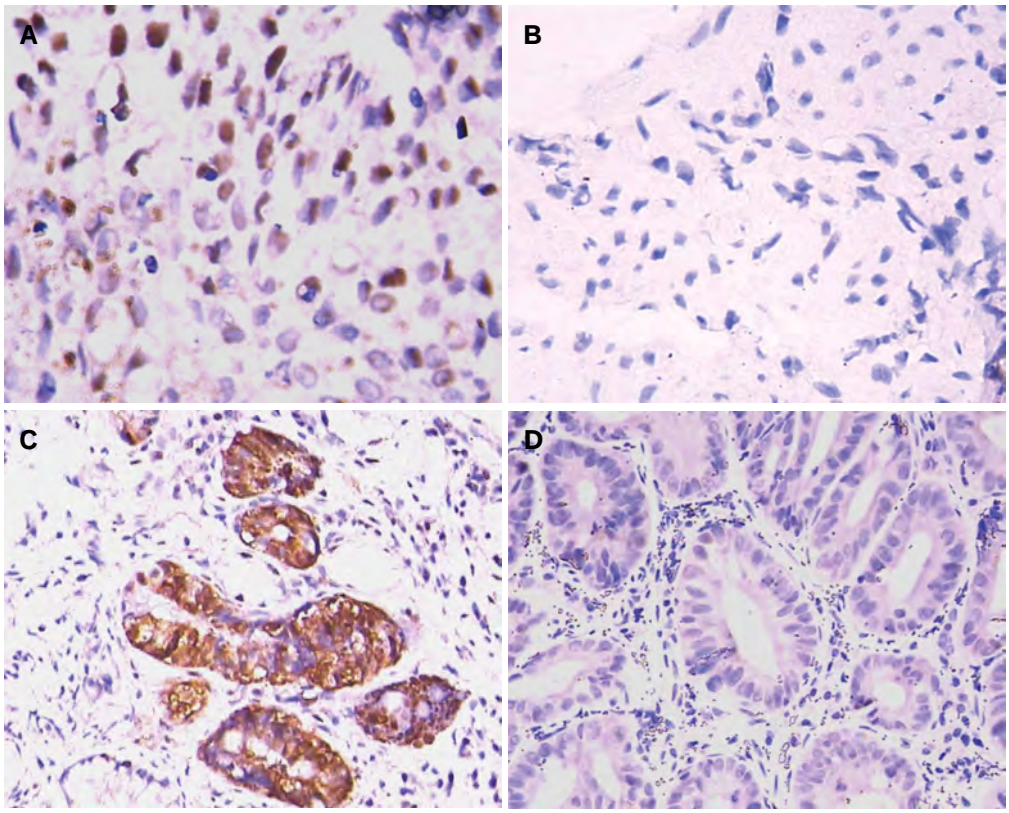


Figure 2 Immunohistochemical results. A: Expression of p53 in gastric pits in chronic atrophic gastritis, and nuclei were stained dark brown; B: p53 expression was undetectable in gastric pits in chronic superficial gastritis; C: Expression of proliferating cell nuclear antigen (PCNA) was detected in gastric pits in chronic atrophic gastritis, and nuclei were stained dark brown; D: PCNA expression was undetectable in gastric pits in chronic superficial gastritis.

which there are many spot- or rod-like gastric pits. When gastric mucosal lesions occur, morphological changes of gastric pits often appear first. Under an ordinary microscope, it is difficult to observe the morphological changes of gastric pits. In contrast, a magnifying endoscope has a magnification comparable to that of a stereomicroscope and allows observing gastric pit patterns clearly. Dye staining is an important auxiliary method for magnifying endoscopy. Magnifying chromoendoscopy can more clearly show the extent and surface conditions of lesions, which is conducive to clearer observation of lesions and more accurate biopsy. In recent years, domestic and foreign scholars have divided gastric mucosal pit patterns into four fundamental types: spot-like, reticular, granular, and villous[4,5]. Yang *et al*[4] and Huang *et al*[6] discovered that gastric pit morphology correlates with the severity of mucosal inflammation. They further divided gastric pits into five fundamental types: A (round spot-like pits), B (short rod-like pits), C (elongated and tortuous pits), D (reticular pits), and E (villus-like pits). This classification can more accurately reflect the evolution from the normal mucosa to superficial gastritis and atrophic gastritis. The presence of type E pits is a characteristic change of intestinal metaplasia. Wang *et al*[7] believe that epithelial and gland hyperplasia and intestinal metaplasia are main factors leading to morphological changes of gastric pits in atrophic gastritis. Endo *et al*[8] examined intestinal metaplasia of Barrett's esophagus and gastric cardia and found that villus-like pits are a characteristic change of intestinal metaplasia.

Gastric mucosal erosions are common lesions that show a special pattern during endoscopy, and some of them have malignant potential with the progression of inflammation. Therefore, it is particularly important to detect and determine the nature of mucosal erosions by endoscopy. By carefully observing gastric erosions, we found that the evolution of six fundamental types of gastric pits reflects the evolution from the normal mucosa to superficial gastritis, atrophic gastritis, intestinal metaplasia, and dysplasia. Type F pits evolve gradually from type E pits. They have obscure or disappearing structures and an extremely irregular arrangement. The boundaries between bowl-shaped defects on the erosion surface and neighboring structures are unclear. The erosive areas may show granular protrusions and irregular thickened capillaries. Histopathological analysis indicates that gastric mucosal lesions that have these subtle structural features are closely related to dysplasia or even early gastric cancer. Tajiri *et al*[9] observed the lesions of depressed early gastric cancer and divided the patterns of microvessels on the lesion surface into six types. They found that microvessels with branches of different calibers, spiral microvessels, and microvessels of different calibers were more commonly seen in undifferentiated carcinoma. Liu *et al*[10] applied magnifying endoscopy to the diagnosis of gastric mucosal erosions and found that there was an indirect or direct causal relationship between gastric mucosal erosions and gastric cancer. The presence of mucosal protrusions on the erosion surface is an endoscopic feature of malignant erosions, and this is initially caused by the replacement of cancerous tissue defects by regenerative non-cancerous mucosa. As time goes on, regenerative mucosa can be replaced by cancerous tissue to form pink mucosal protrusions[11]. In our study, about 80.6% of cases of type E mucosa showed intestinal metaplasia. Under a magnifying endoscope, type F mucosa usually contains no bowl-shaped defects on the erosion surface, which are commonly found in types C-E mucosa. However, the erosion surface has become rough, and the mucosa shows granular hyperplasia or irregular changes. The boundaries between the erosion surface and surrounding structures are unclear, and gastric pits in the periphery of the lesion are sparse, irregularly arranged type E pits. Type F mucosa often suggests varying degrees of dysplasia, which has a coincidence rate of 86.7% compared with pathological results. In addition, some cases of type F mucosa that is proved to have dysplasia by histopathology show varying degrees of capillary dysplasia, which manifests as the presence of irregular, thick, disordered capillaries on the erosive mucosal surface. This characteristic change is similar to tumor vascular change described by Otsuka *et al*[12] and Tajiri *et al*[9] in early gastric cancer. Thus, the presence of types E and F mucosa can be regarded as a subtle structural feature of gastric precancerous lesions. In this study, comparison of the findings of magnifying chromoendoscopy and histopathological analysis results indicates that the presence of types E and F mucosa suggests the emergence of intestinal metaplasia and dysplasia.

The *p53* gene is a tumor suppressor gene that is located on the short arm of human chromosome 17. It encodes a 53-kDa nuclear phosphoprotein that has transcription factor activity and plays an important role in the control of the cell cycle and apoptosis[13]. p53 mediates G1 arrest in response to DNA damage for DNA excision and repair to maintain the stability of the genome[14]. Wild-type p53 acts as not only a transcriptional activator but also a transcription suppressor[15]. Mutant-type p53 (mt-p53) can interfere with intracellular growth factor signaling to promote cell proliferation, suppress apoptosis, and eventually lead to cell transformation and tumor development and progression. In addition, mt-p53 can confer cell resistance to radiotherapy and chemotherapy by inhibiting apoptosis. Therefore, the biological behavior of tumors expressing mt-p53 is worse than that of tumors not expressing mt-p53[16].

Wild-type p53 induces cell differentiation. After transformed cells or cancer cells that do not express wild-type p53 were transfected with wild-type p53, the malignant phenotypes of cells were suppressed, cell growth and division were inhibited, cells were arrested at G1 phase, and *in vivo* tumorigenicity was decreased after inoculation into nude mice. On the other hand, introduction of p53 antisense RNA into the colon cancer cell line SW1116 significantly decreased proliferation rate and blocked cells in G0/1 phase, as revealed by flow cytometry[17].

Wild-type p53 induces apoptosis. After myeloid leukemia cell lines that do not express wild-type p53 were transfected with wild-type p53, typical apoptosis occurred, and cells lost viability. Treatment of tumors with radiation or anticancer agents not only induces DNA damage, blocks DNA metabolism, and leads to necrosis, but also induces apoptosis. The expression of transfected wild-type *p53* gene can increase anticancer treatment-induced apoptosis. In contrast, tumor cells transfected with the *mt-p53* gene or tumor cells not expressing wild-type *p53* gene are resistant to anticancer treatment-induced apoptosis. A study has proved that p53 induces apoptosis *via* the mitochondrial pathway in activated thymocytes[18].

The expression of p53 protein is detectable in almost all types of somatic cells. In normal cells, wild-type p53 protein has a half-life of about 20-30 min and shows little accumulation. Finlay *et al*[19] found that, in actively growing tumor cells, mt-p53 protein, but not wild-type p53 protein, can form a complex with heat shock protein 70. This complex extends the half-life of mt-p53 protein, which significantly increases the accumulation of mt-p53 protein in the nucleus[20]. The *p53* gene is an important tumor suppressor gene, and many human tumors, such as breast cancer, lung cancer, colorectal cancer, and gastric cancer, carry various *p53* gene mutations. High expression of the *mt-p53* gene is often detectable in many types of tumors and transformed cells. This study found that the expression of p53 protein in precancerous lesions (intestinal metaplasia and dysplasia) was higher than that in chronic gastritis.

PCNA was first discovered by Miyachi *et al*[21] in 1978 in the sera of patients with systemic lupus erythematosus. It was named as such because it is only expressed in normal proliferating cells and tumor cells. PCNA is a 36-kDa nuclear protein that is the auxiliary protein of DNA polymerase delta. Two types of nuclear PCNA exist, soluble and insoluble. Soluble PCNA is expressed in various phases of the cell cycle, and its quantity does not change significantly in the process of DNA synthesis. It is susceptible to detergent extraction and destruction by methanol. Insoluble PCNA is more stable and insusceptible to detergent elution and destruction by methanol. No obvious expression of soluble PCNA is detected in G0-Gl phase. However, its expression shows a significant increase in the late Gl phase, peaks in S phase, and decreases significantly in G2-M phase. Since the expression of soluble PCNA correlates well with DNA synthesis, it can be used as a marker for evaluation of cell proliferation. Given that cancer cells have a strong proliferative activity and that PCNA can be used as a cell proliferation marker, many domestic and foreign studies have investigated the relationship between PCNA expression and tumor development, grading, staging, radiation sensitivity, prognosis, recurrence and metastasis, death causes, and tumor markers in a variety of tumor types, and drawn many conclusions, although some of them are still controversial among different studies. In addition, some conclusions were drawn from analyses of one or several tumor types, and it remains to be studied whether they are applicable to all tumor types.

PCNA, as the auxiliary protein of DNA polymerase δ, plays an important role in the regulation of DNA replication and is closely related to the proliferative state of cells. Wu *et al*[22] examined the expression of PCNA in gastric cancer and precancerous lesions and found that the positive rate of PCNA in the cancer group was significantly higher than that in the control group (80.77% *vs* 55.36%, $P < 0.01$), suggesting that high PCNA expression correlates positively with cancerous transformation. These authors therefore believe that cell proliferation activity significantly increases in gastric dysplasia and intestinal metaplasia. In a study of 133 patients with early gastric cancer, Noda *et al*[23] found that high expression of PCNA was significantly associated with a higher rate of lymph node metastasis and a lower 5-year survival rate, indicating that PCNA expression correlates with the degree of tumor differentiation and distant metastasis. PCNA expression was significantly higher in poorly differentiated gastric cancer or gastric cancer with distant metastasis than in well differentiated gastric cancer or gastric cancer without distant metastasis. In this study, we found that PCNA expression showed an upward trend from types A to F pits. The number of cases that showed strong PCNA positivity increased significantly with the increase in the severity of lesions.

In conclusion, this study demonstrates that, compared with ordinary endoscopy, magnifying chromoendoscopy can provide more detailed information about fine mucosal morphology and has a significant advantage in the diagnosis of minute lesions. Magnifying chromoendoscopy in combination with p53 and PCNA detection is particularly helpful for the diagnosis of gastric precancerous lesions. The presence of types E and F pits, in which p53 and PCNA are highly expressed, is highly suggestive of the occurrence of early cancer, and patients developing these changes should be closely followed. If necessary, surgery or endoscopy can be considered.

COMMENTS

Background

Early diagnosis and treatment are important for improving the prognosis of gastric cancer. However, the widespread use of gastroscopy does not significantly improve the detection rate of early gastric cancer. Worldwide efforts are being made to improve the sensitivity of endoscopy in the detection of early cancer and precancerous lesions.

Research frontiers

This study assessed the diagnostic value of magnifying chromoendoscopy combined with detection of proliferating cell nuclear antigen (PCNA) and p53 in the detection of gastric precancerous lesions.

Innovations and breakthroughs

Compared with ordinary endoscopy, magnifying chromoendoscopy can provide more detailed information about fine mucosal morphology and has a significant advantage in the diagnosis of minute lesions.

Applications

Magnifying chromoendoscopy is better than ordinary endoscopy; it can provide more information about mucosal morphology and has a significant advantage in

the diagnosis of minute lesions. Magnifying chromoendoscopy in combination with p53 and PCNA detection is particularly helpful for the diagnosis of gastric precancerous lesions.

Peer review

An interesting manuscript assessing the diagnostic value of magnifying chromoendoscopy combined with detection of PCNA and p53 in gastric precancerous lesions to increase the detection rate of early gastric cancer.

REFERENCES

1 **Parker SL**, Tong T, Bolden S, Wingo PA. Cancer statistics, 1997. *CA Cancer J Clin* 1997; **47**: 5-27 [PMID: 8996076 DOI: 10.3322/canjclin.47.1.5]

2 **Endo M**, Kawano T. Detection and classification of early squamous cell esophageal cancer. *Dis Esophagus* 1997; **10**: 155-158 [PMID: 9280071]

3 **Hosokawa O**, Tsuda S, Kidani E, Watanabe K, Tanigawa Y, Shirasaki S, Hayashi H, Hinoshita T. Diagnosis of gastric cancer up to three years after negative upper gastrointestinal endoscopy. *Endoscopy* 1998; **30**: 669-674 [PMID: 9865554 DOI: 10.1055/s-2007-1001386]

4 **Yang JM**, Chen L, Fan YL, Li XH, Yu X, Fang DC. Endoscopic patterns of gastric mucosa and its clinicopathological significance. *World J Gastroenterol* 2003; **9**: 2552-2556 [PMID: 14606095]

5 **Chen X,** Cen R, Xu FX, Xia J, Luo C, Cheng FL. Magnifying endoscopic classification of gastric pits and their clinicopathologic analysis. *Zhongguo Neijing Zazhi* 2002; **8:** 37-41

6 **Huang YH,** Zhou LY, Lin SR, Jin Z, Liu JJ, Ding SG, Xia ZW, Duan LP, Chang H. Magnifying endoscopic classification of gastric pits and their clinicopathological significance. *Zhongguo Neijing Zazhi* 2004; **10:** 14-17

7 **Wang AY,** Ye CM, Lin SR. Changes in gastric pits in intestinal metaplasia of gastric mucosa. *Zhonghua Neike Zazhi* 1997; **36:** 315-316

8 **Endo T**, Awakawa T, Takahashi H, Arimura Y, Itoh F, Yamashita K, Sasaki S, Yamamoto H, Tang X, Imai K. Classification of Barrett's epithelium by magnifying endoscopy. *Gastrointest Endosc* 2002; **55**: 641-647 [PMID: 11979244 DOI: 10.1067/mge.2002.123420]

9 **Tajiri H**, Doi T, Endo H, Nishina T, Terao T, Hyodo I, Matsuda K, Yagi K. Routine endoscopy using a magnifying endoscope for gastric cancer diagnosis. *Endoscopy* 2002; **34**: 772-777 [PMID: 12244497 DOI: 10.1055/s-2002-34267]

10 **Liu BY,** Yuan LL, Chen X, Wang R. Diagnosis of gastric mucosal erosion by magnifying endoscopy. *Xiandai Xiaohua Ji Jieru Zhenliao* 2004; **9:** 64-66

11 **Huang YH,** Zhou LY, Lin SR, Jin Z, Liu JJ, Ding SG, Xia ZW, Duan LP, Chang H. Magnifying endoscopic manifestation of gastric atrophy, intestinal metaplasia or dysplasia and its diagnostic value. *Zhonghua Xiaohua Neijing Zazhi* 2005; **22:** 231-235

12 **Otsuka Y**, Niwa Y, Ohmiya N, Ando N, Ohashi A, Hirooka Y, Goto H. Usefulness of magnifying endoscopy in the diagnosis of early gastric cancer. *Endoscopy* 2004; **36**: 165-169 [PMID: 14765314 DOI: 10.1055/s-2004-814184]

13 **Dahiya R**, Deng G, Chen KM, Chui RM, Haughney PC, Narayan P. P53 tumour-suppressor gene mutations are mainly localised on exon 7 in human primary and metastatic prostate cancer. *Br J Cancer* 1996; **74**: 264-268 [PMID: 8688333 DOI: 10.1038/bjc.1996.349]

14 **Kastan MB**, Onyekwere O, Sidransky D, Vogelstein B, Craig RW. Participation of p53 protein in the cellular response to DNA damage. *Cancer Res* 1991; **51**: 6304-6311 [PMID: 1933891]

15 **Raycroft L**, Wu HY, Lozano G. Transcriptional activation by wild-type but not transforming mutants of the p53 anti-oncogene. *Science* 1990; **249**: 1049-1051 [PMID: 2144364 DOI: 10.1126/science.2144364]

16 **Aas T**, Børresen AL, Geisler S, Smith-Sørensen B, Johnsen H, Varhaug JE, Akslen LA, Lønning PE. Specific P53 mutations are associated with de novo resistance to doxorubicin in breast cancer patients. *Nat Med* 1996; **2**: 811-814 [PMID: 8673929 DOI: 10.1038/nm0796-811]

17 **Cao J**, Teng L, Cai X. [Inhibition effect of p53 antisense RNA on malignant phenotype of colorectal cancer cells]. *Zhonghua Zhongliu Zazhi* 1997; **19**: 123-126 [PMID: 10743076]

18 **Kakeji Y**, Korenaga D, Tsujitani S, Baba H, Anai H, Maehara Y, Sugimachi K. Gastric cancer with p53 overexpression has high potential for metastasising to lymph nodes. *Br J Cancer* 1993; **67**: 589-593 [PMID: 8439509 DOI: 10.1038/bjc.1993.108]

19 **Finlay CA**, Hinds PW, Tan TH, Eliyahu D, Oren M, Levine AJ. Activating mutations for transformation by p53 produce a gene product that forms an hsc70-p53 complex with an altered half-life. *Mol Cell Biol* 1988; **8**: 531-539 [PMID: 2832726]

20 **Milner J**, Medcalf EA. Cotranslation of activated mutant p53 with wild type drives the wild-type p53 protein into the mutant conformation. *Cell* 1991; **65**: 765-774 [PMID: 2040013 DOI: 10.1016/0092-8674(91)90384-B]

21 **Miyachi K**, Fritzler MJ, Tan EM. Autoantibody to a nuclear antigen in proliferating cells. *J Immunol* 1978; **121**: 2228-2234 [PMID: 102692]

22 **Wu YQ**, Wang MW, Long WD. Expression of p53, bcl-2 and PCNA in gastric precancerous lesions. *Academic Journal of PLA Postgraduate Medical School* 2001; **22**: 286

23 **Noda H**, Maehara Y, Irie K, Kakeji Y, Yonemura T, Sugimachi K. Increased proliferative activity caused by loss of p21(WAF1/CIP1) expression and its clinical significance in patients with early-stage gastric carcinoma. *Cancer* 2002; **94**: 2107-2112 [PMID: 11932915 DOI: 10.1002/cncr.10417]

P- Reviewers Pereiralima JG, Benz C **S- Editor** Gou SX
L- Editor Logan S **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i3.411

World J Gastroenterol 2013 January 21; 19(3): 411-414
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Gastric ischemia after epinephrine injection in a patient with liver cirrhosis

Su Young Kim, Seung-Hee Han, Kyung Han Kim, Sang Ock Kim, Sang-Young Han, Sung-Wook Lee, Yang Hyun Baek

Su Young Kim, Seung-Hee Han, Kyung Han Kim, Sang Ock Kim, Sang-Young Han, Sung-Wook Lee, Yang Hyun Baek, Department of Internal Medicine, Dong-A University College of Medicine, Busan 602-715, South Korea

Author contributions: Kim SY and Baek YH contributed equally to this work; Han SH, Kim KH, Kim SO, Han SY and Lee SW contributed to drafting the report; Kim SY and Baek YH wrote and revised the paper; and all authors contributed to analyzing the patient's data.

Correspondence to: Yang Hyun Baek, MD, Department of Internal Medicine, Dong-A University College of Medicine, 1, 3-ga, Dongdaeshin-dong, Seo-gu, Busan 602-715, South Korea. p100100@dau.ac.kr
Telephone: +82-51-2402983 Fax: +82-51-2402087
Received: September 13, 2012 Revised: October 15, 2012
Accepted: November 11, 2012
Published online: January 21, 2013

Abstract

Endoscopic epinephrine injection is relatively easy, quick and inexpensive. Furthermore, it has a low rate of complications, and it is widely used for the management of nonvariceal upper gastrointestinal bleeding. There have been several case reports of gastric ischemia after endoscopic injection therapy. Inadvertent intra-arterial injection may result in either spasm or thrombosis, leading to subsequent tissue ischemia or necrosis, although the stomach has a rich vascular supply and the vascular reserve of the intramural anastomosis. In addition to endoscopic injection therapy, smoking, hypertension and atherosclerosis are risk factors of gastric ischemia. We report a case of gastric ischemia after submucosal epinephrine injection in a 51-year-old woman with hypertension and liver cirrhosis.

© 2013 Baishideng. All rights reserved.

Key words: Hematemesis; Epinephrine; Gastric ischemia; Liver cirrhosis; Hypertension

Kim SY, Han SH, Kim KH, Kim SO, Han SY, Lee SW, Baek YH. Gastric ischemia after epinephrine injection in a patient with liver cirrhosis. *World J Gastroenterol* 2013; 19(3): 411-414 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i3/411.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i3.411

INTRODUCTION

Acute gastric ischemia rarely occurs due to the rich vascular supply of the stomach and the vascular reserve of the intramural anastomosis[1]. Therefore, there have been few case reports of gastric ischemia worldwide[2,3]. In the rare cases that have been reported, the risk factors for gastric ischemia were smoking, hypertension, atherosclerosis and endoscopic injection therapy[3]. Endoscopic epinephrine injection is a widely used therapy for the management of nonvariceal gastrointestinal bleeding, and it promotes initial hemostasis to stop the bleeding[4].

We report a case of gastric ischemia and necrosis after endoscopic epinephrine injection at the site of the lesion of a biopsy in a patient with liver cirrhosis and hypertension

CASE REPORT

A 51-year-old woman with hypertension and liver cirrhosis was admitted with hematemesis and hematochezia. One day prior to admission, an upper gastrointestinal endoscopy was performed, revealing an elevated lesion with a central depression of the gastric antrum. A variceal lesion was not observed. She underwent an endoscopic biopsy of the lesion of the antrum (Figure 1A), and submucosal injection was performed with a 1:10 000 solution of epinephrine due to bleeding after the endoscopic biopsy. Injection boluses of 2 mL were used for a total of 6 mL. The patient returned home safely after hemostasis. However, she presented to the emergency

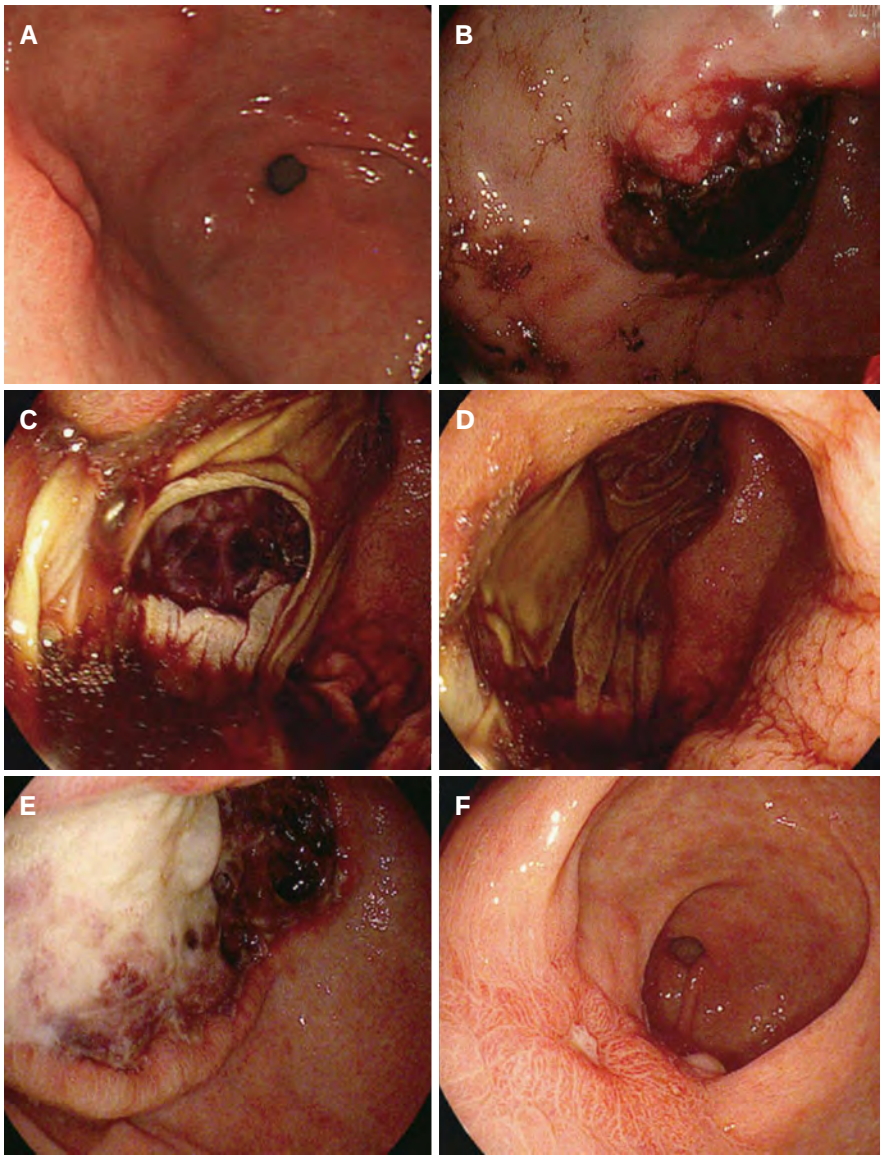


Figure 1 Upper gastrointestinal endoscopy. A: Mucosal elevated lesion with a central depression of the gastric antrum anterior wall. We took an endoscopic biopsy of the elevated lesion; B: Endoscopy revealed the oozing bleeding covered by a hemorrhagic clot in the distal antrum after the endoscopic biopsy. We tried the hemostasis by submucosal injection of a 1:10 000 solution of epinephrine; C: Mucosal dissection with extensive ulcerated; D: Necrotic areas from the distal to the proximal antrum. We did the conservative treatment with proton pump inhibitor, wide spectrum antibiotics and parenteral nutrition; E: Three days after the conservative treatment, a large extensive ulcer with fibrinous and hemorrhagic exudates surrounded by a clear elevated margin. The conservative care was continued; F: Two months later, the decreased size of the ulcer was surrounded by regenerative epithelia with a clear margin.

department reporting massive hematemesis and hematochezia the next day.

In the emergency room, she was in a severe distress with a heart rate of 90 beats/min, an arterial blood pressure of 100/70 mmHg and a respiratory rate of 20 breaths/min. Her abdomen was tender to palpation without guarding. A digital rectal examination was positive for bloody stools. The chest X-ray film and electrocardiogram were normal, as was the plain film of the abdomen. A complete blood count revealed anemia (hemoglobin level, 10.4 g/100 mL; hematocrit level, 29.7%) leukopenia (2360 cells/mm^3 with 56.3% polymorphonuclear cells) and thrombocytopenia (53 000 cells/mm^3). The Child-Pugh classification was A with a mildly prolonged prothrombin time of 14.4 s and hypoalbuminemia (3.0 g/dL). The upper endoscopy revealed oozing bleeding on the site of a previous biopsy, and the lesion was covered by a hemorrhagic clot (Figure 1B). We considered hemoclipping at first, but it was difficult to target the site of clipping because the hemorrhagic clot blocked our view, although vigorous irrigation and precise vessel exposure were not observed. Therefore, we again attempted hemostasis by submucosal injection of a 1:10 000 solution of epinephrine (a total dose of 8 mL). We were not concerned that epinephrine injection would result in gastric ischemia or necrosis at that time. Two days later, the patient presented with severe epigastric pain and melena. The hemoglobin level was 7.7 g/dL. An upper endoscopy

was performed. The endoscopy revealed a mucosal dissection with extensive ulcerated and necrotic areas from the distal to the proximal antrum (Figure 1C and D). We believed that this condition represented mucosal necrosis and severe ischemia. Emergency abdominal computed tomography ruled out arterial thrombosis and perforation, and it revealed an edematous mural thickening with mild mucosal enhancement in the antrum of the stomach. We decided to use conservative treatment because the signs of perforation or sepsis were not observed and the patient had underlying liver cirrhosis. The patient was treated with proton pump inhibitor perfusion, wide spectrum antibiotics and parenteral nutrition. Three days later, endoscopy was performed again to identify changes in the lesion. Endoscopy showed a large extensive ulcer with fibrinous and hemorrhagic exudates surrounded by a clear elevated margin (Figure 1E). We thought that the ischemic ulcer was in a healing state, and conservative care was continued. Her condition was gradually improved, and there was no sign of bleeding. A liquid diet was started, and the patient was discharged from the hospital after 2 wk. A follow-up endoscopy 2 mo later showed the decreased size of the ulcer surrounded by regenerative epithelia with a clear margin (Figure 1F).

DISCUSSION

The rich blood supply of the stomach protects the stomach from ischemia and necrosis[1]. Acute gastric ischemia, an emergency condition associated with high mortality, is rare[3]. Possible risk factors of gastric ischemia include smoking, hypertensive and atherosclerotic vascular disease, trauma, infection, and epinephrine injection[3]. We performed epinephrine injection to control the bleeding after a biopsy. Most cases of post-biopsy bleeding stop on their own without endoscopic therapy. However, we performed the injection therapy because of underlying cirrhosis and the strength of the injection procedure. Injection of epinephrine is effective for controlling upper gastrointestinal bleeding[5]. This technique is relatively easy, quick, and inexpensive. The complication rate associated with epinephrine injection was minimal[2].

However, a few cases have been reported of ischemic necrosis of the stomach and/or duodenum after injection of epinephrine (with or without a sclerosant) to control ulcer bleeding[2,4]. There are multiple mechanisms of action invoked to explain the efficacy of epinephrine, including vasoconstriction, a vascular tamponade effect, and enhanced platelet aggregation[5]. We postulated that gastric ischemia in this case resulted from the injection of a 1:10 000 solution of epinephrine as a vasoconstrictive drug. First, the epinephrine injection might have caused vascular contraction and opening of the intramural arteriovenous shunts, which could have contributed to gastric ischemia. Second, direct mechanical distress of the gastric artery caused by the needle followed by immediate obliteration of the arterial lumen was most likely the cause of the ischemia. The double injection of epinephrine most likely aggravated the gastric ischemia in this patient. Furthermore, morphological alterations in the gastric microcirculation in cirrhosis might be the cause of ischemia. One study reported that increased arteriovenous anastomoses in the gastric wall were related to the occurrence of acute gastric mucosal lesions in patients with liver cirrhosis[6]. Another study showed arteriovenous anastomoses of 50% in the gastric wall and a straight pattern of the arterioles with dilation of the precapillaries, capillaries, and submucosal and subserosal veins in cirrhotic patients, although the sample size was small[7]. In addition, the long-term history of hypertension, might have caused atherosclerotic vascular changes. Therefore, the abnormal mucosal structure resulting from liver cirrhosis and hypertension was an important risk factor, in addition to the injection of epinephrine, for gastric ischemia in this patient.

Clinically, gastric infarction presents as an acute abdominal emergency with diarrhea or hematemesis that rapidly progresses to acute peritonitis, irreversible septic shock, and death if untreated[8]. The treatment of acute necrotizing ischemic gastritis is emergency laparotomy with resection when necessary. In most cases, gastric ischemic necrosis has been treated with surgery[3,4,9]. In our case, the patient was only treated with conservative therapy with total parenteral nutrition, intravenous antibiotics, and a proton pump inhibitor. The results of this conservative treatment were favorable.

This is first report of the occurrence of gastric ischemia after epinephrine injection in a patient with liver cirrhosis. We report a case of fatal gastric ischemia and postulate that accidental intra-arterial injection may be responsible for this event. Inadvertent intra-arterial injection may result in either spasm or thrombosis and lead to subsequent tissue ischemia or necrosis, and underlying liver cirrhosis might influence this rare complication. Therefore, we should pay more attention to the risk of bleeding in underlying vascular disease or cirrhosis during endoscopic hemostasis.

REFERENCES

1 **Jacobson ED**. The circulation of the stomach. *Gastroenterology* 1965; **48**: 85-109 [PMID: 14252756]
2 **Lee TH,** Lee JS, Cho KH, Kim SH, Kim JO, Cho JY, Shim CS. A case of ischemic gastric necrosis after submucosal epinephrine injection. *Korean J Med* 2009; **77**: 488-492
3 **Richieri JP**, Pol B, Payan MJ. Acute necrotizing ischemic gastritis: clinical, endoscopic and histopathologic aspects. *Gastrointest Endosc* 1998; **48**: 210-212 [PMID: 9717792 DOI: 10.1016/S0016-5107(98)70168-3]
4 **Hilzenrat N**, Lamoureux E, Alpert L. Gastric ischemia after epinephrine injection for upper GI bleeding in a patient with unsuspected amyloidosis. *Gastrointest Endosc* 2003; **58**: 307-308 [PMID: 12872113 DOI: 10.1067/mge.2003.352]
5 **Park WG**, Yeh RW, Triadafilopoulos G. Injection therapies for nonvariceal bleeding disorders of the GI tract. *Gastrointest Endosc* 2007; **66**: 343-354 [PMID: 17643711 DOI: 10.1016/j.gie.2006.11.019]

6 **Hasumi A**, Aoki H, Shimazu M, Kawata S, Yoshimatsu Y. Causative relationship between a hyperdynamic state due to increased A-V anastomoses in the gastric wall and AGML in patients with liver cirrhosis. *J Gastroenterol Hepatol* 1989; **4** Suppl 1: 143-145 [PMID: 2519033]
7 **Hashizume M**, Tanaka K, Inokuchi K. Morphology of gastric microcirculation in cirrhosis. *Hepatology* 1983; **3**: 1008-1012 [PMID: 6629315 DOI: 10.1002/hep.1840030619]
8 **Cohen EB**. Infarction of the stomach; report of three cases of total gastric infarction and one case of partial infarction. *Am J Med* 1951; **11**: 645-652 [PMID: 14894464 DOI: 10.1016/0002-9343(51)90096-4]
9 **Dorta G**, Michetti P, Burckhardt P, Gillet M. Acute ischemia followed by hemorrhagic gastric necrosis after injection sclerotherapy for ulcer. *Endoscopy* 1996; **28**: 532 [PMID: 8886654 DOI: 10.1055/s-2007-1005548]

P- Reviewers Atta HM, De Gottardi A **S- Editor** Gou SX
L- Editor A **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i3.415

World J Gastroenterol 2013 January 21; 19(3): 415-417
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Primary aortoduodenal fistula: A case report

Abdu Hassan Alzobydi, Shaista Salman Guraya

Abdu Hassan Alzobydi, Department of Surgery, King Khalid Hospital, Najran 21431, Saudi Arabia
Shaista Salman Guraya, Department of Radiology, Taibah University, Almadinah Almunawwarah 41477, Saudi Arabia
Author contributions: Guraya SS contributed to drafting the report, analyzing the patient's data and writing the paper; Alzobydi AH revised the paper and approved the final version to be published.
Correspondence to: Shaista Salman Guraya, FRCR, PhD, Cert MedEd, Assistant Professor, Department of Radiology, Taibah University, Almadinah Almunawwarah 41477, Saudi Arabia. drss76@yahoo.com
Telephone: +966-4-8460008 Fax: +966-4-8461407
Received: July 26, 2012 Revised: September 12, 2012
Accepted: October 19, 2012
Published online: January 21, 2013

Abstract

Primary abdominoaortic fistula is an extremely rare cause of upper gastrointestinal (GI) bleeding. The diagnosis is frequently delayed due to the rarity of the disease and low index of suspicion by physicians. A range of invasive and non-invasive diagnostic tools are available, but helical computer tomography (CT) remains the mainstay. Surgery offers the only hope for survival. This case report presents a 47-year-old male with massive upper GI bleeding. Various diagnostic tests and an exploratory laparotomy failed to identify the diagnosis. Later, a primary aortoduodenal fistula was confirmed by CT scan which necessitated surgical repair of the fistula and a Goretex graft for the abdominal aortic aneurysm. The patient made an uneventful recovery and remained well to the first postoperative visit in the clinic 2 wk after surgery.

© 2013 Baishideng. All rights reserved.

Key words: Abdominal aortic aneurysm; Aortoduodenal fistula; Computer tomography; Mycotic aneurysm

Alzobydi AH, Guraya SS. Primary aortoduodenal fistula: A case report. *World J Gastroenterol* 2013; 19(3): 415-417 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i3/415.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i3.415

INTRODUCTION

Primary aortoduodenal fistula (PADF) is a rare, challenging complication of abdominal aortic aneurysm (AAA). PADF is an abnormal communication between the infrarenal aorta and duodenum, whereas secondary aortoduodenal fistula usually results from a previously implanted endovascular stent-graft procedure[1]. Since their first description about 100 years ago, more than 200 PADFs have been reported in the world literature[2-4]. They are usually found between the 3rd part of the duodenum and infrarenal aorta, caused by evolutionary complication of an aortic aneurysm[5]. Although less frequent, this abnormal communication also occurs between the aorta and other parts of the gastrointestinal (GI) tract, namely the esophagus, jejunum, ileum, or colon. The rarity of PADFs poses major diagnostic and therapeutic difficulties to the treating physicians.

The present case report describes a PADF between the infrarenal AAA and fourth part of the duodenum, which was successfully managed at King Khalid Hospital, Najran, Saudi Arabia.

CASE REPORT

A 47-year-old Saudi male presented to the emergency room of King Khalid Hospital Najran, Saudi Arabia with a 6-h history of massive fresh upper GI bleeding. He was a heavy smoker with no notable medical history. On examination, he had normal sensorium, pulse 113 beats/min, blood pressure 90/70 mmHg, and a cold sweaty skin. The laboratory investigations showed hemoglobin (Hb) 9.3 g/dL, white cell count 7100/mm^3, while electrolytes, renal and coagulation profiles, serum amylase and liver function testes were normal. The abdominal examination was unremarkable except for tenderness in the epigastrium. After initial resuscitation,

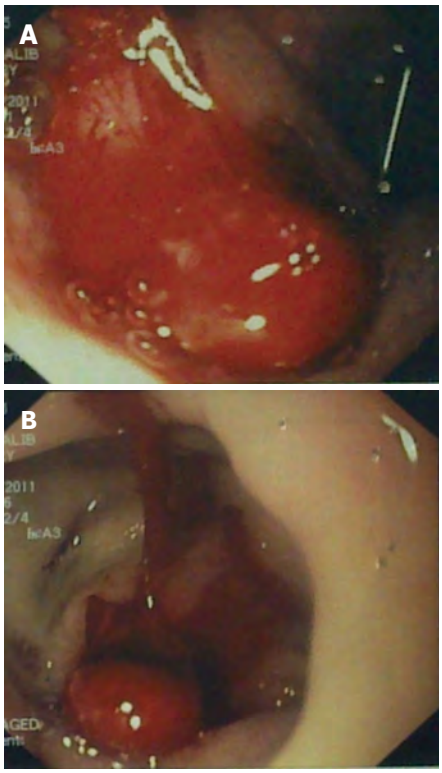


Figure 1 Endoscopic views of the stomach (A) and duodenum (B) showing a huge amount of fresh blood emerging from a concealed source.

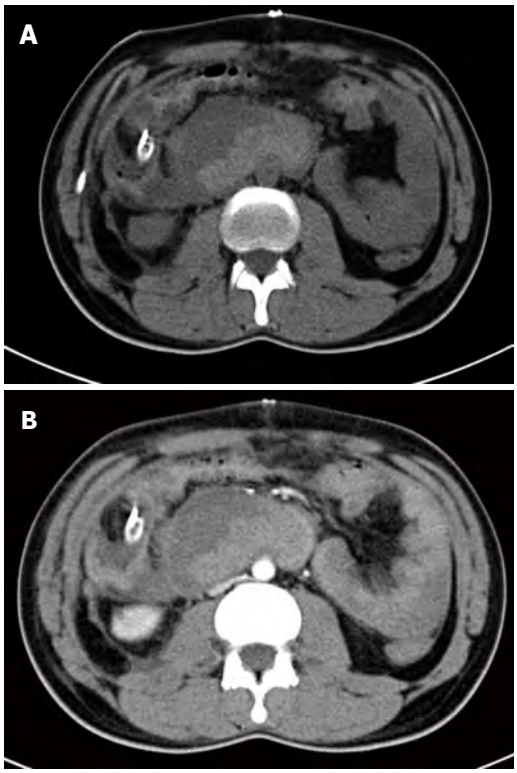


Figure 2 Computer tomography scan showing a primary aortoduodenal fistula between the infrarenal aorta and 4th part of the duodenum. A: Computer tomography (CT) scan without contrast showing a ruptured aortic aneurysm; B: Arterial phase of the CT scan showing the partially thrombosed abdominal aortic aneurysm.

upper GI endoscopy was performed and showed a huge amount of fresh blood emanating from the duodenum (Figure 1). No definite source of the bleeding could be identified. The patient was admitted in the intensive care unit, where, despite massive blood transfusion and active fluid resuscitation, the patient remained hemodynamically unstable, fresh bleeding continued in the nasogastric tube, and Hb dropped to 6.7 g/dL. On the 2nd post-admission day, the responsible consultant surgeon performed an exploratory laparotomy which revealed a distended duodenum and no blood in the peritoneum. A duodenotomy was performed which drained fresh and clotted blood, but no active source of the bleeding could be delineated. Perioperative upper GI endoscopy could not provide any additional information. Duodenotomy was closed over a tube drain followed by mass closure of the abdomen. Postoperatively, the patient continued to have fresh bleeding in the nasogastric tube. A computer tomography (CT) scan at that stage showed a PADF between the infrarenal aorta and fourth part of the duodenum (Figure 2). The vascular team was consulted and a second exploratory laparotomy was undertaken where the AAA was repaired with a prosthetic Goretex tube graft, and the duodenal fistula was closed in two layers. An omentopexy was performed between the aorta-graft anastomosis and duodenum. The culture and sensitivity report of the aortic wall reported the growth of *Klebsiella*. The patient made an uneventful recovery and was discharged home in a stable condition. His first follow-up visit in the surgical outpatient clinic, 2 wk after discharge, showed normal parameters.

DISCUSSION

The term PADF was first coined by Cooper[6], and the first case report was described by Salman[7]. The incidence of primary aorto-enteric fistulas is very low and, according to autopsy findings, ranges between 0.04% and 0.07%[8]. Up to December 2006, 366 cases of primary aorto-enteric fistulae have been reported[9]. Regarding the anatomical distribution, the 3rd part of the duodenum is involved in two-thirds of cases while the 4th part is affected in one-third of cases. Because of the close approximation and fixed nature of the duodenum, the expanding nature of the AAA causes irritation and inflammation, resulting in eventual fistulization over the passage of time.

The proposed mechanisms for the development of PADF are direct wear and inflammatory destruction triggered by infection, foreign bodies, or erosion[5]. Eighty percent of PADFs have been estimated to arise from an AAA. Gad reported 73% of PADFs developed from atherosclerotic aneurysms and 26% from traumatic or mycotic aneurysms[10]. The remaining 1% are caused by radiation, pancreatic carcinoma, metastases, ulcers, gallstones, diverticulitis, and appendicitis. The classical presentations

of PADFs are upper GI bleeding (64%), abdominal pain (32%), and a pulsatile abdominal mass (25%)[11,12]. Other rare symptoms include intermittent back pain, fever, and sepsis, melena, and syncope. Quite frequently an expansile abdominal mass and a bruit is noticed on abdominal examination.

The most valuable diagnostic tool for the diagnosis of PADF is considered to be helical CT scan with intravenous contrast[13]. Rapid scanning, high resolution, non-invasive procedure, image quality, and data processing are the outright benefits of CT scanning. Loss of the aneurysmal wall, air in the retroperitoneum or thrombus, or destruction of the fat plane between the aneurysm and duodenum strongly suggest a PADF. In addition, visualization of the contrast within the GI tract is a striking CT finding. Other diagnostic modalities include upper GI endoscopy and angiography. Endoscopy is useful in diagnosing upper GI bleeding, although a negative report does not exclude the possibility of a PADF. The visualization of the fistula below the 3rd part of the duodenum is quite difficult due to acute angulation between 3rd and 4th parts of the duodenum[12]. Angiography is not reliable in diagnosing PADF. In a retrospective study, 13 out of 36 aorto-enteric fistulas were diagnosed by angiography before surgery[14]. Magnetic resonance imaging, tagged white blood cell scans, colonoscopy, and ultrasound are of limited value[15].

The mortality from untreated PADF is almost 100%. The survival after surgery ranges from 18% to 93%[16]. The recommended surgical procedure consists of closure of the duodenal fistula, aortic ligation to exclude the aneurysm, and an extraluminal bypass. In the case of an infected AAA, antibiotics should be given for 4-6 wk after surgery, if a positive culture is obtained[17]. The most commonly encountered cultured organisms in primary mycotic PADF are *Salmonella* and *Klebsiella*. In our case, third generation cephalosporins were administered for 1 mo with promising results.

To conclude, PADF are an extremely uncommon cause of massive upper GI bleeding and abdominal mass. A CT scan is the gold standard for diagnosis of this entity, and surgical intervention offers the only treatment for survival.

REFERENCES

1 **Biancari F**, Romsi P, Perälä J, Koivukangas V, Cresti R, Juvonen T. Staged endovascular stent-grafting and surgical treatment of a secondary aortoduodenal fistula. *Eur J Vasc Endovasc Surg* 2006; **31**: 42-43 [PMID: 16269256 DOI:10.1016/j.ejvs.2005.09.007]

2 **Reckless JP**, McColl I, Taylor GW. Aorto-enteric fistulae: an uncommon complication of abdominal aortic aneurysms. *Br J Surg* 1972; **59**: 458-460 [PMID: 4537432 DOI:10.1002/bjs.1800590613]

3 **Saers SJ**, Scheltinga MR. Primary aortoenteric fistula. *Br J Surg* 2005; **92**: 143-152 [PMID: 15685700 DOI:10.1002/bjs.4928]

4 **Lemos DW**, Raffetto JD, Moore TC, Menzoian JO. Primary aortoduodenal fistula: a case report and review of the literature. *J Vasc Surg* 2003; **37**: 686-689 [PMID: 12618713 DOI:10.1067/mva.2003.101]

5 **Lozano FS**, Muñoz-Bellvis L, San Norberto E, Garcia-Plaza A, Gonzalez-Porras JR. Primary aortoduodenal fistula: new case reports and a review of the literature. *J Gastrointest Surg* 2008; **12**: 1561-1565 [PMID: 18301952 DOI:10.1007/s11605-008-0499-6]

6 **Cooper A.** Lectures on principles and practice of surgery with additional notes and cases by F Tyrrell. 5th ed. Philadelphia, PA: Haswell, Barrington, and Haswell, 1839

7 **Salman M**. Aneurysme de l'aorte ventrale mort gur rupture de la poche attriele dars leduodenum. *Bull Soc Anat* 1843; **18:** 283

8 **Voorhoeve R**, Moll FL, de Letter JA, Bast TJ, Wester JP, Slee PH. Primary aortoenteric fistula: report of eight new cases and review of the literature. *Ann Vasc Surg* 1996; **10**: 40-48 [PMID: 8688296 DOI:10.1007/BF02002340]

9 **Aksoy M**, Yanar H, Taviloglu K, Ertekin C, Ayalp K, Yanar F, Guloglu R, Kurtoglu M. Rupture of abdominal aortic aneurysm into sigmoid colon: a case report. *World J Gastroenterol* 2006; **12**: 7549-7550 [PMID: 17167850]

10 **Gad A**. Aortoduodenal fistula revisited. *Scand J Gastroenterol Suppl* 1989; **167**: 97-100 [PMID: 2694333 DOI:10.3109/00365528909091322]

11 **Sweeney MS**, Gadacz TR. Primary aortoduodenal fistula: manifestation, diagnosis, and treatment. *Surgery* 1984; **96**: 492-497 [PMID: 6236571]

12 **Korkut AK**, Arpinar E, Yasar T, Guney D. Primary aortoduodenal fistula complicated by abdominal aortic aneurysm. *J Cardiovasc Surg* (Torino) 2000; **41**: 113-115 [PMID: 10836235]

13 **Hill SL, Kaot LH, Alexander RH.** Recurrent aortoduodenal fistula: a lesion in management. *Ann Surg* 1982; **48:** 137-140

14 **Peck JJ**, Eidemiller LR. Aortoenteric fistulas. *Arch Surg* 1992; **127**: 1191-1193; discussion 1193-1194 [PMID: 1417484]

15 **Chuter TA,** Lukaszewicz GC, Reilly LM, Kerlan RK, Faruqi R, Sawhney R, Wall SD, Canto C, LaBerge JM, Gordon RL, Messina LM. Endovascular repair of a presumed aortoenteric fistula: late failure due to recurrent infection. *J Endovasc Ther* 2000; **7:** 240-244 [PMID: 10883963 DOI:10.1583/1545-1550(2000)007<0240:EROAPA>2.3.CO;2]

16 **Kavanagh DO**, Dowdall JF, Younis F, Sheehan S, Mehigan D, Barry MC. Aorto-enteric fistula: changing management strategies. *Ir j Med Sc* 2006; **175:** 40-44

17 **Tambyraja AL**, Murie JA, Chalmers RT. Ruptured inflammatory abdominal aortic aneurysm: insights in clinical management and outcome. *J Vasc Surg* 2004; **39**: 400-403 [PMID: 14743143 DOI:10.1016/j.jvs.2003.07.029]

P- Reviewers Karmy-Jones R, Sun ZH **S- Editor** Jiang L
L- Editor Cant MR **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i3.418

World J Gastroenterol 2013 January 21; 19(3): 418-421
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Emergency caudate lobectomy for ruptured hepatocellular carcinoma with multiple primary cancers

Long-Hao Sun, Hong-Qiu Han, Peng-Zhi Wang, Wei-Jun Tian

Long-Hao Sun, Hong-Qiu Han, Peng-Zhi Wang, Wei-Jun Tian, Department of General Sugery, Tianjin Medical University General Hospital, Tianjin 300052, China

Author contributions: Han HQ and Tian WJ performed the operation; Sun LH and Wang PZ collected case data; Sun LH and Tian WJ wrote the paper.

Correspondence to: Wei-Jun Tian, Professor, Department of General Sugery, Tianjin Medical University General Hospital, Tianjin 300052, China. jonathontian@163.com

Telephone: +86-22-60362365 Fax: +86-22-60362365

Received: November 2, 2012 Revised: December 17, 2012
Accepted: December 22, 2012
Published online: January 21, 2013

Abstract

We report a case of metachronous multiple primary malignancies involving both rectum and liver with colonic metastasis from hepatocellular carcinoma (HCC) through hematogenous pathway. A 72-year-old woman was admitted to the emergency department with right upper abdominal pain for 4 h. Considering her surgical history of Mile's procedure plus liver resection for rectal cancer with liver metastasis three years ago and the finding of urgent computed tomography scan on admission, the preoperative diagnosis was spontaneous rupture of rectal liver metastasis located in caudate lobe and colonic metastasis from rectal cancer. The patient underwent an emergency isolated caudate lobectomy at a hemorrhagic shock status. Pathology reported a primary HCC in the caudate lobe and colonic metastasis of HCC with tumor embolus in the surrounding vessels of the intestine. No regional lymph node involvement was found. It is hypothesized that HCC may disseminate hematogenously to the ascending colon, thus making it a rare case.

© 2013 Baishideng. All rights reserved.

Key words: Emergency isolated caudate lobectomy; Multiple primary malignancies; Rectal adenocarcinoma; Hepatocellular carcinoma; Hematogenous metastasis

Sun LH, Han HQ, Wang PZ, Tian WJ. Emergency caudate lobectomy for ruptured hepatocellular carcinoma with multiple primary cancers. *World J Gastroenterol* 2013; 19(3): 418-421 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i3/418.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i3.418

INTRODUCTION

The caudate lobe of the liver is located deep in the hepatic parenchyma and surrounded by some major vessels. Because of its surgically difficult-to- approach anatomic location and perception of early involvement of the inferior vena cava (IVC) or portal vein by tumors, caudate lobectomy was once used infrequently. With more precise anatomical knowledge, development of surgical technique, and improvement in perioperative care, it has become more common recently. But in order to succeed in the procedure, the optimal operative approach should be planned and determined carefully before operation. Emergency isolated caudate lobectomy was rarely reported worldwide, especially for the ruptured primary hepatocellular carcinoma (HCC). The incidence of colorectal cancers has increased in recent years. As the overall survival rate has improved due to the development of multiple therapies, it is not surprising to see more patients with multiple primary malignancies involving the colorectum and other organs. For colorectal cancer, stomach was the most common extracolonic site of both the synchronous and metachronous multiple primary malignancies, but liver is very rarely involved. Extrahepatic metastasis of HCC occurs occasionally and increases with prolonged survival. However, HCC with gastrointestinal (GI) tract metastasis is seldom reported, and it is only found in less than 6% of the cases in autopsy series. GI metastasis through hematogenous pathway is especially rare.

CASE REPORT

A 72-year-old woman was admitted with right upper abdominal pain. The pain started in the epigastrium. A few hours before admission, the pain disseminated to the upper right abdominal quadrant and right back region and became severe.

The patient had a previous history of rectal adenocarcinoma with liver metastasis. Three years earlier she underwent the Miles procedure and partial hepatectomy. Following the operation, she received combined chemotherapy with 5-fluorouracil, folinic acid and oxaliplatin. Six months ago, computerized tomography (CT) scan of the abdomen showed a 5 cm × 5 cm × 5 cm, diverticulum like, exophytic mass located in the ascending colon near the ileocecus, without lymph node enlargement. It was considered a metastasis of rectal cancer. The mass showed no obvious response to systemic adjuvant chemotherapy.

On admission, her temperature was 36.9 ℃, pulse was 112 beats/min and blood pressure was 90/50 mmHg. The oxygen saturation was 95% while the patient was breathing ambient air. On physical examination, the patient was ill-looking, conscious with skin and conjunctival pallor. The abdomen was distended, and bowel sounds were absent. There was tenderness in the right upper abdominal quadrant, with guarding and rebound tenderness. Lungs and heart sounds were normal.

Her blood work-up on admission showed a hemoglobin level of 6.8 g/L, white cell count of 8.9 × 10^9/L and neutrophils of 72%. Coagulation tests, liver function, renal function and electrolytes were all within the normal range. Urgent CT scan of the abdomen showed multiple masses localized in the liver, a 6 cm × 6 cm × 6 cm mass in the caudate lobe of the liver (Couinaud's segment 1), and signs of abdominal haemorrhage (Figure 1). The preoperative diagnoses were hemorrhagic shock, spontaneous rupture of rectal liver metastasis, colonic metastasis of rectal cancer or multiple primary cancers.

Hemodynamic stability was achieved following rehydration with intravenous fluid and multiple blood transfusions. At the same time, the patient was promptly sent to the operating room. Open exploratory laparotomy was performed *via* a J-shaped skin incision, with more than 2 liters of blood and clots and intra-abdominal adhesions due to the last operation visualized in the peritoneum. Inspection of the liver revealed a ruptured mass of 6 cm × 6 cm × 6 cm in the hepatic segment I with active bleeding. A 5 cm × 5 cm × 5 cm erosive tumor lesion was localized in the beginning of the ascending colon and nearly occupied the whole lumen. Isolated caudate lobectomy was performed *via* bilateral approach, in addition to the resection of ileocecum. The operation time was 290 min and blood loss was 2000 mL. Ten units of packed red blood cells and 2000 mL of fresh frozen plasma were transfused during the perioperative period. Histological examination demonstrated a primary HCC located in the caudate lobe and a colonic metastasis of HCC with tumor embolus in the surrounding vessels of the intestine (Figure 2). No regional lymph node metastasis was found. Immunohistochemical findings revealed that polyclonal alpha fetoprotein and hepatocyte antigen were positive and carcinombryonic antigen was negative. The patient made a full recovery from the surgery and was subsequently discharged 10 d after admission. Eight months later, the patient died of cachexia.

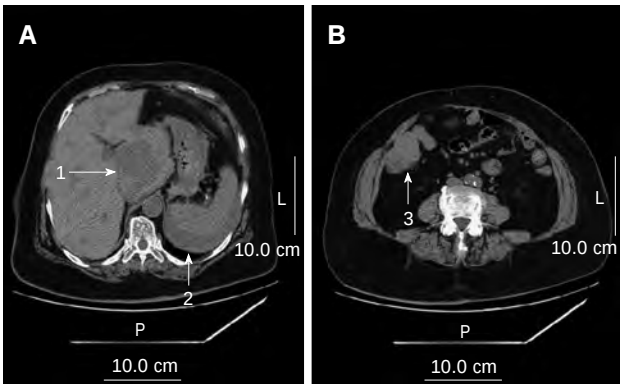


Figure 1 Urgent computed tomography. A: Sagittal view; B: Transverse view. 1: A ruptured mass of 6 cm × 6 cm × 6 cm localized in the I hepatic segment; 2: Perisplenic hemoperitoneum; 3: A 5 cm × 5 cm × 5 cm mass abutting the ileocecus without lymph node enlargement. L: Left; P: Posterior.

DISCUSSION

The caudate lobe of the liver is located deep in the hepatic parenchyma behind both major lobes and between the confluence of the three main hepatic veins, IVC, and hepatic hilum. It is generally divided into three parts, as described by Kumon: the left Spiegel lobe (Couinaud's segment 1), the caudate process, and the paracaval portion (Couinaud's segment 9). Because of its surgically difficult-to-approach anatomic location and perception of early involvement of the IVC or portal vein by tumors, caudate lobectomy was once called a forbidden zone of hepatic surgery and not performed frequently until it was first described by Lerut. In the early days, patients underwent resection of the caudate lobe along with removal of adjacent portions of the liver, i.e., right or left lobectomy, in order to get a better exposure. But for patients who concurrently had chronic liver disease, removal of too much normal hepatic parenchyma would delay recovery or even cause liver failure. With more precise anatomical knowledge, development of surgical technique, and improvement in perioperative care, isolated caudate lobectomy[1] has become common recently. Nevertheless, clinical assessment should be conducted strictly before operative period[2]. Laboratory investigations, including tests of complete blood count, coagulation, liver function, renal function, electrolytes, indocyanine green retention rate at 15 min, and virology, should be carried out to evaluate the patient's surgical fitness; and radiological studies, including ultrasonography, CT scan, and three dimensional reconstructive CT, should be undertaken to evaluate the hepatic lobe size, extent and location of the tumor, vascular involvement, lymph node affection, and the severity

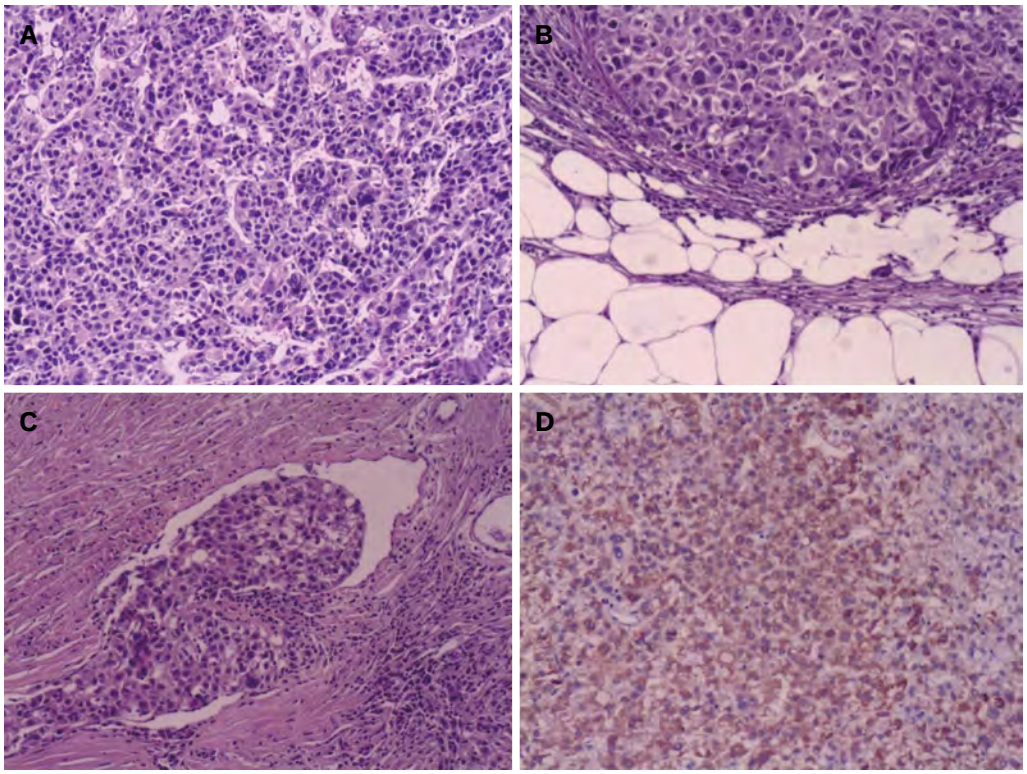


Figure 2 Histopathology. A: Hepatocellular carcinoma (HCC) located in the caudate lobe [hematoxylin and eosin (HE), ×100]; B: Colonic metastasis of HCC (HE, × 100); C: Cancer embolus in the vessels surrounding the intestine (HE, ×100); D: Polyclonal hepatocyte antigen was positive by immunohistochemistry, ×100.

of cirrhosis, and to rule out intra-abdominal metastasis. The most appropriate operative procedure should be planned and determined preoperatively[3]. Consequently, emergency isolated caudate lobectomy was rarely reported worldwide, especially for the ruptured primary HCC. For these patients, routine treatments included compression and packing hemostasis, transcatheter arterial chemoembolization (TACE), and partial hepatectomy. Because the caudate lobe requires the arterial blood supply from the two major lobes of the liver, TACE cannot achieve the goal of hemostasis. The necrotic tumor tissue and important vessels surrounding the caudate lobe also affect the compression and packing hemostasis. As a result, emergency isolated caudate lobectomy is considered to be the best choice for these patients. There are four approaches for the resection of the caudate lobe of the liver, left side approach, right side approach[4], bilateral approach, and anterior transhepatic approach by splitting parenchyma of the liver. The approach to the caudate lobe depends on the location of the tumor and the expertise of the surgeon. Hepatic surgery is a very bloody procedure, especially the caudate lobectomy, because of the unique anatomical relationship between the caudate lobe and IVC and the portal triad. Vascular control combined with positioning and central venous pressure control is recommended to minimize intraoperative blood loss. Full mobilization of liver is indicated before the dissection of the caudate lobe. Dividing the fibrous retrocaval ligament is extremely important in fully mobilizing the caudate lobe to expose the hepatic veins along the anterior surface of the IVC. Once the left lateral edge of the caudate lobe is free, ligation of the short hepatic veins draining directly into IVC is performed. Our patient underwent a partial hepatectomy three years ago for rectal liver metastasis resulting in complicated intra-abdominal adhesions, which further increased the difficulties of the operative procedure[5]. Likewise, the pathophysiological changes of hemorrhagic shock also make it more difficult to keep the stability of the haemodynamics of the patient.

Considering the history of rectal cancer three years ago in this case, the preoperative diagnosis was spontaneous rupture of rectal liver metastasis and colonic metastasis of rectal cancer. But histological examination demonstrated a primary HCC located in the caudate lobe and a colonic metastasis of HCC. Multiple primary cancers are relatively rare[6]. The overall incidence rate is between 0.73% and 11.7%. However, more multiple primary cancers have been encountered due to an increase in the number of elderly patients and advancement in diagnostic techniques. In addition, cancer patients are assumed to be at an increased risk of developing cancers in other organs due to genetic alterations or exposure to the same environmental carcinogens[7]. It is generally defined according to the criteria of Warren and Gates: (1) each tumor must be clearly malignant on histologic examination; (2) each tumor must be distinct; and (3) the possibility that the second tumor represents a metastasis must be excluded. Multiple primary cancers can be synchronous or metachronous depending on the interval between their diagnosis. Synchronous malignancies are secondary

cancers occurring simultaneously or within 6 mo after the diagnosis of primary cancers, while metachronous malignancies are secondary cancers that developed more than 6 mo after the diagnosis of primary cancers. The incidence of colorectal cancers has increased in recent years. As the overall survival rate has improved due to the development of multiple therapies, it is not surprising to see more patients with multiple primary malignancies involving the colorectum and other organs[8]. For colorectal cancer, stomach is the most common extracolonic site of the synchronous multiple primary cancers. The thyroid gland is the second organ involved. The stomach is also the most common extracolonic site of the metachronous multiple primary malignancies, followed by the cervix, lung, and skin. The metachronous multiple primary malignancies involving both rectum and liver are very rare. Although the mechanisms underlying the development of multiple primary cancers are not fully understood, some factors such as sustaining environmental carcinogens, susceptibility to carcinogen, cancer cell immunodeficiency, and immunosuppression caused by radiotherapy and chemotherapy have been implicated. Under these circumstances, it is expected that as people live longer, they have more chances to develop multiple primary malignancies. The prognosis of the patients with multiple primary cancers can be predicted independently by the stage of each tumor.

HCC is one of the most common malignancies worldwide[9]. Extrahepatic metastasis of HCC occurs occasionally and increases with prolonged survival. The most frequent sites of metastasis are lungs, bones, regional lymph nodes, and adrenal glands. However, HCC with GI tract metastasis is very rare, being found in only less than 6% of cases in autopsy series[10]. The metastasis sites only reported in some case reports included duodenum, stomach, colon and jejunum. The major pathway of the metastasis is direct invasion to the contiguous GI tract via adhesion to the serosal side by a bulky tumor mass. However, the frequency of metastatic colon cancer arising from HCC is low. In this case, the solitary metastasis from HCC appeared in ileocecus, separated from other liver tissues. The mode of the metastasis to the colon is still unclear. It is hypothesized that HCC may disseminate hematogenously to the distant GI tracts. Tumor embolus in the vessels surrounding the intestine may be the key evidence of hematogenous metastasis. Absence of regional lymph node metastasis may indicate low possibility of lymphatic metastasis. The prognosis of GI metastasis from HCC is known to be poor with a median survival period of seven months.

In conclusion, we report a rare case of metachronous multiple primary malignancies involving both rectum and liver with colonic metastasis through hematogenous pathway. This patient was manifested by spontaneous rupture of HCC located in the caudate lobe, and underwent an emergency isolated caudate lobectomy, with a full recovery after surgery.

REFERENCES

1 **Sakoda M**, Ueno S, Kubo F, Hiwatashi K, Tateno T, Kurahara H, Mataki Y, Shinchi H, Natsugoe S. Surgery for hepatocellular carcinoma located in the caudate lobe. *World J Surg* 2009; **33**: 1922-1926 [PMID: 19582505 DOI: 10.1007/s00268-009-0110-7]

2 **Lee SG**, Hwang S, Jung JP, Lee YJ, Kim KH, Ahn CS. Outcome of patients with huge hepatocellular carcinoma after primary resection and treatment of recurrent lesions. *Br J Surg* 2007; **94**: 320-326 [PMID: 17205495 DOI: 10.1002/bjs.5622]

3 **Pawlik TM**, Poon RT, Abdalla EK, Zorzi D, Ikai I, Curley SA, Nagorney DM, Belghiti J, Ng IO, Yamaoka Y, Lauwers GY, Vauthey JN. Critical appraisal of the clinical and pathologic predictors of survival after resection of large hepatocellular carcinoma. *Arch Surg* 2005; **140**: 450-457; discussion 457-458 [PMID: 15897440 DOI: 10.1001/archsurg.140.5.450]

4 **Chaib E**, Ribeiro MA, Silva Fde S, Saad WA, Cecconello I. Caudate lobectomy: tumor location, topographic classification, and technique using right- and left-sided approaches to the liver. *Am J Surg* 2008; **196**: 245-251 [PMID: 18571618 DOI: 10.1016/j.amjsurg.2007.11.020]

5 **Ishizawa T**, Hasegawa K, Ikeda M, Aoki T, Sano K, Imamura H, Kokudo N, Makuuchi M. Transhepatic approach for a small paracaval tumor in repeat resection. *Dig Surg* 2007; **24**: 409-412 [PMID: 17855778 DOI: 10.1159/000108322]

6 **Ueno M**, Muto T, Oya M, Ota H, Azekura K, Yamaguchi T. Multiple primary cancer: an experience at the Cancer Institute Hospital with special reference to colorectal cancer. *Int J Clin Oncol* 2003; **8**: 162-167 [PMID: 12851840 DOI: 10.1007/s10147-003-0322-z]

7 **Yamamoto S**, Yoshimura K, Ri S, Fujita S, Akasu T, Moriya Y. The risk of multiple primary malignancies with colorectal carcinoma. *Dis Colon Rectum* 2006; **49**: S30-S36 [PMID: 17106813 DOI: 10.1007/s10350-006-0600-8]

8 **Ahmed F**, Goodman MT, Kosary C, Ruiz B, Wu XC, Chen VW, Correa CN. Excess risk of subsequent primary cancers among colorectal carcinoma survivors, 1975-2001. *Cancer* 2006; **107**: 1162-1171 [PMID: 16838312 DOI: 10.1002/cncr.22013]

9 **Greten TF**, Papendorf F, Bleck JS, Kirchhoff T, Wohlberedt T, Kubicka S, Klempnauer J, Galanski M, Manns MP. Survival rate in patients with hepatocellular carcinoma: a retrospective analysis of 389 patients. *Br J Cancer* 2005; **92**: 1862-1868 [PMID: 15870713 DOI: 10.1038/sj.bjc.6602590]

10 **Nozaki Y**, Kobayashi N, Shimamura T, Akiyama T, Inamori M, Iida H, Endo H, Fujita K, Yoneda M, Takahashi H, Abe Y, Kirikoshi H, Kubota K, Saito S, Sasaki T, Nakajima A. Colonic metastasis from hepatocellular carcinoma: manifested by gastrointestinal bleeding. *Dig Dis Sci* 2008; **53**: 3265-3266 [PMID: 18415678 DOI: 10.1007/s10620-008-0273-3]

P- Reviewer Wong GLH **S- Editor** Gou SX **L- Editor** A **E- Editor** Li JY



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i3.422

World J Gastroenterol 2013 January 21; 19(3): 422-425
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Primary histiocytic sarcoma of the stomach: A case report with imaging findings

Xun-Ze Shen, Fang Liu, Rui-Jun Ni, Bo-Yin Wang

Xun-Ze Shen, Fang Liu, Rui-Jun Ni, Bo-Yin Wang, Department of Radiology, Shaoxing People's Hospital, Shaoxing Hospital of Zhejiang University, Shaoxing 332100, Zhejiang Province, China
Fang Liu, Department of Pathology, Shaoxing People's Hospital, Shaoxing Hospital of Zhejiang University, Shaoxing 332100, Zhejiang Province, China
Author contributions: Shen XZ designed the research; Shen XZ and Ni RJ wrote the paper; Shen XZ and Wang BY revised the paper; Liu F analyzed the pathological figures.
Correspondence to: Bo-Yin Wang, Chief Physician, Department of Radiology, Shaoxing People's Hospital, Shaoxing Hospital of Zhejiang University, Shaoxing 332100, Zhejiang Province, China. sxsrmyyfsk@163.com
Telephone: +86-575-88228548 Fax: +86-575-88228899
Received: September 11, 2012 Revised: November 30, 2012
Accepted: December 15, 2012
Published online: January 21, 2013

Abstract

Histiocytic sarcoma (HS) is a rare malignant neoplasm that originates from a histiocytic hematopoietic lineage characterized by histiocytic differentiation and its corresponding immunophenotypic features. We herein reported a case of primary HS of the stomach which was confirmed through histopathologic examination and immunohistochemical staining. A 52-year-old woman presented with progressive difficulty in feeding and dull pain in the epigastric region. Gastroscopy, endoscopic ultrasonography, double contrast examination, and computed tomography revealed a mass located on the posterior wall of fundus and lesser curvature of the stomach. Microscopically, the cytoplasm of the tumor cells was abundant and eosinophilic. Immunohistochemical staining revealed that the tumor cells were positive for CD45RO and CD68. It is difficult to differentiate HS of stomach from other gastric malignancies by radiological evaluation alone. However, HS may be considered when a protruding and ulcerated mass in stomach shows heterogeneous hypervascular features. To the best of our knowledge, this is the first report in English language literature that emphasizes the imaging findings of human gastric HS.

© 2013 Baishideng. All rights reserved.

Key words: Histiocytic sarcoma; Stomach; Primary; Endoscopic ultrasonography; Gastroscope; Double contrast examination; Computed tomography

Shen XZ, Liu F, Ni RJ, Wang BY. Primary histiocytic sarcoma of the stomach: A case report with imaging findings. *World J Gastroenterol* 2013; 19(3): 422-425 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i3/422.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i3.422

INTRODUCTION

Histiocytic sarcoma (HS) is an exceedingly rare lymphohematopoietic malignant neoplasm composed of tumor cells showing morphologic and immunophenotypic features of mature histiocytes[1,2]. Most patients are adults (median age 46 years). Male predilection is found in some studies. About one-third of cases present in lymph nodes, about one-third in skin, and about one-third in a variety of other extranodal sites, most commonly the intestinal tract[3].

HS of the stomach is extremely rare and it has rarely been described in the English literature[3,4]. The imaging findings of gastric HS are not well known. Thus, it is still indistinguishable from other gastric neoplasms both clinically and radiographically[5]. To the best of our knowledge, only ten cases of gastric HS have been reported in the international medical literature[3-8].

We report herein a case of primary HS of the stomach with an emphasis on the imaging findings of endoscopic ultrasonography (EUS), gastroscope, double contrast examination, and computed tomography (CT).

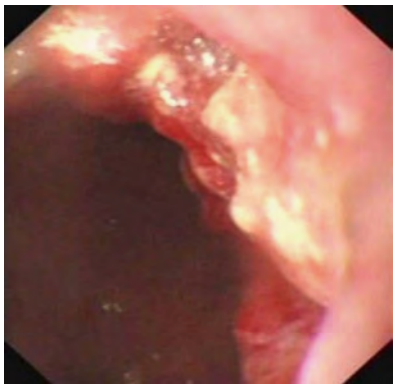


Figure 1 Gastroscopic examination revealed a large irregular ulcer with apophysis and erosive hemorrhage located on the posterior wall of fundus and lesser curvature.

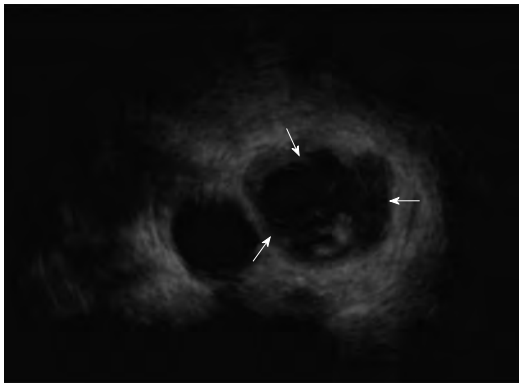


Figure 2 Endoscopic ultrasonography showed thickening about the semi-perimeter of the gastric fundal mucosa and a heterogenous hypoechoic mass, the whole layer of the local wall involved (arrows).

CASE REPORT

A 52-year-old woman presented with a one-month history of progressive difficulty in feeding and dull pain in the epigastric region for 3 d, without haematemesis or black dejection. At physical examination, she was slightly tender in the middle of the upper abdomen, without abdominal distension. There was no history of previous known stomach disease.

Gastroscopic examination revealed a large irregular ulcer with apophysis and erosive hemorrhage located on the posterior wall of fundus and lesser curvature of the stomach (Figure 1).

EUS detected thickening about the semi-perimeter of the gastric fundal mucosa, and a heterogenous hypoechoic mass, and the whole layer of the local gastric wall was infiltrated (Figure 2).

The upper digestive pneumobarium double imaging demonstrated a filling defect and a shadow of soft tissue mass around the cardia and lesser curvature of the stomach, and the abdominal part of esophagus was involved. There was destruction of the mucosa, rigidity of the gastric wall, and intracavitary niches in the lesion area (Figure 3).

Plain and contrast-enhanced CT scans of the abdomen showed a focal irregular thickened gastric wall and a poorly marginated, ulcerated soft tissue mass formation with heterogeneously obvious enhancement in the lesser gastric curvature and fundus area (Figure 4). Swelling of the adjacent regional lymph nodes was also found.

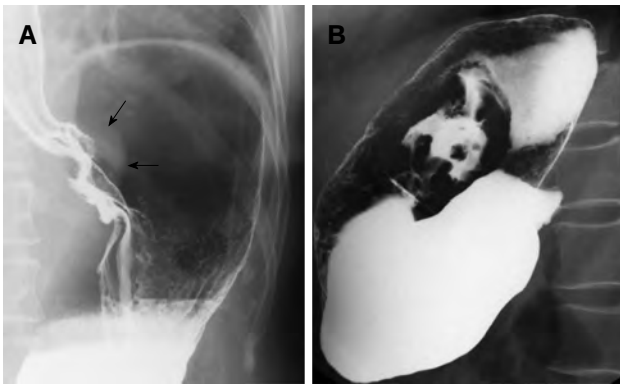


Figure 3 Double contrast examination demonstrated the shadow of soft tissue mass around the cardia and lesser curvature of stomach (arrows) (A), and **filling defect, intracavitary niches, destruction of mucosa, as well as rigidity of** the local wall were seen (B). The abdominal part of esophagus was involved.

Radical total gastrectomy was performed, and a 7 cm × 6 cm irregularly protruded tumor in the gastric curvature and fundus with the distal esophagus involved was found during the operation, associated with enlargement of the 9th and 11th groups of perigastric lymph nodes.

The surgical specimens were sent to histopathologic examination. The greater omentum did not show any tumor invasion, and tumor metastasis was not found in any of the 22 examined perigastric lymph nodes. The tumors showed infiltrative borders and areas of ulceration, with invasion into the serosa. The tumor cells were monomorphic to pleomorphic in shape. The cytoplasm was abundant and eosinophilic. The nuclei were large, and round to oval. Bizarre multinuclear giant cells were scattered. Immunohistochemical analysis indicated that the tumor cells expressed CD68, CD45RO, CD31 and Cyclin D1, and approximately 90% of the cells stained for Ki-67, but Bcl-2, Bcl-6, CD10, CD23, CD35, CD56, EMA, CK, Mum-1 and Pax-5 were negative (Figure 5). The histological and immunohistochemical diagnosis for the malignant tumor of the stomach was histiocytic sarcoma.

The patient received three cycles of chemotherapy after radical surgery. She has been free of recurrence or metastasis for 4 mo.

DISCUSSION

Histiocytic sarcoma, also known as true histiocytic lymphoma, is an exceedingly rare lymphohematopoietic malignant neoplasm composed of tumor cells showing morphologic and immunophenotypic features of mature histiocytes[2]. These malignant tumors most commonly present in lymph nodes or at extranodal sites, such as the gastrointestinal (GI) system, bone marrow and skin. The vast majority of GI HS are located in the intestinal tract; only few cases are found in the stomach. Hornick *et al*[3]

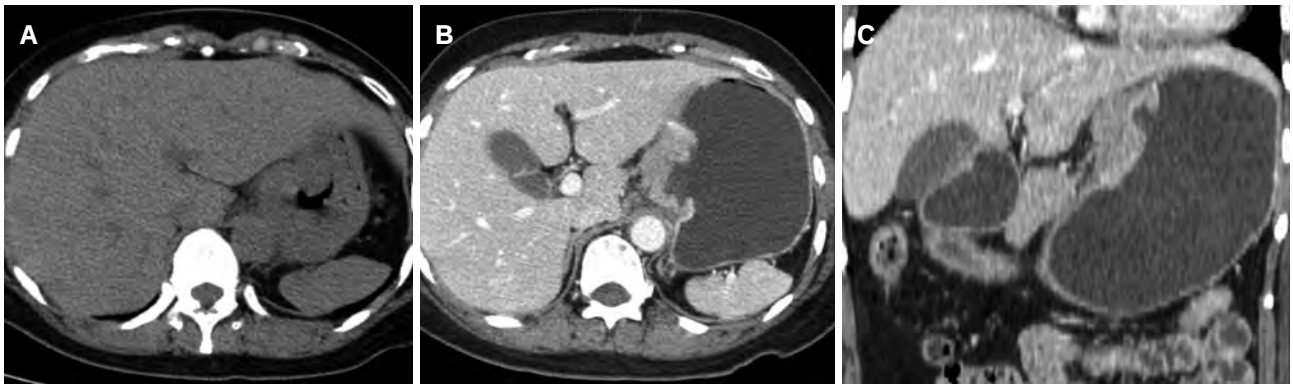


Figure 4 Abdominal computed tomography scan showed focal irregular thickened gastric wall and a ulcerated soft tissue mass formation with heterogeneously obvious enhancement in the lesser gastric curvature and fundus area. A: Plain scan; B: Contrast-enhanced scan; C: Coronal multiplanar reconstruction of enhancement scan.

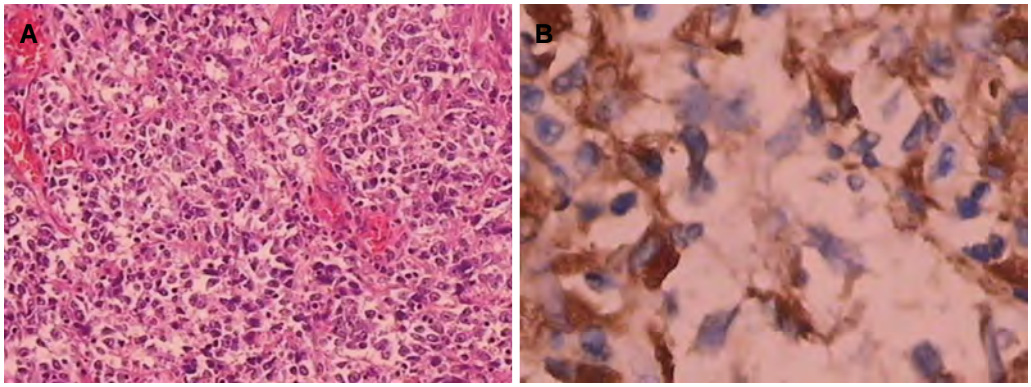


Figure 5 Histopathologic exam showed the tumor cells were marked inequality in the sizes, the cytoplasm was abundant and eosinophilic, the nuclei were large, round to oval, bizarre multinuclear giant cells were scattered. The tumor cells expressed CD68. A: Hematoxylin and eosin ×100; B: Immunohistochemical staining for CD68 ×400.

and Copie-Bergman *et al*[4] once described 14 cases of extranodal HS and 13 cases of true histiocytic lymphomas, respectively; among these cases, a total of 12 lesions in 11 patients were located in the GI tract, but only one lesion arose in the stomach. Gastric HS can be located anywhere in the stomach: the cardia, the fundus, the antrum, the lesser curvature, or the greater curvature, but most arise in the lesser curvature and involve the adjacent area. There were ulcers in almost all lesions, and perigastric regional lymphadenopathies were also the common associated findings in the patients with gastric HS[5-8].

Sundersingh *et al*[7] reported a multifocal HS of the gastrointestinal tract, and a CT scan revealed multifocal, circumferential gastrointestinal wall thickening involving the stomach and jejunal loops. Gastric HS can also exist together with HS of the colon[2]. In our case, the distal esophagus had also been infiltrated. Akiba *et al*[9] reported a case of HS arising in the parotid gland region; the patient had recurrent lesions in the pelvis and stomach 5 mo after parotidectomy.

On CT or magnetic resonance imaging enhanced scans, extranodal HS lesions often present with notably heterogeneous contrast enhancement, because generally the neoplasm has a plentiful blood supply[10,11]. Fant *et al*[12] reported one case of primary gastric HS in a dog, a large, protruding and ulcerated mass was observed on tomographic and endoscopic examinations, moreover, the lesion was also apparently strengthened on enhanced CT scan. The CT manifestations in our case were exceedingly similar with those of the above case.

The imaging features of gastric HS are nonspecific. Abdominal radiographs may show a soft tissue mass with an irregular ulcer and heterogeneous hypervascular features, mostly arising in the lesser curvature and involving the adjacent area.

In conclusion, HS of the stomach is extremely rare, and, to our knowledge, its imaging features have not been previously detailed and summarized in the English-language literature in humans[5-8]. The gastric HS can be primary or secondary, but the primary tumor seems to be more prevalent, and it can also coexist with other sites of HS. It is difficult to differentiate HS of the stomach from other gastric malignancies by radiological evaluation alone. However, HS may be considered when a protruding and ulcerated mass in the stomach shows heterogeneous hypervascular features, particularly when it arises in the lesser curvature.

REFERENCES

1 World Health Organization Classification of Tumors: Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissue. Lyon: IARC Press, 2001: 274-289

2 **Grogan TM**, Pileri SA, Chan JK, Weiss LM, Fletcher CD. Histiocytic sarcoma. In: Swerdlow SH, Campo E, Harris NL, Jaffe ES, Pileri SA, Stein H. WHO Classification of Tumours of Haematopoeitic and Lymphoid Tissues. Lyon: IARC Press, 2008: 356-357

3 **Hornick JL**, Jaffe ES, Fletcher CD. Extranodal histiocytic sarcoma: clinicopathologic analysis of 14 cases of a rare epithelioid malignancy. *Am J Surg Pathol* 2004; **28**: 1133-1144 [PMID: 15316312 DOI: 10.1097/01.pas.0000131541.95394.23]

4 **Copie-Bergman C**, Wotherspoon AC, Norton AJ, Diss TC, Isaacson PG. True histiocytic lymphoma: a morphologic, immunohistochemical, and molecular genetic study of 13 cases. *Am J Surg Pathol* 1998; **22**: 1386-1392 [PMID: 9808131 DOI: 10.1097/00000478-199811000-00009]

5 **Yoo KD**, Han DS, Chung SM, Kim SM, Bae JH, Eun CS, Paik SS, Oh YH. A case of extranodal histiocytic sarcoma of stomach mimicking gastric adenocarcinoma. *Korean J Gastroenterol* 2010; **55**: 127-132 [PMID: 20168059 DOI: 10.4166/kjg.2010.55.2.127]

6 **Congyang L**, Xinggui W, Hao L, Weihua H. Synchronous histiocytic sarcoma and diffuse large B cell lymphoma involving the stomach: a case report and review of the literature. *Int J Hematol* 2011; **93**: 247-252 [PMID: 21286876 DOI: 10.1007/s12185-011-0773-3]

7 **Sundersingh S**, Majhi U, Seshadhri RA, Tenali GS. Multifocal histiocytic sarcoma of the gastrointestinal tract. *Indian J Pathol Microbiol* 2012; **55**: 233-235 [PMID: 22771652 DOI: 10.4103/0377-4929.97891]

8 **Feng T**, He MX, Gu WY, Bai CG, Ma DL, Zheng JM, Zhu MH. Histiocytic sarcoma of stomach: report of a case. *Zhonghua Binglixue Zazhi* 2012; **41**: 130-131 [PMID: 22455895 DOI: 10.3760/cma.j.issn.0529-5807.2012.02.016]

9 **Akiba J**, Harada H, Kawahara A, Arakawa F, Mihashi H, Mihashi R, Ohshima K, Yano H. Histiocytic sarcoma of the parotid gland region. *Pathol Int* 2011; **61**: 373-376 [PMID: 21615614 DOI: 10.1111/j.1440-1827.2011.02671.x]

10 **Bell SL**, Hanzely Z, Alakandy LM, Jackson R, Stewart W. Primary meningeal histiocytic sarcoma: a report of two unusual cases. *Neuropathol Appl Neurobiol* 2012; **38**: 111-114 [PMID: 21711377 DOI: 10.1111/j.1365-2990.2011.01205.x]

11 **Makis W**, Ciarallo A, Derbekyan V, Lisbona R. Histiocytic sarcoma involving lymph nodes: imaging appearance on gallium-67 and F-18 FDG PET/CT. *Clin Nucl Med* 2011; **36**: e37-e38 [PMID: 21552013 DOI: 10.1097/RLU.0b013e3182173979]

12 **Fant P**, Caldin M, Furlanello T, De Lorenzi D, Bertolini G, Bettini G, Morini M, Masserdotti C. Primary gastric histiocytic sarcoma in a dog--a case report. *J Vet Med A Physiol Pathol Clin Med* 2004; **51**: 358-362 [PMID: 15533119 DOI: 10.1111/j.1439-0442.2004.00645.x]

P- Reviewers Greenawalt DM, Okita NT, Polymeros D
S- Editor Song XX **L- Editor** A **E- Editor** Li JY



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i3.426

World J Gastroenterol 2013 January 21; 19(3): 426-428
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

Inflammatory bowel disease: A proposal to facilitate the achievement of an unequivocal diagnosis

Gabriella Canavese, Gabrio Bassotti, Marco Astegiano, Isabella Castellano, Paola Cassoni, Anna Sapino, Vincenzo Villanacci

Gabriella Canavese, Isabella Castellano, Paola Cassoni, Anna Sapino, Ⅲ Service of Pathology, Department of Laboratory Diagnosis, Azienda Ospedaliera Città della Salute e della Scienza di Torino, Department of Medical Sciences, University of Torino, 10100 Torino, Italy
Gabrio Bassotti, Gastroenterology and Hepatology Section, Department of Clinical and Experimental Medicine, University of Perugia, 06100 Perugia, Italy
Marco Astegiano, Gastroenterology and Hepatology Section Azienda Ospedaliera Città della Salute e della Scienza di Torino, 10100 Torino, Italy
Vincenzo Villanacci, Department of Pathology, Spedali Civili of Brescia, 25100 Brescia, Italy
Author contributions: Canavese G conceived the proposal and wrote the manuscript; Bassotti G and Astegiano M collaborated in the proposal design; Castellano I and Cassoni P critically revised the manuscript; Sapino A collaborated to the proposal design and drafting of the article; and Villanacci V conceived the proposal.
Supported by Fondazione Malattie Infiammatorie Intestinali (IBD) Onlus - Torino; Compagnia San Paolo, Torino
Correspondence to: Anna Sapino, Professor, Ⅲ Service of Pathology, Department of Laboratory Diagnosis, Azienda Ospedaliera Città della Salute e della Scienza di Torino, Department of Medical Sciences University of Torino, 10100 Torino, Italy. gcanavese@molinette.piemonte.it
Telephone: +39-11-6334273 Fax: +39-11-6635267
Received: September 25, 2012 Revised: December 18, 2012
Accepted: December 22, 2012
Published online: January 21, 2013

Abstract

Following the international guidelines criteria an adequate "diagnostic conclusion" of inflammatory bowel disease (IBD) can be achieved only if clinical, endoscopic and laboratory findings, together with sample technical adequacy and unequivocal histomorphological signs of the disease are available. Thus, a conclusive diagnosis requires a complex combination of clinical, endoscopic and histological data. A considerable number of endoscopic biopsies obtained from IBD patients do not meet the above-mentioned requirements. The aim of the present proposal is to introduce a binary system of evaluation in the "diagnostic conclusion" of the histopathological report that will help to simplify the clinical decisions and consequent patient management. In patients with no history of disease, the pathologist should classify the biopsies in "Diagnostic", when the criteria established by the international guidelines are satisfied and "not diagnostic" when one or more of the above-mentioned criteria are not met. The term "not diagnostic" should replace "highly suggestive" and "probable". This new terminology could avoid ambiguous expressions that encourage the clinician to classify the patient as affected by IBD without fulfilling all of the requirements for an accurate diagnostic approach.

© 2013 Baishideng. All rights reserved.

Key words: Inflammatory bowel disease; Diagnosis; Biopsy

Canavese G, Bassotti G, Astegiano M, Castellano I, Cassoni P, Sapino A, Villanacci V. Inflammatory bowel disease: A proposal to facilitate the achievement of an unequivocal diagnosis. *World J Gastroenterol* 2013; 19(3): 426-428 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i3/426.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i3.426

TO THE EDITOR

Various guidelines have defined the criteria used for the diagnostic evaluation of endoscopic biopsies as chronic idiopathic inflammatory bowel disease (IBD)[1-5].

Most of these guidelines include a "preliminary section" of the histological report that includes the evaluation of the different morphological parameters of the

biopsy specimen and a final section with the "diagnostic conclusion" derived from the previous synoptic analyses. In practice, the adequate diagnosis of colon biopsy specimens for non-neoplastic disease requires the following parameters: (1) clinical, endoscopic, and laboratory findings; and (2) technical adequacy of endoscopic sampling and histological procedures, particularly a proper orientation of the biopsies[3]; and unequivocal evidence of specific morphological signs of the disease in histological analysis[5-7].

Thus, a conclusive diagnosis requires a complex combination of clinical, endoscopic and histological data. Consequently, a considerable number of endoscopic biopsies obtained from IBD patients do not meet the above-mentioned requirements and cannot receive a definite histological diagnosis. In the more recent guidelines, the categories "highly suggestive[1] and "probable"[6] in the final section of the diagnostic report encompass all of these cases. Although these terms are ambiguous, they are often interpreted as a definitive diagnosis for the clinical management of patients with endoscopy suggestive for IBD.

The aim of the present proposal is to introduce a binary system of evaluation in the "diagnostic conclusion" section that will help to simplify the clinical decisions and consequent patient management. For this purpose, patients without a previous histological diagnosis of IBD and patients with a previous unequivocal diagnosis of IBD should be approached differently.

In patients with no history of disease, the pathologist should use the following terms to classify the biopsies.

Diagnostic, when the criteria established by the international guidelines[1,2] for the diagnosis of IBD are satisfied: (1) complete or extensive mapping of ileo-colonic segments; (2) adequate specimens, correctly oriented; (3) adequate information, including clinical history, laboratory data, and previous histological and/or endoscopic reports; and (4) histological examination of specimens shows unequivocal microscopic signs of the disease (diagnostic for IBD) or definitely rules out the diagnosis of IBD (negative for IBD). If possible, the pathologist should specify the type of the non-IBD colitis (infective, drug-induced, lymphocytic, collagenous, *etc.*).

Not diagnostic, when one or more of the above-mentioned criteria are not met. The term "not diagnostic" should replace "highly suggestive"[1] and "probable"[6]. After discussion with the gastroenterologist and the endoscopist, these patients should be re-evaluated. In cases with persistent symptoms, the patient should be sent back to endoscopy with the goal of achieving diagnostic biopsies. IBD shows a distinctive microscopic morphology only after 2-3 wk[8], which should be considered in the timing of subsequent biopsies.

In cases of a previous unequivocal diagnosis of IBD performed in the same institution (or in a different center, if specimens are available for revision), the sampling requirements are less stringent and more dependent on endoscopy. The categories "diagnostic/not diagnostic" in the final section of the report are not required, but the report should include an evaluation of disease activity, the presence or absence of dysplasia, and the presence or absence of Cytomegalovirus (follow-up evaluation). Figure 1 provides an example of an algorithm used for this classification.

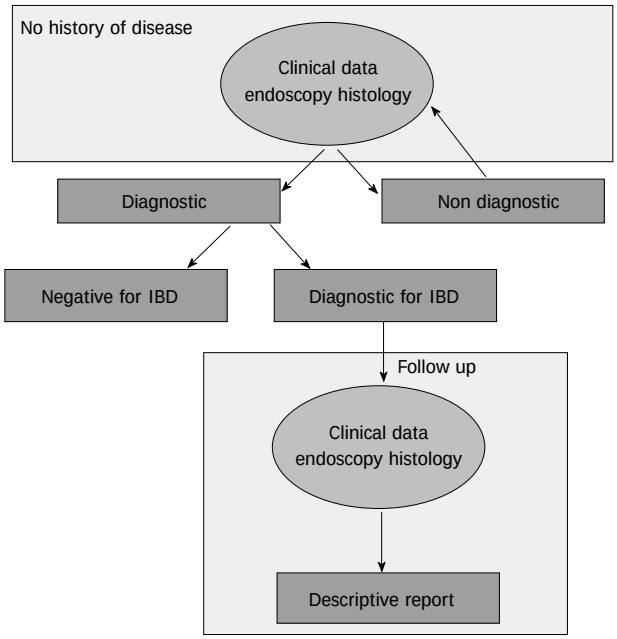


Figure 1 A graphic representation of the described terms (boxes) with suggestions for clinical management. IBD: Inflammatory bowel disease.

In our opinion, the advantages of using this binary system to approach endoscopic biopsies in IBD are the following: (1) this terminology could avoid ambiguous expressions that encourage the clinician to classify the patient as affected by IBD without fulfilling all of the requirements for an accurate diagnostic approach; (2) a second set of biopsies after a "not diagnostic" result should simplify the differential diagnosis from non-IBD colitis. Moreover, after the first set of biopsies, the clinician may be able to collect other data (laboratory data, response to therapy, *etc.*) that are useful for diagnosis; (3) this approach will reduce the use of inappropriate treatments that might cause mucosal changes, which can complicate the histological evaluation and compromise the correct assessment of the patient[8]; and (4) last, but not least, the quality of clinical and biological studies should be improved by including only case series with a definitive diagnosis.

The final goal of our proposal is to seek comments and suggestions about this topic and share this approach with other authors.

REFERENCES

1 **Cornaggia M**, Leutner M, Mescoli C, Sturniolo GC, Gullotta R. Chronic idiopathic inflammatory bowel diseases: the histology report. *Dig Liver Dis* 2011; **43** Suppl 4: S293-S303 [PMID: 21459335 DOI: 10.1016/S1590-8658(11)60585-9]

2 **Van Assche G**, Dignass A, Panes J, Beaugerie L, Karagiannis J, Allez M, Ochsenkühn T, Orchard T, Rogler G, Louis E,

Kupcinskas L, Mantzaris G, Travis S, Stange E. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Definitions and diagnosis. *J Crohns Colitis* 2010; **4**: 7-27 [PMID: 21122488]

3 **Bryan F**, Warren, Neil A. Sheperd. What are the controversies in histopathological diagnosis ? In: Jewell DP, Warren BF, Mortensen NJ. Challenges in Inflammatory Bowel Disease. 2001. Available from: URL: http: //trove.nla.gov.au/work/20355368?selectedversion=NBD28674755

4 **Jenkins D**, Balsitis M, Gallivan S, Dixon MF, Gilmour HM, Shepherd NA, Theodossi A, Williams GT. Guidelines for the initial biopsy diagnosis of suspected chronic idiopathic inflammatory bowel disease. The British Society of Gastroenterology Initiative. *J Clin Pathol* 1997; **50**: 93-105 [PMID: 9155688]

5 **Vilela EG**, Torres HO, Martins FP, Ferrari Mde L, Andrade MM, Cunha AS. Evaluation of inflammatory activity in Crohn's disease and ulcerative colitis. *World J Gastroenterol* 2012; **18**: 872-881 [PMID: 22408345 DOI: 10.3748/wjg.v18.i9.872]

6 **Tanaka M**, Saito H, Fukuda S, Sasaki Y, Munakata A, Kudo H. Simple mucosal biopsy criteria differentiating among Crohn disease, ulcerative colitis, and other forms of colitis: measurement of validity. *Scand J Gastroenterol* 2000; **35**: 281-286 [PMID: 10766322 DOI: 10.1080/003655200750024155]

7 **Tanaka M**, Riddell RH, Saito H, Soma Y, Hidaka H, Kudo H. Morphologic criteria applicable to biopsy specimens for effective distinction of inflammatory bowel disease from other forms of colitis and of Crohn's disease from ulcerative colitis. *Scand J Gastroenterol* 1999; **34**: 55-67 [PMID: 10048734 DOI: 10.1080/00365529950172844]

8 **Geboes K**. Pathology of inflammatory bowel diseases (IBD): variability with time and treatment. *Colorectal Dis* 2001; **3**: 2-12 [PMID: 12791013 DOI: 10.1111/j.1463-1318.2001.00187]

P- Reviewers Rocha R, Tanaka T, Ukleja A, Wirth S
S- Editor Song XX **L- Editor** A **E- Editor** Li JY



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i3.429

World J Gastroenterol 2013 January 21; 19(3): 429-430
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

Association of inducible nitric oxide synthetase genotype and *Helicobacter pylori* infection gastric cancer risk may be due to faulty primer design

Amin Talebi Bezmin Abadi, Johannes G Kusters

Amin Talebi Bezmin Abadi, Johannes G Kusters, Department of Medical Microbiology, University Medical Center Utrecht, 3584 CX Utrecht, The Netherlands
Author contributions: Abadi ATB and Kusters JG contributed to primary concept, in silico analysis of primers, sequences and writing the manuscript.
Correspondence to: Dr. Johannes G Kusters, Department of Medical Microbiology, University Medical Center Utrecht, Heidelberglaan 100, 3584 CX Utrecht,
The Netherlands. h.kusters@umcutrecht.nl
Telephone: +31-88-7553687 Fax: +31-88-7555426
Received: September 27, 2012 Revised: December 11, 2012
Accepted: December 20, 2012
Published online: January 21, 2013

Abstract

Rafiei *et al* recently described an association between the presence of the C150T polymorphism of the inducible nitric oxide synthase (*iNOS*) gene and *Helicobacter pylori* (*H. pylori*) induced gastric cancer. When we used primer-BLAST to find the polymerase chain reaction (PCR) product that would be generated by the primers used by these authors no products against any of the sequences present in the GenBank database were found. Further analysis of the *iNOS* sequences present in the GenBank suggest that the result from their study might come from a faulty primer design and may thus represent an artifact. Alternatively they may be correct and have identified a truly interesting explanation for the mechanism whereby *H. pylori* induces gastric cancer but some additional experiments would be in order to exclude the possibility of a PCR artifact.

© 2013 Baishideng. All rights reserved.

Key words: *Helicobacter pylori*; Polymorphism; Inducible nitric oxide synthase

Abadi ATB, Kusters JG. Association of inducible nitric oxide synthetase genotype and *Helicobacter pylori* infection gastric cancer risk may be due to faulty primer design. *World J Gastroenterol* 2013; 19(3): 429-430 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i3/429.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i3.429

TO THE EDITOR

Rafiei *et al*[1] recently published an interesting article in the *World Journal of Gastroenterology* entitled "Inducible nitric oxide synthetase genotype and *Helicobacter pylori* infection affect gastric cancer risk". In this study, they investigated the possible association between inducible nitric oxide synthase (*iNOS*) genotype and gastric cancer among the 329 patients from northern Iran. They found a clear association between the presence of C150T polymorphism of the *iNOS* gene and the presence of gastric cancer in *Helicobacter pylori* (*H. pylori*) infected patients. Strikingly they found an overall frequency of the 150T allele of approximately 25% of the individuals (both controls and cases) tested[1], which came as a surprise to us for the original description by Shen *et al*[2] mentions a frequency of 13%. This discrepancy could obviously be explained by genetic differences in the ethnic populations but is not discussed by Rafiei *et al*[1] in their paper and this prompted us to carefully study the methods used to establish the C150T polymorphism. In order to establish the polymorphism the authors used the method that was described by Shen *et al*[3]. Briefly, a polymerase chain reaction (PCR) was used to produce a 255 bp amplicon of the *iNOS* gene that was subsequently digested with *Tsp* 509 I for determination of the C150T polymorphism. When we used primer-BLAST (http://www.ncbi.nlm.nih.gov/tools/primer-blast) to find the PCR product that would be generated by these primers this program was unable to find any products against

any of the sequences present in the GenBank database. Performing a manual search against the Assembled RefSeq human Genomes database (Build 37.3) did only result in two *iNOS* hit for the forward primer (database entry ref NT 010799.15 and NW 001838430.2) and no hits for the reverse primer. When we subsequently performed a basic BLAST search with the separate primers we only found hits with the forward primer in 7/9 *iNOS* sequences in the Genbank database version 25 September 2012 and no hits with the reverse primer. Further analysis of the 9 *iNOS* sequences present in the GenBank revealed that a *Tsp* 509 I site at the expected distance from the forward primer in all but one sequence (GenBank accession no DQ149843.1). This sequence (DQ149843.1) was submitted by Shen *et al*[3], the group who originally identified the C150T polymorphism. Interestingly the 3' end of this 288 bp sequence (supposedly containing the binding site for the reverse primer) differs considerably from all but one of the other *iNOS* sequences in the database; i.e., an 288 *iNOS* sequence with accession no X85772.1. This closely homologous sequence (X85772.1) differs only in the C150T position and was submitted to the database by Xu *et al*[4]. A possible explanation for the aberrant 3' end of this sequence is that the sequence from Xu *et al*[4] is assembled from two cosmid clones and as the sequence differences seem to be at the link region of these two cosmids it may represent a cloning artifact. Careful analysis of these two sequences in fact does show some similarity to the reverse primer at the expected position, but the two 3' bases clearly differ, making it almost impossible to produce a PCR product with this primer. Conclusively, it looks like the unique results of these two studies[1,2] might come from a faulty primer design and may thus represent an artifact. Alternatively they may be correct and present a truly interesting clue for the mechanism whereby *H. pylori* induces gastric cancer but we feel that some additional experiments would be in order to exclude the possible artifact as mentioned above.

REFERENCES

1 **Rafiei A**, Hosseini V, Janbabai G, Fazli B, Ajami A, Hosseini-Khah Z, Gilbreath J, Merrell DS. Inducible nitric oxide synthetase genotype and Helicobacter pylori infection affect gastric cancer risk. *World J Gastroenterol* 2012; **18**: 4917-4924 [PMID: 23002365 DOI: 10.3748/wjg.v18.i35.4917]

2 **Shen J**, Wang R, Wang L, Wang Z, Xing H, Wang B, Li M, Hua Z, Wang J, Guo C, Wang X, Xu X. Study on the Relationship between susceptibility of stomach neoplasm cancer and polymorphism of inducible nitric oxide synthase gene. *Zhonghua Liuxingbingxue Zazhi* 2002; **23**: 374-377 [PMID: 12482371]

3 **Shen J**, Wang RT, Wang LW, Xu YC, Wang XR. A novel genetic polymorphism of inducible nitric oxide synthase is associated with an increased risk of gastric cancer. *World J Gastroenterol* 2004; **10**: 3278-3283 [PMID: 15484300]

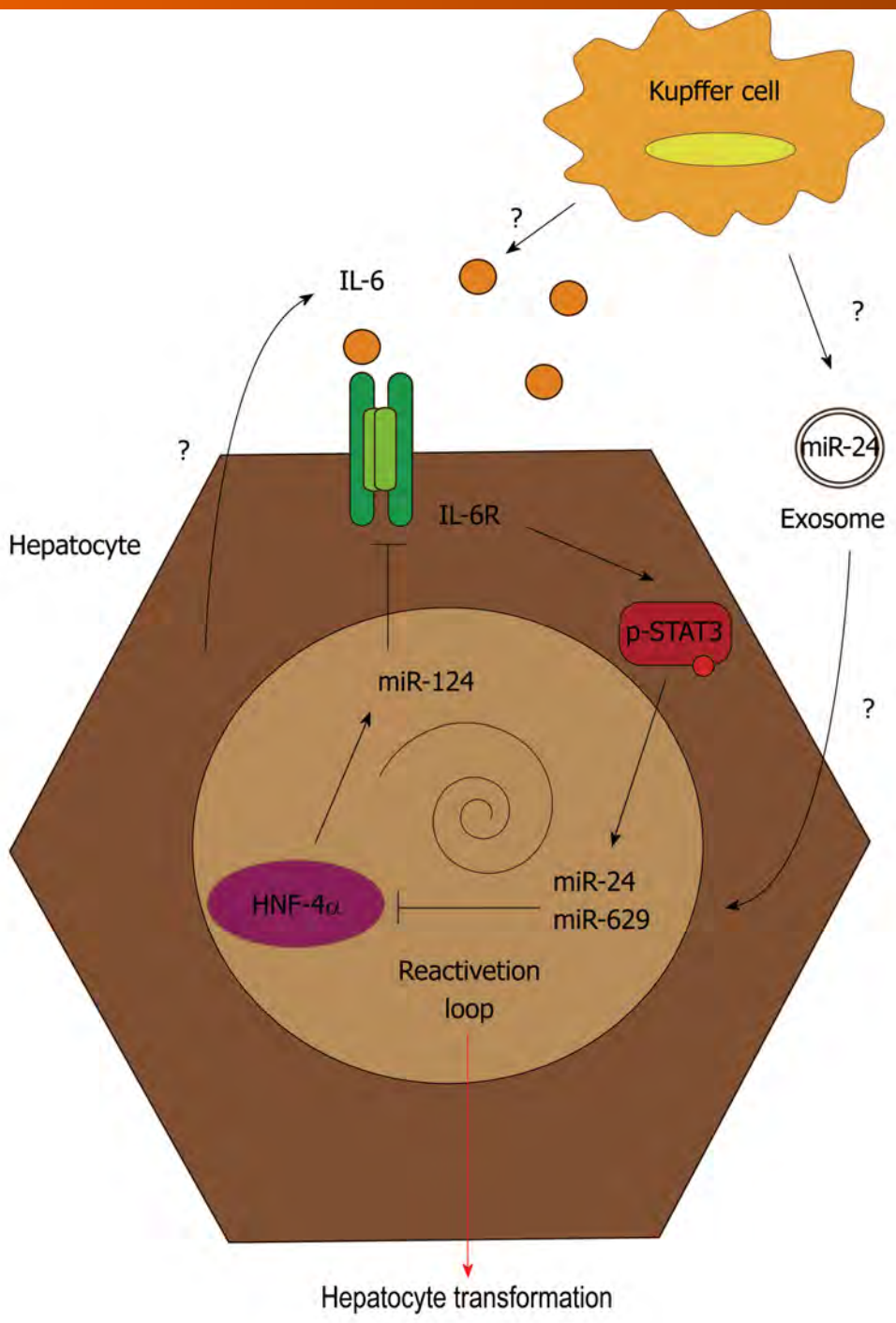
4 **Xu W**, Charles IG, Liu L, Moncada S, Emson P. Molecular cloning and structural organization of the human inducible nitric oxide synthase gene (NOS2). *Biochem Biophys Res Commun* 1996; **219**: 784-788 [PMID: 8645258]

P- Reviewers Day AS, Tosetti C **S- Editor** Song XX
L- Editor A **E- Editor** Li JY

ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

World J Gastroenterol 2013 January 28; 19(4): 431-606



Baishideng Publishing Group
www.wjgnet.com

World Journal of Gastroenterology

Contents

Weekly Volume 19 Number 4 January 28, 2013

EDITORIAL

431 Advanced endoscopic technologies for colorectal cancer screening
Obstein KL, Valdastri P

FIELD OF VISION

440 MicroRNA-feedback loop as a key modulator of liver tumorigenesis and inflammation
Gougelet A, Colnot S

TOPIC HIGHLIGHT

445 Update on adrenal insufficiency in patients with liver cirrhosis
Trifan A, Chiriac S, Stanciu C

REVIEW

457 Obesity and cholangiocarcinoma
Parsi MA

463 Sedation in gastrointestinal endoscopy: Current issues
Triantafillidis JK, Merikas E, Nikolakis D, Papalois AE

ORIGINAL ARTICLE

482 Age-dependent slowing of enteric axonal transport in insulin-resistant mice
LePard KJ, Cellini J

492 Combined early fluid resuscitation and hydrogen inhalation attenuates lung and intestine injury
Liu W, Shan LP, Dong XS, Liu XW, Ma T, Liu Z

BRIEF ARTICLE

503 Development and validation of a registry-based definition of eosinophilic esophagitis in Denmark
Dellon ES, Erichsen R, Pedersen L, Shaheen NJ, Baron JA, Sørensen HT, Vyberg M

511 Endoscopic ultrasound evaluation in the surgical treatment of duodenal and peri-ampullary adenomas
Azih LC, Broussard BL, Phadnis MA, Heslin MJ, Eloubeidi MA, Varadarajulu S, Arnoletti JP

516 Effect of alcohol consumption on liver stiffness measured by transient elastography
Bardou-Jacquet E, Legros L, Soro D, Latournerie M, Guillygomarc'h A, Le Lan C, Brissot P, Guyader D, Moirand R

523 Role of Ki-67 as a prognostic factor in gastrointestinal stromal tumors
Belev B, Brčić I, Prejac J, Golubić ZA, Vrbanec D, Božikov J, Alerić I, Boban M, Razumović JJ

528 Can endoscopic submucosal dissection be safely performed in a smaller specialized clinic?
Sohara N, Hagiwara S, Arai R, Iizuka H, Onozato Y, Kakizaki S

536 Cdx2 expression and its promoter methylation during metaplasia-dysplasia-carcinoma sequence in Barrett's esophagus
Makita K, Kitazawa R, Semba S, Fujiishi K, Nakagawa M, Haraguchi R, Kitazawa S

542 Relationship between diversion colitis and quality of life in rectal cancer
Son DN, Choi DJ, Woo SU, Kim J, Keom BR, Kim CH, Baek SJ, Kim SH

550 Colon transit time according to physical activity and characteristics in South Korean adults
Cho KO, Jo YJ, Song BK, Oh JW, Kim YS

556 New endoscopic classification of achalasia for selection of candidates for peroral endoscopic myotomy
Li HK, Linghu EQ

561 Pilot study on efficacy of reduced cathartic bowel preparation with polyethylene glycol and bisacodyl
Chen ZY, Shen HS, Luo MY, Duan CJ, Cai WL, Lu HB, Zhang GP, Liu Y, Liang JZ

569 Retrospective study of steroid therapy for patients with autoimmune pancreatitis in a Chinese population
Liu B, Li J, Yan LN, Sun HR, Liu T, Zhang ZX

575 Increased expression of tyrosine phosphatase SHP-2 in *Helicobacter pylori*-infected gastric cancer
Jiang J, Jin MS, Kong F, Wang YP, Jia ZF, Cao DH, Ma HX, Suo J, Cao XY

CASE REPORT

581 Acute hepatitis C virus infection in a nurse trainee following a needlestick injury
Scaggiante R, Chemello L, Rinaldi R, Bartolucci GB, Trevisan A

586 Duplication of the transverse colon in an adult: Case report and review
Banchini F, Delfanti R, Begnini E, Tripodi MC, Capelli P

590 Steroid-refractory ulcerative colitis and associated primary sclerosing cholangitis treated with infliximab
Duca I, Ramírez de la Piscina P, Estrada S, Calderón R, Spicakova K, Urtasun L, Marra-López C, Zabaleta S, Bengoa R, Marcaide MA, García-Campos F

594 Iatrogenic esophago-tracheal fistula: Challenges in diagnosis and management
Hovde Ø, Lie ØH, Johansson PA, Stubhaug Ø, Johnson E, Hofstad B, Hauge T

597 Fulminant gastrointestinal graft-versus-host disease concomitant with cytomegalovirus infection: Case report and literature review
Okubo H, Nagata N, Uemura N

604 Emphysematous cholecystitis with massive gas in the abdominal cavity
Miyahara H, Shida D, Matsunaga H, Takahama Y, Miyamoto S

World Journal of Gastroenterology
Volume 19 Number 4 January 28, 2013

Contents

APPENDIX	**I-VI**	Instructions to authors
ABOUT COVER		Gougelet A, Colnot S. MicroRNA-feedback loop as a key modulator of liver tumorigenesis and inflammation. *World J Gastroenterol* 2013; 19(4): 440-444 http://www.wjgnet.com/1007-9327/full/v19/i4/440.htm
AIMS AND SCOPE		*World Journal of Gastroenterology* (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. *WJG* was established on October 1, 1995. It is published weekly on the 7th, 14th, 21st, and 28th each month. The *WJG* Editorial Board consists of 1352 experts in gastroenterology and hepatology from 64 countries. The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.
INDEXING/ABSTRACTING		*World Journal of Gastroenterology* is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, Digital Object Identifier, and Directory of Open Access Journals. ISI, Journal Citation Reports®, Gastroenterology and Hepatology, 2011 Impact Factor: 2.471 (32/74); Total Cites: 16951 (7/74); Current Articles: 677 (1/74); and Eigenfactor® Score: 0.06035 (5/74).
FLYLEAF	**I-IX**	Editorial Board

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Shuai Ma*
Responsible Electronic Editor: *Li Xiong*
Proofing Editor-in-Chief: *Lian-Sheng Ma*
Responsible Science Editor: *Huan-Huan Zhai*
Proofing Editorial Office Director: *Jin-Lei Wang*

NAME OF JOURNAL
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

EDITOR-IN-CHIEF
Ferruccio Bonino, MD, PhD, Professor of Gastroenterology, Director of Liver and Digestive Disease Division, Department of Internal Medicine, University of Pisa, Director of General Medicine 2 Unit University Hospital of Pisa, Via Roma 67, 56124 Pisa, Italy

Myung-Hwan Kim, MD, PhD, Professor, Head, Department of Gastroenterology, Director, Center for Biliary Diseases, University of Ulsan College of Medicine, Asan Medical Center, 388-1 Pungnap-2dong, Songpa-gu, Seoul 138-736, South Korea

Kjell Öberg, MD, PhD, Professor, Department of Endocrine Oncology, Uppsala University Hospital, SE-751 85 Uppsala, Sweden

Matt D Rutter, MBBS, MD, FRCP, Consultant Gastroenterologist, Senior Lecturer, Director, Tees Bowel Cancer Screening Centre, University Hospital of North Tees, Durham University, Stockton-on-Tees, Cleveland TS19 8PE, United Kingdom

Andrzej S Tarnawski, MD, PhD, DSc (Med), Professor of Medicine, Chief Gastroenterology, VA Long Beach Health Care System, University of California, Irvine, CA, 5901 E. Seventh Str., Long Beach, CA 90822, United States

EDITORIAL OFFICE
Jian-Xia Cheng, Director
Jin-Lei Wang, Vice Director
World Journal of Gastroenterology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Co., Limited
Room 1701, 17/F, Henan Building,
No.90 Jaffe Road, Wanchai, Hong Kong, China
Fax: +852-31158812
Telephone: +852-58042046
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

PUBLICATION DATE
January 28, 2013

COPYRIGHT
© 2013 Baishideng. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT
All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i4.431

World J Gastroenterol 2013 January 28; 19(4): 431-439
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

EDITORIAL

Advanced endoscopic technologies for colorectal cancer screening

Keith L Obstein, Pietro Valdastri

Keith L Obstein, Department of Medicine, Division of Gastroenterology, Hepatology and Nutrition, Vanderbilt University Medical Center, Nashville, TN 37232, United States
Pietro Valdastri, STORM Lab, Department of Mechanical Engineering, Vanderbilt University, Nashville, TN 37235, United States
Author contributions: Obstein KL and Valdastri P contributed equally to this work.
Supported by The National Science Foundation, Grant No. CNS-1239355; the National Center for Research Resources, Grant No. UL1 RR024975-01; and the National Center for Advancing Translational Sciences, Grant No. 2 UL1 TR000445-06 (the content is solely the responsibility of the authors and does not necessarily represent the official views of the NIH)
Correspondence to: Pietro Valdastri, PhD, Assistant Professor, Director of the STORM Lab, Department of Mechanical Engineering, Vanderbilt University, 2301 Vanderbilt Place PMB 351592, Nashville, TN 37235,
United States. p.valdastri@vanderbilt.edu
Telephone: +1-615-8756955 Fax: +1-615-3436687
Received: June 20, 2012 Revised: August 23, 2012
Accepted: August 26, 2012
Published online: January 28, 2013

Abstract

Colorectal cancer is the third most common cancer in men and the second most common cancer in women worldwide. Diagnosing colorectal has been increasingly successful due to advances in technology. Flexible endoscopy is considered to be an effective method for early diagnosis and treatment of gastrointestinal cancer, making it a popular choice for screening programs. However, millions of people who may benefit from endoscopic colorectal cancer screening fail to have the procedure performed. Main reasons include psychological barriers due to the indignity of the procedure, fear of procedure related pain, bowel preparation discomfort, and potential need for sedation. Therefore, an urgent need for new technologies addressing these issues clearly exists. In this review, we discuss a set of advanced endoscopic technologies for colorectal cancer screening that are either already available or close to clinical trial. In particular, we focus on visual-inspection-only advanced flexible colonoscopes, interventional colonoscopes with alternative propulsion mechanisms, wireless capsule colonoscopy, and technologies for intraprocedural bowel cleansing. Many of these devices have the potential to reduce exam related patient discomfort, obviate the need for sedation, increase diagnostic yield, reduce learning curves, improve access to screening, and possibly avert the need for a bowel preparation.

© 2013 Baishideng. All rights reserved.

Key words: Endoscopy; Technology; Capsule colonoscopy; Colorectal cancer; Screening

Obstein KL, Valdastri P. Advanced endoscopic technologies for colorectal cancer screening. *World J Gastroenterol* 2013; 19(4): 431-439 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i4/431.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i4.431

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer in men and the second most common cancer in women worldwide with approximately 608 000 people dying each year[1]. In the United States alone there are approximately 1.14 million people alive who have a history of CRC and 1 in 20 will be diagnosed with cancer of the colon or rectum in their lifetime[2]. Unfortunately, this number is projected to increase by 62% by the year 2030[3].

Diagnosing CRC has been increasingly successful due to numerous advances in technology. One of the paramount technological advances has been the ability to directly visualize the gastrointestinal (GI) tract (and provide therapy) with the flexible endoscope. The earliest flexible endoscope, completely based on optical fibers, was

presented at the American Gastroscopy Society annual meeting in May 1957 by Hirschowitz[4]. This achievement was inspired by a paper published in 1954, entitled "a flexible fiberscope using static scanning", by Hopkins *et al*[5] at the Imperial College of Science and Technology in London. Building on this history of technological innovation, and driven by breakthroughs in electronics, material science, computational capabilities, sensing, and actuation strategies, many novel GI devices and diagnostic techniques have emerged. In this review, we will discuss a set of advanced endoscopic technologies for CRC screening that are either already available or close to clinical trial; and have the potential to reduce procedure related patient discomfort and increase diagnostic yield.

LIMITATION OF STANDARD GI ENOSCOPY

Flexible endoscopy is considered to be an effective method for early diagnosis and treatment of GI cancer, thus is a primary choice for screening programs. Few complications are associated with this technique, with cardiorespiratory problems related to sedation and analgesia being the most common (0.03%-20% incidence[6]). Less frequent complications include, infection (0.2% incidence[7]), bleeding (0.2%-2.1% incidence) or perforation (0.1% incidence[8]), which potentially require subsequent medications, transfusions, or endoscopic/surgical intervention to correct.

Based on the efficacy and low complication rate of flexible endoscopy, it is clear that the main clinical challenge facing GI endoscopy is one of distribution. Millions of people who may benefit from endoscopic CRC screening fail to have the procedure performed. The reasons cited include psychological barriers due to the indignity of the procedure, fear of procedure related pain, bowel preparation discomfort, and potential need for sedation[9].

How rational are these fears? From a mechanical perspective, the endoscope consists of a long and fairly stiff (compared to the compliant colon) tube with a steerable head. The colonoscope must easily navigate the colon curves and traverse the intestinal environment efficiently - meaning that a colonoscope must be simultaneously stiff and compliant. If the colonoscope is too stiff, it will deform the colon wall significantly at turns; yet, if it is too compliant there will be undesired buckling[10]. Since the colonoscope must be pushed from the back, while the tip is aimed along the lumen center, when the intestine bends, the shaft pushes against the colon wall until the lumen and its surroundings provide sufficient counter pressure to force the endoscope shaft to bend. This stretches the colon and often leads to "loop" formation, thus potentially causing substantial discomfort. In particular, looping occurs when the colonoscope continues to be advanced into the colon without a corresponding progression of the tip. This displaces the colon from its native configuration and stretches mesentery muscles. Looping of the endoscope has been shown to be responsible for 90% of the pain episodes in colonoscopy and increases the chance of tissue damage and perforation[11]. Special maneuvers can be performed to minimize this effect, making colonoscopy a procedure that requires a great degree of training, technical skill, and experience to safely perform[12,13]. Despite these techniques, even expert endoscopists can not always prevent all challenges or complications; partially because the flexible endoscope design is one of compromise and is not perfect for its intended purpose[10]. Additionally, the need for a bowel preparation acts as a potential deterrent due to the unpleasantness of ingesting powerful medications to clean the intestine. A detailed discussion on bowel preparation is beyond the scope of this review and will only be discussed briefly.

TOWARD MECHANISMS ENABLING PAINLESS COLONOSCOPY

Several colonoscope modifications have recently been presented with the common goal of preventing excessive force application to the colon wall and consequent looping. These devices can be categorized into two groups: those designed purely for visual inspection (visual inspection devices), and those that contain internal channels through which interventional devices (i.e., biopsy, snare, needle, *etc.*) can be passed (interventional devices). In addition to flexible colonoscope modifications, wireless capsule colonoscopy is emerging as a "patient-friendly" alternative technique for visual inspection of the colon. In this section we will provide an overview of recent advancements in these three device categories.

Visual-inspection-only advanced flexible colonoscopes

An example diagnostic-only device is the CathCam, whose development has been supported by Ethicon Endo Surgery, Inc., represented in Figure 1A. The CathCam is a nonsterile, disposable, multilumen catheter with a working length of 1.8 m and a diameter of 11 mm. Vision and illumination are provided by a 3-mm camera with six light-emitting diodes. The catheter is designed for single use, while the camera is reusable and is mounted into the catheter tip prior to the procedure. The channels of the CathCam accommodate the cables of the video camera and enable the system to provide suction, irrigation, and visualization. An accessory channel (2.8 mm in diameter) is provided for a looped guide wire (0.024-inch hinged lumen-seeking guide wire) that is advanced through the channel and into the lumen of the colon. The guide wire then serves to guide the catheter forward when the catheter is pushed[14]. This device has demonstrated 30%-40% peak force reduction in benchtop experiments and in live pigs, in comparison to standard colonoscopes[15] (Table 1).

Another advanced diagnostic flexible endoscope is the Aer-O-Scope™ (GI View Ltd., Ramat Gan, Israel), which is a pneumatic, skill-independent, self-propelling, and self-navigating disposable colonoscope[16], repre-

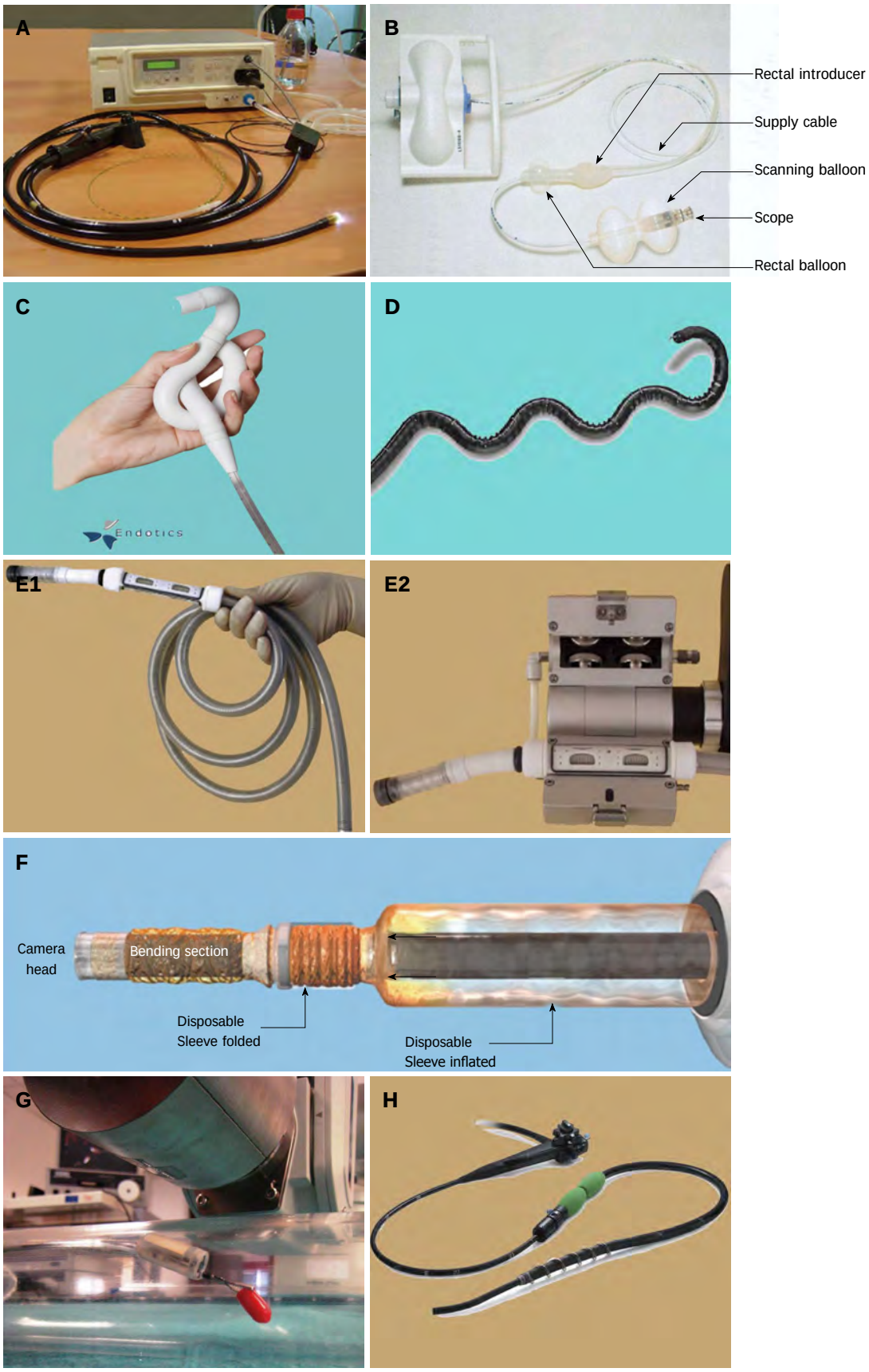


Figure 1 Figures of the devices. A: The CathCam system, reprinted from[15], with permission from Elsevier; B: The Aer-O-Scope™ system, reprinted from[18], with permission from Elsevier; C: The Endotics™ system, image courtesy of Era Endoscopy; D: The NeoGuide™ system, reprinted by permission from Macmillan Publisher Ltd.: The *American Journal of Gastroenterology*[11], copyright 2007; E: The Invendoscope™ system, reprinted from[24] with permission from Elsevier; F: The ColonoSight™ system, reprinted from[26] with permission from Elsevier; G: Magnetic endoscopic device described in[27]; H: The Endo-Ease™ overtube over a pediatric colonoscope, reprinted from[28], with permission from Elsevier.

Table 1 Main characteristics of advanced flexible colonoscopes

Technology	Advantages	Disadvantages	Human studies
CathCam	Disposable; peak force reduction with respect to standard colonoscope	No instrument channel; no steering	No
Aer-O-Scope™	Disposable; "tip pulling" locomotion; computer-aided control	No instrument channel; no steering	Yes[18]
Endotics™	Disposable; Steerable head; "tip pulling" locomotion; computer-aided control; thin tail	No instrument channel; average procedure time longer than colonoscopy	Yes[20,21]
NeoGuide™	Instrument channel; shape retention; 3D map of the device; computer-aided control	Reusable (need for cleaning); large diameter	Yes[11]
Invendoscope™	Disposable; instrument channel; "tip pulling" locomotion; computer-aided control	Diameter similar to a colonoscope	Yes[24,25]
ColonoSight™	Disposable; instrument channel; "tip pulling" locomotion	Diameter similar to a colonoscope	Yes[26]
Magnetic endoscopic device	Instrument channel; "tip pulling" locomotion; computer-aided control; thin tail	Complexities related to magnetic control	No
Endo-Ease™	Facilitates cecal intubation	Large diameter	Yes[29]
ScopeGuide™	3D real-time visualization of the device	Standard colonoscope	Yes[32,33]

sented in Figure 1B. The device is composed of a rectal introducer, a supply cable, and an endoscope embedded within a scanning balloon that serves as its vehicle. The rectal introducer is a hollow silicon tube (1.7 m in length, 19 mm in diameter) with a silicone balloon (80 mm in diameter) attached to its outer surface. The introducer is inserted into the rectum with its outer balloon, and the endoscope and its vehicle balloon are passed through the hollow tube of the introducer. The silicone balloon on the introducer seals the anus to prevent gas leakage. CO_2 is insufflated between the two inflated balloons, and gas pressure advances the vehicle balloon, endoscope, and a trailing supply cable. The supply cable on the endoscope/scanning balloon is a flexible polyurethane multi-lumen catheter, 5.5 mm in diameter, coated with a hydrophilic material, which supplies the electro-optical capsule (the scope) and its vehicle balloon with electricity, air, water, and suction. The volume of the vehicle/scanning balloon is pressure-regulated. The balloon is designed to adapt to the shape of the colon as it travels forward. The balloon has a large predetermined maximal volume and thin wall (8 μm) that help it adapt to the shape of the colon in response to pressure exerted by the colonic walls. These features allow the balloon to maintain the seal with the colon wall, so that gas introduced on one side of the balloon does not escape to the other side. This allows a pressure gradient to develop from behind the balloon to in front of it during forward propulsion, and vice versa during withdrawal. Since the tip has no steering capabilities, an omnidirectional colonoscopic viewing system has been installed on the tip of the latest prototype[17]. The Aer-O-Scope™ is intended to be used for diagnostic purposes and therefore does not have a working channel for therapeutic instruments. In a preliminary pilot feasibility study[18], the Aer-O-Scope™ effectively intubated the cecum in 10 out of 12 subjects (83%) with an average time to cecum of 14 min ± 7 min.

Another approach, inspired by Geometer moths, is inchworm locomotion[19]. Inchworm locomotion is similar to the motion utilized in double balloon endoscopy where there is lengthening and shortening along two anchor points (distal anchor and proximal anchor). The Endotics™ system (Era Endoscopy s.r.l., Pisa, Italy) effectively utilized inchworm locomotion and consists of a disposable robotic probe as a head, a steerable tip, a flexible body (17 mm in diameter), a thin tail (7.5 mm in diameter), and a control box with an electro-pneumatic connector. The Endotics™ endoscopic device is represented in Figure 1C. The head hosts both a vision system, including camera, light emitting diode light source and channels for a water jet and air insufflation/suction. The operator can steer the head of the robotic colonoscope 180° in every direction, elongate the body of the probe in order to move it forward along the intestine, and can control rinsing, insufflation, and suction. A semiautomatic sequence of actions is implemented to move the probe like an inchworm, wherein two vacuum anchors located in the proximal and distal ends of the probe are sequentially actuated between extensions and retractions of the central body. Experiments in a phantom model with embedded force sensors demonstrated 90% lower force application than conventional colonoscopy[20]. A human study[21] with 71 unsedated patients demonstrated that this system has a comparable diagnostic accuracy to colonoscopy, and does not require sedation. The frequency of successful procedures (i.e., reaching the cecum), the total procedure time, and the need for sedation were recorded in this study. In 13 cases (18%), the device was unable to reach the cecum and the average procedure time was 45.1 ± 18.5 min for the Endotics™ system compared to 23.7 ± 7.2 min for traditional colonoscopy ($P < 0.0001$).

Currently, the lack of tissue interaction makes it improbable that diagnostic-only devices will completely replace traditional colonoscopes. Only prospective comparative outcome trials will be able to conclusively determine whether a diagnostic-only device (followed by conventional colonoscopy for potential therapeutic intervention) will be preferable to conventional colonoscopy with both diagnostic and therapeutic capabilities.

Interventional colonoscopes with alternative propulsion mechanisms

A first subset of this class of colonoscopes consists of shape retention devices, which are essentially tubes that

are initially flexible and can be stiffened when desired. While several examples of devices utilizing this principle are described in literature[10], only one has reached the clinical trial stage. This is the NeoGuide™ from NeoGuide Systems Inc. (a company based in San Jose, California, United States that was acquired in 2009 by Intuitive Surgical of Sunnyvale, CA, United States). The NeoGuide™, represented in Figure 1D, consists of a 173 cm-long endoscope composed of sixteen 8 cm-long independent vertebrae. Each segment can be directed to assume a right, left, up, down, circular curve, or a combination of these motions. During manual insertion of the device, the position and angle of the scope tip are encoded into a computer algorithm. As the colonoscope is advanced, the computer directs each successive vertebra to take the same shape that the tip had at a given insertion depth. The insertion tube thus changes its shape at different insertion depths in a "follow-the-leader" manner. By controlling the shape of the insertion tube, the NeoGuide™ does not rely on conventional pushing against the colon wall to maneuver. The cross-sectional diameter of the NeoGuide™ insertion tube is approximately 14 mm at the tip, increasing to approximately 20 mm at the proximal shaft of the scope (the working channel is 3.2 mm). The mechanical valves that control insufflation, suction, or water irrigation are the same as in conventional endoscopes. Biopsies and therapeutic maneuvers are conducted with the scope in passive mode; where the shape and stiffness of the endoscope is the same as that of a standard colonoscope. *In vitro* evaluation of the NeoGuide™ system[22] showed significantly less looping and lateral force required for advancement than procedures with a standard colonoscope. An initial clinical trial of 10 sedated patients demonstrated a looping rate of 40%. Although looping was defined as extensive in three of four cases, it was successfully straightened under computerized 3D imaging and the cecum was reached in all patients[11]. The 3D map images generated by the NeoGuide™ endoscopy system provide information regarding tip position, insertion tube position, and colonic looping[23]. These additional pieces of information may contribute to a safer and more comfortable procedure for the patient.

Another computer-aided colonoscope is the disposable Invendoscope™ (Invendo Medical GmbH, Kissing, Germany). The colonoscope, represented in Figure 1E, has a working length of 210 cm and the internal functional endoscope is covered by several layers, starting with a 10-mm diameter sheath. The sheath is covered by double layers of an "inverted sleeve" that provides the propulsion mechanism. Eight drive wheels in the driving unit grip the inner layer of the inverted sleeve and rotate, causing it to move forward. The "inverted sleeve" mechanism causes the colonoscope to "grow" at a position 10 cm below the distal end. Similarly, when the colonoscope is being retracted, the drive wheels rotate in the opposite direction and the endoscope "shrinks". This technology, combined with a small bending radius, was designed to reduce the forces exerted on the walls of the colon, with the goal of minimizing patient discomfort - even without sedation. Other than these mechanisms, the Invendoscope™ functions in a manner similar to conventional endoscopes, allowing for insufflation, rinsing, and suction. It also has a 3.2-mm working channel, allowing for therapeutic procedures to be performed.

A pilot study of 34 healthy volunteers (19 men; mean age 49.7 years) demonstrated that the Invendoscope was able to reach the cecum without any sedation in 80%-90% of cases[24]. This result was supported by a prospective single-arm study of 61 healthy volunteers (34 men and 27 women; mean age 57.5 years) undergoing screening colonoscopy, with a cecal intubation rate of 98.4% with less than 5% of patients requiring sedation[25].

The ColonoSight™, a disposable, self-propelling device based on IntraPull and ProtectiScope technologies by Stryker, Inc., has three working channels: a 3.7-mm-wide channel for suction and insertion of accessory tools, a channel for irrigation, and a channel for insufflation (Figure 1F). A disposable sleeve anchored at the proximal end of the device envelopes the endoscope, protecting it from contamination. The IntraPull mechanism generates a force close to the tip of the scope by pumping compressed air inside the sleeve controlled by depressing a foot pedal. The material of the sleeve does not allow expansion, and therefore the increased pressure inside the sleeve creates a force directed toward the tip of the colonoscope, thus pushing the tip forward as the folded part of the sleeve is deployed. The maximum force generated is 4.9 N (0.5 kgf) whereby the maximum force generated with a standard colonoscope is approximately 44 N (4.5 kgf). Once the endoscopist releases the foot pedal, the sleeve is deflated and the applied force is removed. A pilot prospective study of 178 participants (48 women and 130 men; age 51.8 ± 10.7 years) reported a success rate of 90% in reaching the cecum without complication of bleeding or perforation. All patients in the study were sedated using midazolam, mepiridine, or propofol; therefore, no data on patient discomfort was reported[26].

With the exception of the Endotics™ system, tip deflection of the above devices are accomplished by wires travelling the length of the device - effectively creating a minimum outer endoscope diameter (lower boundary) of approximately 10 mm. If however, one could "pull" rather than "push" the endoscope, the outer diameter lower boundary may be reduced further; limited by the space needed for the therapeutic channel and the electrical connections to the vision module[21]. Magnetic steering and control was applied to an endoscopic device containing a tip-mounted magnetic camera (diameter 11 mm, length 26 mm) connected to an external control box by a 5.4-mm diameter multi-lumen soft tether[27]. This connection was used for insufflation, passage of a therapeutic instrument, activation of a lens cleaning mechanism, and for operating the vision module. The external magnet was held by a 7 degree-of-freedom (DoF) robotic arm that was controlled in real-time by the endoscopist. The external magnet driving the endoscopic device is represented in Figure 1G.

A magnetic field sensor was also embedded in the device head to enable localization and closed loop control. The magnet capsule system allowed for "pulling" of the device throughout the colon thus eliminating the need for structural cables traveling the length of the device and the need for pushing the endoscope at the base in order to forward advance the scope. This approach would appear to prevent looping and reduce stretching of the colon wall. These advantageous characteristics are enhanced by dramatic reduction in both the bending stiffness of the shaft and in the mass of the proposed device (from 1240 g for a standard colonoscope to 34 g, including the soft tether), while retaining the therapeutic capabilities provided by a conventional colonoscope. The device has been tested in *ex-vivo* colon models and animal trials with encouraging results. Human trials are planned, but have not yet been conducted.

Another noteworthy mechanism for locomotion, designed to improve cecal intubation rate when standard colonoscopy fails, is the Spirus Endo-Ease™ system[28]. The Endo-Ease™ system, represented in Figure 1H, consists of a 90 cm disposable flexible plastic overtube with a 5-mm soft spiral thread at its tip. The overtube is designed with a 13-mm inner diameter to host a variable-stiffness pediatric colonoscope. Clockwise rotation of the overtube, which mimics the motion of a corkscrew, pleats the bowel onto the external surface of the tube. Bowel pleating is accomplished without apparent twisting of the lumen because the mesentery attachment resists the rotation. In a preliminarily clinical trial[29] of 22 patients (the median age 68 years, 58% men) with incomplete colonoscopy secondary to redundancy of their colon, the cecal intubation rate was reported in 22 patients (92%) with a median time to cecum of 14.2 min (range 6-30 min). No complications were reported.

The same principle was used in a computer-aided colonoscope[30] where two sections with a spiral thread rotate to achieve forward motion. While preliminary qualitative data about *in vivo* experience shows the feasibility of this approach (forward speed of 11 mm/min), clinical trials have not been reported to date.

A common theme in reducing discomfort associated with standard colonoscopy appears to be shifting the location of the propulsive force from the base of the device outside of the patient (i.e., "pushing", typical force profile available in literature[31]) to the tip of the colonoscope inside the patient (i.e., "pulling"). The latter can result in improved alignment of the direction of the applied force with the desired direction of forward tip motion. Several devices have demonstrated that this technique can reduce forces applied to the colon wall during insertion, which is believed to correlate with a reduction in patient discomfort and the risk of colon perforation. Diverse methods for reducing tip forces have been proposed, ranging from pneumatic pressure[22,25,26] to robotic locomotion[21] to magnetic fields[27]. While it remains to be seen which of these technologies will achieve market penetration and widespread clinical use; the thin tether of the last two devices described previously appears advantageous.

An alternative approach to minimize looping of the endoscope may lie in the endoscopists ability to "visualize" the shape of the endoscope as she or he advances it throughout the colon. A commercially available endoscopic system from Olympus, the ScopeGuide™ (CF-H180DL), integrates electromagnetic tracking into a standard colonoscope and provides a real-time rendering of the endoscope shape in free space on a display. A recent study[32] comparing this platform with standard colonoscopy reported reduced cecal intubation time (181 s *vs* 216 s; $P < 0.05$) and reduced patient discomfort on a 10-point visual analog scale (2.44 *vs* 1.85; $P < 0.05$). Unfortunately, other studies[33] of expert endoscopists utilizing this device have not reproduced these results; leading one to believe that the application of the system may be limited to novices.

In regard to reducing the learning curve, computer-aided devices or image guided techniques[18,21,23,25,27,32] have the potential to make colonoscopy less technically challenging for novices. Haptics has also been explored with promising results to facilitate colonoscopy[34]. In particular, lumen detection by real-time image analysis was used to provide a feedback force at the user interface that should aid the physician to steer the endoscope tip toward the lumen. Given the increasing role of technology in healthcare, one day it may be possible for examinations to be carried out remotely (i.e., patient and provider are not in the same physical location) by health care professionals in the community in order to meet the increased demand for screening colonoscopy.

Wireless capsule colonoscopy

Trends in consumer electronics such as miniaturization, low power operation, and wireless technologies have paved the way to the introduction of wireless capsule endoscopy (WCE) in 2000[35]. This technology has now become essential for small bowel inspection[36] and it is trying to expand its reach for use in the large intestine with purposely developed capsule devices, such as the PillCam™ Colon by Given Imaging (Yokneam, Israel). The effectiveness of the second generation of PillCam™ Colon[37] as an alternative to colonoscopy for CRC screening has been analyzed in a recent editorial[38] that appeared in this journal on the basis of recent clinical trials. Although the sensitivity for polyp detection for the PillCam™ Colon is close to 90%, specificity remains disappointingly low due to a substantial rate of false-positive results[39]. Therefore, despite clear patient comfort benefits provided by WCE, colonoscopy is still the gold-standard for CRC screening[40].

A main research stream for WCE toward the final goal of replacing flexible endoscopy consists in providing the capsule with the possibility to be remotely controlled and manipulated. Magnetic coupling is one of the few physical phenomena capable of transmitting actuation forces across a physical barrier. Remote magnetic ma-

nipulation has been adopted to steer capsule endoscopes by several research groups worldwide. Given imaging has investigated the use of a handheld external magnet to translate and orient a capsule in the upper GI tract using a modified version of PillCam™ Colon, which was half-filled with magnets[41]. This demonstrated the feasibility of magnetic steering, but revealed that more research was required to increase the reliability and accuracy of magnetic control[42]. An alternative technique for generating the external magnetic field, jointly developed by Olympus and Siemens, involved use of a magnetic resonance imaging scanner to create the field and field gradients[43]. In a recent nonrandomized blinded pilot study comparing a capsule device to traditional endoscopy for evaluation of the stomach, the overall diagnostic yield was similar for both methods[44].

A robotic navigation system - commonly used for cardiovascular clinical procedures (Niobe™, Stereotaxis, Inc, United States) - was used to control the orientation of a wireless capsule endoscope throughout the entire GI tract in several *ex-vivo* and *in-vivo* animal trials[45-47]. This technique was validated by equipping a PillCam™ with a custom-made coaxial magnetic shell[48] glued to its external surface. *In vivo* tests performed with 3D fluoroscopic localization demonstrated an accuracy of 1° in orientation[47]; however, the Niobe™ does not allow for field-gradient control, thus controlled translations of the capsule are not possible - this is an important limitation for this approach.

Magnetic control over position and orientation of a capsule inside the colon was demonstrated by coupling permanent magnets - one embedded inside the endoscopic capsule and the other connected as the end effector of a 6 DoF industrial robotic arm in an animal pilot study[49]. The same kind of magnetic coupling was used by the first ever reported wireless therapeutic endoscopic capsule[50]. This pilot study in a porcine model demonstrated the feasibility of wireless controlled deployment of a surgical clip to close an iatrogenic bleed in the colon. In a different study[51], the combined use of external static magnetic fields and internal actuation to move small embedded permanent magnets allowed for wirelessly controlled precise camera steering. This approach was used to obtain a full 360° view in the gastric cavity and a 45° span inside the colon.

Overall, this body of literature indicates that precise capsule manipulation can be achieved by means of magnetic coupling and that, if relevant clinical evidence will further support this approach, the next generation of wireless capsule endoscopes may eventually have a concrete potential to replace traditional colonoscopy, at least for CRC screening.

TOWARD MECHANISMS ENABLING UNPREPARED COLONOSCOPY

A significant common disadvantage of both conventional and novel colonoscopes is the need for bowel preparation. An innovative device addressing this challenge is the ClearPath™ system[52] that consists of a control cabinet and a disposable unit. The control cabinet includes a peristaltic pump, a controller, and a pinch valve that enables control of suction. The disposable element consists of a multi-lumen, custom-made, extruded tube. The tube has two channels: one that supplies water for irrigation and one that provides suction. When attached to the standard colonoscope, the ClearPath™ system adds an additional 6 mm to the outer diameter of the standard colonoscope. Water for irrigation flows through four 0.6 mm nozzles in the distal head and debris is evacuated through a single 18 mm^2 cross-sectional aperture. Preliminary animal trials on partially prepared domestic swine demonstrated effective intraprocedural colon cleaning with no immediate mucosal damage, acute complication (i.e., perforation), or delayed adverse consequence.

An alternative approach[53] for cleaning the colon during colonoscopy consists of a disposable soft-tipped catheter with a water jet spray that can be advanced, under direct vision, through the accessory channel of the standard colonoscope and into fecal matter. When the water jet is activated the solid stool can be broken apart for removal by suction. The water jet catheter tip has 4 radial nozzles through which the water is pumped. Feasibility studies in the unprepared colons of anesthetized pigs demonstrated effectiveness; however, mucosal trauma, bleeding, perforation, clogging of the colonoscope suction channel, and electrolyte imbalances may limit the overall impact.

A similar approach for intraprocedural bowel cleansing[54], still based on a disposable catheter, has been developed by Medjet Ltd., Tel Aviv, Israel. The MedJet digestive tract lumen cleaning device provides controlled delivery of a supersonic two-phase jet of micro-droplets consisting of minimal amounts of saline solution and CO_2. The solution is accelerated through the catheter, and enhances visibility by clearing away stool, secretions, or blood; and by disintegrating small particles for suctioning through the working channel of the endoscope during colonoscopy. In a recent clinical study[54], the MedJet was reported to be effective - offering significant improvement in bowel cleansing in 32 patients with no device-related adverse events.

In summary, there have been promising advances in the development of devices utilized for CRC screening with the devices designed to achieve a common goal to improve the way we directly view the colon (and possible intervene on any pathology present). The main principle that must be kept in mind with any new device is patient safety. Secondary principles that continue to be addressed include ease of use, reduction of the learning curve, improvement in colonic visualization, improvement in patient procedural comfort, better access to endoscopic screening, and possibly obviation of the need for a bowel preparation. With technology improving at a rapid pace, it may not be long before a disruptive innovation takes hold and we see the conventional colonoscope as an item on the shelf in a medical museum.

REFERENCES

1 **Ferlay J**, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; **127**: 2893-2917 [PMID: 21351269 DOI: 10.1002/ijc.25516]

2 **Howlader N,** Noone AM, Krapcho M, Neyman N, Aminou R, Altekruse SF, Kosary CL, Ruhl J, Tatalovich Z, Cho H, Mariotto A, Eisner MP, Lewis DR, Chen HS, Feuer EJ, Cronin KA, editors. SEER Cancer Statistics Review, 1975-2009 (Vintage 2009 Populations). Bethesda, MD: National Cancer Institute. Available from: ULR: http: //seer.cancer.gov/csr/1975_2009_pops09

3 **O'Callaghan T**. Introduction: The prevention agenda. *Nature* 2011; **471**: S2-S4 [PMID: 21430716 DOI: 10.1038/471S2a]

4 **Classen M,** Tytgat GNJ, Lightdale CJ. Gastroenterological Endoscop. Stuttgart, Germany: George Thieme Verlag, 2002

5 **Hopkins HH,** Kapany NS. A flexible fiberscope, using static scanning. *Nature* 1954; **173:** 39-41 [DOI: 10.1038/173039b0]

6 **Nelson DB**, McQuaid KR, Bond JH, Lieberman DA, Weiss DG, Johnston TK. Procedural success and complications of large-scale screening colonoscopy. *Gastrointest Endosc* 2002; **55**: 307-314 [PMID: 11868001 DOI: 10.1067/mge.2002.121883]

7 **Dafnis G**, Ekbom A, Pahlman L, Blomqvist P. Complications of diagnostic and therapeutic colonoscopy within a defined population in Sweden. *Gastrointest Endosc* 2001; **54**: 302-309 [PMID: 11522969 DOI: 10.1067/mge.2001.117545]

8 **Varadarajulu S**, Banerjee S, Barth BA, Desilets DJ, Kaul V, Kethu SR, Pedrosa MC, Pfau PR, Tokar JL, Wang A, Wong Kee Song LM, Rodriguez SA. GI endoscopes. *Gastrointest Endosc* 2011; **74**: 1-6.e6 [PMID: 21704803 DOI: 10.1016/j.gie.2011.01.061]

9 **Bujanda L**, Sarasqueta C, Zubiaurre L, Cosme A, Muñoz C, Sánchez A, Martín C, Tito L, Piñol V, Castells A, Llor X, Xicola RM, Pons E, Clofent J, de Castro ML, Cuquerella J, Medina E, Gutierrez A, Arenas JI, Jover R. Low adherence to colonoscopy in the screening of first-degree relatives of patients with colorectal cancer. *Gut* 2007; **56**: 1714-1718 [PMID: 17400596 DOI: 10.1136/gut.2007.120709]

10 **Loeve A**, Breedveld P, Dankelman J. Scopes too flexible...and too stiff. *IEEE Pulse* 2010; **1**: 26-41 [PMID: 21097368 DOI: 10.1109/MPUL.2010.939176]

11 **Eickhoff A**, van Dam J, Jakobs R, Kudis V, Hartmann D, Damian U, Weickert U, Schilling D, Riemann JF. Computer-assisted colonoscopy (the NeoGuide Endoscopy System): results of the first human clinical trial ("PACE study"). *Am J Gastroenterol* 2007; **102**: 261-266 [PMID: 17156149 DOI: 10.1111/j.1572-0241.2006.01002.x]

12 **Lee SH**, Chung IK, Kim SJ, Kim JO, Ko BM, Hwangbo Y, Kim WH, Park DH, Lee SK, Park CH, Baek IH, Park DI, Park SJ, Ji JS, Jang BI, Jeen YT, Shin JE, Byeon JS, Eun CS, Han DS. An adequate level of training for technical competence in screening and diagnostic colonoscopy: a prospective multicenter evaluation of the learning curve. *Gastrointest Endosc* 2008; **67**: 683-689 [PMID: 18279862 DOI: 10.1016/j.gie.2007.10.018]

13 **Obstein KL**, Patil VD, Jayender J, San José Estépar R, Spofford IS, Lengyel BI, Vosburgh KG, Thompson CC. Evaluation of colonoscopy technical skill levels by use of an objective kinematic-based system. *Gastrointest Endosc* 2011; **73**: 315-321 [PMID: 21111413 DOI: 10.1016/j.gie.2010.09.005]

14 **Fritscher-Ravens A**, Fox S, Swain CP, Milla P, Long G. Cath Cam guide wire-directed colonoscopy: first pilot study in patients with a previous incomplete colonoscopy. *Endoscopy* 2006; **38**: 209-213 [PMID: 16528644 DOI: 10.1055/s-2006-925138]

15 **Long G**, Fritscher-Ravens A, Mosse CA, Mills T, Swain P. The Cath-Cam: a new concept in colonoscopy. *Gastrointest Endosc* 2006; **64**: 997-1001 [PMID: 17140912 DOI: 10.1016/j.gie.2006.06.033]

16 **Pfeffer J**, Grinshpon R, Rex D, Levin B, Rösch T, Arber N, Halpern Z. The Aer-O-Scope: proof of the concept of a pneumatic, skill-independent, self-propelling, self-navigating colonoscope in a pig model. *Endoscopy* 2006; **38**: 144-148 [PMID: 16479421 DOI: 10.1055/s-2006-925089]

17 **Arber N**, Grinshpon R, Pfeffer J, Maor L, Bar-Meir S, Rex D. Proof-of-concept study of the Aer-O-Scope omnidirectional colonoscopic viewing system in ex vivo and in vivo porcine models. *Endoscopy* 2007; **39**: 412-417 [PMID: 17516347 DOI: 10.1055/s-2007-966452]

18 **Vucelic B**, Rex D, Pulanic R, Pfefer J, Hrstic I, Levin B, Halpern Z, Arber N. The aer-o-scope: proof of concept of a pneumatic, skill-independent, self-propelling, self-navigating colonoscope. *Gastroenterology* 2006; **130**: 672-677 [PMID: 16530508 DOI: 10.1053/j.gastro.2005.12.018]

19 **Phee L**, Accoto D, Menciassi A, Stefanini C, Carrozza MC, Dario P. Analysis and development of locomotion devices for the gastrointestinal tract. *IEEE Trans Biomed Eng* 2002; **49**: 613-616 [PMID: 12046707 DOI: 10.1109/TBME.2002.1001976]

20 **Cosentino F**, Tumino E, Passoni GR, Morandi E, Capria A. Functional evaluation of the endotics system, a new disposable self-propelled robotic colonoscope: in vitro tests and clinical trial. *Int J Artif Organs* 2009; **32**: 517-527 [PMID: 19844894]

21 **Tumino E**, Sacco R, Bertini M, Bertoni M, Parisi G, Capria A. Endotics system vs colonoscopy for the detection of polyps. *World J Gastroenterol* 2010; **16**: 5452-5456 [PMID: 21086563 DOI: 10.3748/wjg.v16.i43.5452]

22 **Eickhoff A,** Jakobs R, Kamal A, Mermash S, Riemann JF, van Dam J. In vitro evaluation of forces exerted by a new computer-assisted colonoscope (the NeoGuide Endoscopy System). *Endoscopy* 2006; **38:** 1224-1229 [PMID: 17163323 DOI: 10.1055/s−2006−945014]

23 **Striegel J**, Jakobs R, Van Dam J, Weickert U, Riemann JF, Eickhoff A. Determining scope position during colonoscopy without use of ionizing radiation or magnetic imaging: the enhanced mapping ability of the NeoGuide Endoscopy System. *Surg Endosc* 2011; **25**: 636-640 [PMID: 20730449]

24 **Rösch T**, Adler A, Pohl H, Wettschureck E, Koch M, Wiedenmann B, Hoepffner N. A motor-driven single-use colonoscope controlled with a hand-held device: a feasibility study in volunteers. *Gastrointest Endosc* 2008; **67**: 1139-1146 [PMID: 18355823 DOI: 10.1016/j.gie.2007.10.065]

25 **Groth S**, Rex DK, Rösch T, Hoepffner N. High cecal intubation rates with a new computer-assisted colonoscope: a feasibility study. *Am J Gastroenterol* 2011; **106**: 1075-1080 [PMID: 21386833 DOI: 10.1038/ajg.2011.52]

26 **Shike M**, Fireman Z, Eliakim R, Segol O, Sloyer A, Cohen LB, Goldfarb-Albak S, Repici A. Sightline ColonoSight system for a disposable, power-assisted, non-fiber-optic colonoscopy (with video). *Gastrointest Endosc* 2008; **68**: 701-710 [PMID: 18501356 DOI: 10.1016/j.gie.2007.12.062]

27 **Valdastri P**, Ciuti G, Verbeni A, Menciassi A, Dario P, Arezzo A, Morino M. Magnetic air capsule robotic system: proof of concept of a novel approach for painless colonoscopy. *Surg Endosc* 2012; **26**: 1238-1246 [PMID: 22179445 DOI: 10.1007/s00464-011-2054-x]

28 **Akerman PA**, Agrawal D, Chen W, Cantero D, Avila J, Pangtay J. Spiral enteroscopy: a novel method of enteroscopy by using the Endo-Ease Discovery SB overtube and a pediatric colonoscope. *Gastrointest Endosc* 2009; **69**: 327-332 [PMID: 19100974 DOI: 10.1016/j.gie.2008.07.042]

29 **Schembre DB**, Ross AS, Gluck MN, Brandabur JJ, McCormick SE, Lin OS. Spiral overtube-assisted colonoscopy after incomplete colonoscopy in the redundant colon. *Gastrointest Endosc* 2011; **73**: 515-519 [PMID: 21353848 DOI: 10.1016/j.gie.2010.11.047]

30 **Trovato G**, Shikanai M, Ukawa G, Kinoshita J, Murai N, Lee JW, Ishii H, Takanishi A, Tanoue K, Ieiri S, Konishi K, Hashizume M. Development of a colon endoscope robot

that adjusts its locomotion through the use of reinforcement learning. *Int J Comput Assist Radiol Surg* 2010; **5**: 317-325 [PMID: 20480247 DOI: 10.1007/s11548-010-0481-0]
31 **Dogramadzi S**, Virk GS, Bell GD, Rowland RS, Hancock J. Recording forces exerted on the bowel wall during colonoscopy: in vitro evaluation. *Int J Med Robot* 2005; **1**: 89-97 [PMID: 17518409 DOI: 10.1002/rcs.61]
32 **Szura M**, Bucki K, Matyja A, Kulig J. Evaluation of magne tic scope navigation in screening endoscopic examination of colorectal cancer. *Surg Endosc* 2012; **26**: 632-638 [PMID: 21959687 DOI: 10.1007/s00464-011-1930-8]
33 **Shah SG**, Thomas-Gibson S, Brooker JC, Suzuki N, Williams CB, Thapar C, Saunders BP. Use of video and magnetic endoscope imaging for rating competence at colonoscopy: validation of a measurement tool. *Gastrointest Endosc* 2002; **56**: 568-573 [PMID: 12297780 DOI: 10.1016/S0016-5107(02)70449-5]
34 **Reilink R**, Stramigioli S, Kappers AM, Misra S. Evaluation of flexible endoscope steering using haptic guidance. *Int J Med Robot* 2011; **7**: 178-186 [PMID: 21462290 DOI: 10.1002/rcs.386]
35 **Iddan G**, Meron G, Glukhovsky A, Swain P. Wireless capsule endoscopy. *Nature* 2000; **405**: 417 [PMID: 10839527 DOI: 10.1038/35013140]
36 **Ladas SD**, Triantafyllou K, Spada C, Riccioni ME, Rey JF, Niv Y, Delvaux M, de Franchis R, Costamagna G. European Society of Gastrointestinal Endoscopy (ESGE): recommendations (2009) on clinical use of video capsule endoscopy to investigate small-bowel, esophageal and colonic diseases. *Endoscopy* 2010; **42**: 220-227 [PMID: 20195992 DOI: 10.1055/s-0029-1243968]
37 **Eliakim R**, Yassin K, Niv Y, Metzger Y, Lachter J, Gal E, Sapoznikov B, Konikoff F, Leichtmann G, Fireman Z, Kopelman Y, Adler SN. Prospective multicenter performance evaluation of the second-generation colon capsule compared with colonoscopy. *Endoscopy* 2009; **41**: 1026-1031 [PMID: 19967618 DOI: 10.1055/s-0029-1215360]
38 **Riccioni ME**, Urgesi R, Cianci R, Bizzotto A, Spada C, Costamagna G. Colon capsule endoscopy: Advantages, limitations and expectations. Which novelties? *World J Gastrointest Endosc* 2012; **4**: 99-107 [PMID: 22523610 DOI: 10.4253/wjge.v4.i4.99]
39 **Spada C**, De Vincentis F, Cesaro P, Hassan C, Riccioni ME, Minelli Grazioli L, Bolivar S, Zurita A, Costamagna G. Accuracy and safety of second-generation PillCam COLON capsule for colorectal polyp detection. *Therap Adv Gastroenterol* 2012; **5**: 173-178 [PMID: 22570677 DOI: 10.1177/1756283X12438054]
40 **Spada C**, Hassan C, Galmiche JP, Neuhaus H, Dumonceau JM, Adler S, Epstein O, Gay G, Pennazio M, Rex DK, Benamouzig R, de Franchis R, Delvaux M, Devière J, Eliakim R, Fraser C, Hagenmuller F, Herrerias JM, Keuchel M, Macrae F, Munoz-Navas M, Ponchon T, Quintero E, Riccioni ME, Rondonotti E, Marmo R, Sung JJ, Tajiri H, Toth E, Triantafyllou K, Van Gossum A, Costamagna G. Colon capsule endoscopy: European Society of Gastrointestinal Endoscopy (ESGE) Guideline. *Endoscopy* 2012; **44**: 527-536 [PMID: 22389230 DOI: 10.1055/s-0031-1291717]
41 **Keller J**, Fibbe C, Volke F, Gerber J, Mosse AC, Reimann-Zawadzki M, Rabinovitz E, Layer P, Swain P. Remote magnetic control of a wireless capsule endoscope in the esophagus is safe and feasible: results of a randomized, clinical trial in healthy volunteers. *Gastrointest Endosc* 2010; **72**: 941-946 [PMID: 20855064 DOI: 10.1016/j.gie.2010.06.053]
42 **Keller J**, Fibbe C, Volke F, Gerber J, Mosse AC, Reimann-Zawadzki M, Rabinovitz E, Layer P, Schmitt D, Andresen V, Rosien U, Swain P. Inspection of the human stomach using remote-controlled capsule endoscopy: a feasibility study in healthy volunteers (with videos). *Gastrointest Endosc* 2011; **73**: 22-28 [PMID: 21067740 DOI: 10.1016/j.gie.2010.08.053]
43 **Rey JF**, Ogata H, Hosoe N, Ohtsuka K, Ogata N, Ikeda K, Aihara H, Pangtay I, Hibi T, Kudo S, Tajiri H. Feasibility of stomach exploration with a guided capsule endoscope. *Endoscopy* 2010; **42**: 541-545 [PMID: 20593331 DOI: 10.1055/s-0030-1255521]
44 **Rey JF**, Ogata H, Hosoe N, Ohtsuka K, Ogata N, Ikeda K, Aihara H, Pangtay I, Hibi T, Kudo SE, Tajiri H. Blinded nonrandomized comparative study of gastric examination with a magnetically guided capsule endoscope and standard videoendoscope. *Gastrointest Endosc* 2012; **75**: 373-381 [PMID: 22154417 DOI: 10.1016/j.gie.2011.09.030]
45 **Carpi F**, Galbiati S, Carpi A. Controlled navigation of endoscopic capsules: concept and preliminary experimental investigations. *IEEE Trans Biomed Eng* 2007; **54**: 2028-2036 [PMID: 18018698 DOI: 10.1109/TBME.2007.894729]
46 **Carpi F**, Pappone C. Magnetic maneuvering of endoscopic capsules by means of a robotic navigation system. *IEEE Trans Biomed Eng* 2009; **56**: 1482-1490 [PMID: 19174328 DOI: 10.1109/TBME.2009.2013336]
47 **Carpi F**, Kastelein N, Talcott M, Pappone C. Magnetically controllable gastrointestinal steering of video capsules. *IEEE Trans Biomed Eng* 2011; **58**: 231-234 [PMID: 20952324 DOI: 10.1109/TBME.2010.2087332]
48 **Carpi F**, Galbiati S, Carpi A. Magnetic shells for gastrointestinal endoscopic capsules as a means to control their motion. *Biomed Pharmacother* 2006; **60**: 370-374 [PMID: 16935464 DOI: 10.1016/j.biopha.2006.07.001]
49 **Ciuti G**, Donlin R, Valdastri P, Arezzo A, Menciassi A, Morino M, Dario P. Robotic versus manual control in magnetic steering of an endoscopic capsule. *Endoscopy* 2010; **42**: 148-152 [PMID: 20017088 DOI: 10.1055/s-0029-1243808]
50 **Valdastri P**, Quaglia C, Susilo E, Menciassi A, Dario P, Ho CN, Anhoeck G, Schurr MO. Wireless therapeutic endoscopic capsule: in vivo experiment. *Endoscopy* 2008; **40**: 979-982 [PMID: 19065478 DOI: 10.1055/s-0028-1103424]
51 **Valdastri P**, Quaglia C, Buselli E, Arezzo A, Di Lorenzo N, Morino M, Menciassi A, Dario P. A magnetic internal mechanism for precise orientation of the camera in wireless endoluminal applications. *Endoscopy* 2010; **42**: 481-486 [PMID: 20506065 DOI: 10.1055/s-0029-1244170]
52 **Moshkowitz M**, Hirsch Y, Carmel I, Duvdevany T, Fabian I, Willenz EP, Cohen J. A novel device for rapid cleaning of poorly prepared colons. *Endoscopy* 2010; **42**: 834-836 [PMID: 20886401 DOI: 10.1055/s-0030-1255777]
53 **Fritscher-Ravens A**, Mosse CA, Mills T, Ikeda K, Swain P. Colon cleaning during colonoscopy: a new mechanical cleaning device tested in a porcine model. *Gastrointest Endosc* 2006; **63**: 141-143 [PMID: 16377331 DOI: 10.1016/j.gie.2005.05.023]
54 **Kiesslich R**, Schuster N, Hoffman A, Goetz M, Galle PR, Santo E, Halpern Z. MedJet--a new CO2-based disposable cleaning device allows safe and effective bowel cleansing during colonoscopy: a pilot study. *Endoscopy* 2012; **44**: 767-771 [PMID: 22438188 DOI: 10.1055/s-0031-1291703]

P- Reviewers Bujanda L, Rodriguez DC, Cichoż-Lach H
S- Editor Lv S **L- Editor** A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i4.440

World J Gastroenterol 2013 January 28; 19(4): 440-444
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

MicroRNA-feedback loop as a key modulator of liver tumorigenesis and inflammation

Angélique Gougelet, Sabine Colnot

Angélique Gougelet, Sabine Colnot, Institut Cochin, INSERM, U1016, 75014 Paris, France
Angélique Gougelet, Sabine Colnot, Centre National de la Recherche Scientifique, UMR8104, 75014 Paris, France
Angélique Gougelet, Sabine Colnot, Université Paris Descartes, Sorbonne Paris Cité, 75013 Paris, France
Author contributions: Gougelet A wrote this paper and Colnot S drafted the final version of the manuscript.
Correspondence to: Angélique Gougelet, PhD, Institut Cochin, INSERM U1016, 24 rue du Faubourg Saint-Jacques, 75014 Paris, France. angelique.gougelet@inserm.fr
Telephone: +33-1-44412567 Fax: +33-1-44412421
Received: July 13, 2012 Revised: October 26, 2012
Accepted: December 15, 2012
Published online: January 28, 2013

Abstract

A recent work of Iliopoulos *et al* published in *Cell* highlighted a circuit orchestrated by microRNAs (miRNAs) that results in liver tumorigenesis and inflammation. This feedback loop, governed by miR-24 and miR-629, promotes a hepatocyte nuclear factor-4α transient inhibition resulting in miR-124 induction and signal transducer and activator of transcription 3 activation. These promising data support the use of miRNA mimics or inhibitors as potent therapeutic approaches in liver cancer.

© 2013 Baishideng. All rights reserved.

Key words: Hepatocyte nuclear factor-4α; MicroRNA; Interleukin-6; Signal transducer and activator of transcription 3; Hepatocellular oncogenesis

Gougelet A, Colnot S. MicroRNA-feedback loop as a key modulator of liver tumorigenesis and inflammation. *World J Gastroenterol* 2013; 19(4): 440-444 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i4/440.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i4.440

COMMENTARY ON HOT TOPICS

Hepatocyte nuclear factor-4α (HNF-4α) belongs to the nuclear receptor superfamily of ligand dependent transcription factors NR2A1[1]. It is expressed in the kidney, intestine and pancreas. HNF-4α is most highly expressed in the liver[2-5], where it binds to the promoter of 12% of genes expressed in the adult liver[6]. It plays a key role in liver development during gastrulation[7] and orchestrates the parenchyma formation in the adult liver[8]. It also guides hepatoblasts to hepatocyte differentiation[9]. The conditional disruption of HNF-4α in mouse liver showed that HNF-4α regulates a number of genes involved in lipid, glucose, amino acid and xenobiotic metabolisms[4]. Related to its central role in liver physiology, HNF-4α has been reported to be involved in liver oncogenesis; however, its role is still controversial. A study by Xu *et al*[10] suggested that HNF-4α expression was increased in human hepatocarcinoma (HCC), which contrasted with other studies that reported that the HNF-4α level was decreased in HCC[11-13]. HNF-4α has also been associated with cancer and inflammation in the colon: its intestinal disruption in mouse revealed that HNF-4α protects against colitis and inflammatory disease[14,15] and promotes tumorigenesis[15]. Liver cancer is a clear example of an inflammation-related cancer[16-19]; therefore, it does not seem surprising that HNF-4α could also orchestrate an inflammatory program in the liver, as was shown last year by Wang *et al*[20] in experiments performed on hepatocarcinoma cells exposed to interleukin (IL)-6.

In a recent paper published in *Cell*, D. Iliopoulos' Laboratory aimed to decipher the epigenetic circuit centered on HNF-4α, which promotes liver oncogenesis[21]. They particularly focused on microRNAs (miRNAs), which are small RNAs produced from non-coding DNA regions that control gene expression by binding to the 3' untranslated region (3' UTR) of target messenger RNAs (mRNAs). miRNA binding results in the degradation of the mRNA and/or the repression of its translation[22].

Since their discovery in 1994 in *Caenorhabditis elegans*[23], miRNAs have become an expanding area of research, and evidence supports the view that miRNAs are key regulators of many physiological processes, e.g., growth, proliferation and differentiation[24-27]. They are also implicated in the initiation and progression of various cancers[28-30]. Concerning HCC, microtranscriptomic analyses revealed a miRNA signature of liver cancer, i.e., miR-122 loss[31] or miR-221 upregulation[32], and a number of studies support the view that miRNAs direct HCC progression through the induction of cell proliferation or metastasis and the suppression of apoptosis[33]. Taken together, these data highlight miRNAs as promising diagnostic and therapeutic targets for HCC.

The work of Maria Hatziapostolou is a robust description of the molecular factors underlying HNF-4α loss-mediated hepatocarcinoma based on *in vitro* and *in vivo* experiments and confirmed on HCC human samples. Prior to the precise deciphering of hepatocarcinoma molecular origins, the authors confirmed that the silencing of HNF-4α was sufficient to increase cell colony formation and invasion of nontransformed immortalized human hepatocytes (IMH) and human liver cancer cell lines. On their own, the injection of IMH cells with a preliminary transient inhibition of HNF-4α in immunosuppressed mice resulted in tumor apparition. Interestingly, HNF-4α was maintained at a low level 55 d after cell xenografts, suggesting that transient silencing of HNF-4α initiated a feedback loop that stably maintained cell transformation and suppressed its own expression.

What are the factors involved in HNF-4α silencing? An miRNA screening was performed on a luciferase reporter gene containing the 3' UTR region of HNF-4α, which revealed that miR-24 and, to a lesser extent, miR-629, were HNF-4α repressors. The transient expression of both miRNAs, the equivalent of HNF-4α silencing, transformed IMH cells, increased their invasiveness *in vitro* and promoted the appearance of tumors in mice. Strikingly, both miRNAs were induced following HNF-4α silencing, supporting the involvement of these miRNAs in the HNF-4α- dependent feedback loop.

Interestingly, miR-24 and miR-629 possess a binding motif for signal transducer and activator of transcription 3 (STAT3) in their promoters, a well-known effector of IL-6-dependent cancer inflammation[34] (for a review), particularly in liver cancer[35] (for a review). In brief, the binding of IL-6 to its receptor, IL-6R, activates the Janus kinase family, which phosphorylates STAT3 on tyrosine residues. STAT3 could then form homodimers before translocation into the nucleus to promote the expression of an inflammatory program[36] (for a review). Here, the authors showed that in response to IL-6, STAT3 binding to miR-24 and miR-629 promoters increased, resulting in miRNA expression. In turn, miR-24 expression and HNF-4α silencing enhanced the phosphorylation status of STAT3, and thus its activation. STAT3 and IL-6 are two other members of the HNF-4α feedback circuit promoting liver oncogenesis; an antibody against IL-6 blocked all the HNF-4α silencing effects that they observed.

The next step consisted in screening of miRNAs possessing an HNF-4α binding site, which identified miR-124 as a preferential HNF-4α target. miR-124 was, in fact, an integral part of the feedback loop, because its inhibition by an antisense as-miR-124, or following HNF-4α silencing, enhanced STAT3 phosphorylation coupled to an induction of IL-6 secretion and expression of IL-6R, a direct target of miR-124.

The relevance of the loop miR-24/miR-629/HNF-4α/miR-124/STAT3 was confirmed *in vivo* with a mouse model of HCC induced by diethylnitrosamine. During tumor progression, the HNF-4α level was initially dropped off after four weeks, followed by miR-124 repression and inversely by miR-24 and IL-6R induction, which supports the essential role of the HNF-4α loop in tumor progression. One of the important finding of this study was that an injection of miR-124-mimic encapsulated into liposomes in this model was sufficient to limit tumor growth, even if this mimic was administered four weeks before analysis. miR-124 mimics were also able to prevent tumor development. Taken together, these results argue in favor of miR-124 delivery as a potent strategy for treating or preventing HCC. Although, to date, the efficacy of miRNA mimics have not been demonstrated *in vivo*, this approach could be attractive. The prerequisite for this approach is that the complementary passenger RNA in the double stranded miRNA mimic does not create a new miRNA that could induce off-target effects[37,38].

The second exciting idea from this study relies upon the need for an inflammatory context to optimally disrupt the HNF-4α epigenetic circuit and promote tumorigenesis. If the tumors developed in a mouse model lacked STAT-3 in the liver, the tumors were smaller when the HNF-4α loop was less inhibited. This supports the hypothesis that HNF-4α loss engages an anti-tumorigenic program and a STAT3-dependent anti-inflammatory program, both orchestrated by the trio miR-24/miR-629/miR-124, to induce hepatocyte transformation. Such an epigenetic loop has already been described by the same authors for breast cancers, in which cell transformation was dictated by an epigenetic switch achieved by IL-6, nuclear factor-κB and the miRNA let-7[39].

Finally, the authors demonstrated the relevance of their model for human liver oncogenesis: more than 50% of HCC samples from patients showed a loss of HNF-4α and of miR-124, accompanied by an increase in miR-24 and IL-6R expression; all these parameters were associated with a higher level of phospho-STAT3 status in relation with IL-6 and IL-6R expression. As a confirmation of the HNF-4α circuit's key role in HCC progression, the authors showed that the activity of the HNF-4α circuit becomes more deregulated as the HCC grade increased.

In summary, this work strongly argues for a tumor suppressor role of HNF-4α in liver, as was already sh-

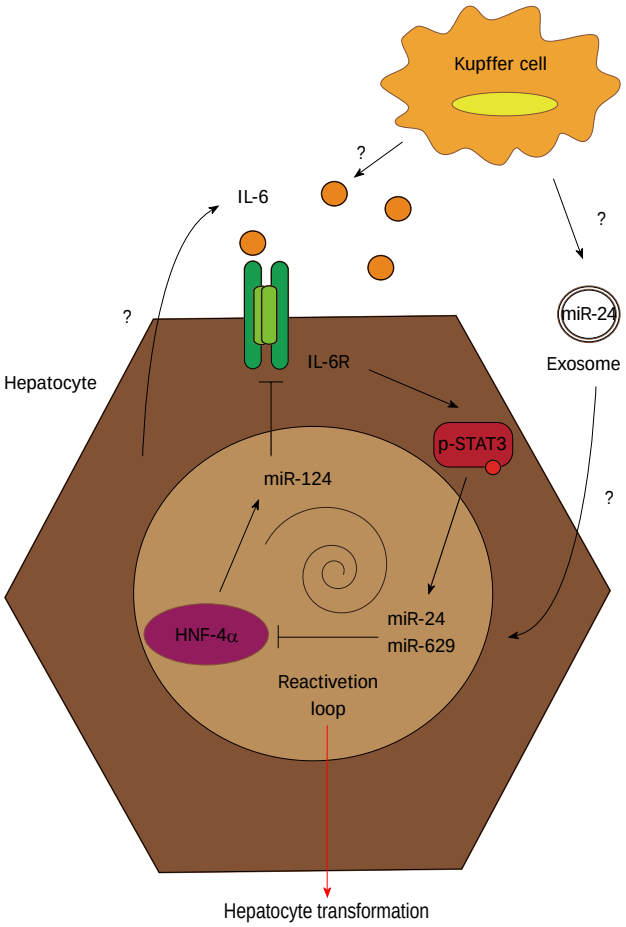


Figure 1 From transient hepatocyte nuclear factor-4α inhibition to stable hepatocyte transformation. The transient inhibition of hepatocyte nuclear factor-4α (HNF-4α) following miR-24 and miR-629 induction promotes stable repression of the pro-tumorigenic HNF-4α feedback circuit. This results in miR-124 inhibition, followed by an increase in interleukin (IL)-6 secretion and IL-6R expression. These two events lead to hyperphosphorylation of signal transducer and activator of transcription 3 (STAT3), which auto-amplifies the process through the re-induction of miR-24 and miR-629.

own[12], and supports the key role of this transcription factor in limiting the tumor inflammatory environment by inhibiting the IL-6/STAT3 pathway. The authors described the interconnection between the trio miR-24/miR-629/miR-124 and HNF-4α as a key regulator of HCC progression (Figure 1). In 2010, Takagi *et al*[40] described a crosstalk between HNF-4α and miR-24 in a liver metabolic loop for the control of bile acid synthesis. In brief, bile acids induce reactive oxygen species generation, resulting in the activation of the mitogen-activated protein (MAP) kinase pathway. In turn, miR-24, induced by the MAP kinases, downregulates HNF-4α, and, thus, the expression of bile acid-synthesizing enzymes. Hepatitis B virus (HBV) and alcohol abuse, two etiological agents associated with HCC development, contribute to oxidative stress in the liver. In consequence, miR-24 inhibition and, thus, reactivation of the HNF-4α feedback circuit in HCC could also be performed using antioxidants, a class of therapeutic agents currently used for HBV patients[41].

The take-home message of this study is that a transient signal at any level of the feedback circuit (HNF-4α or miR-124 silencing, miR-24 overexpression or IL-6 secretion) stably induces hepatocyte transformation, which could be auto-amplified by this feedback loop, and lead to chronic inflammation, predisposing a patient to liver cancer development and immune escape[19]. The activation of the IL-6/STAT3 axis is, at least in part, caused by a hepatocyte source, as shown by the *in vitro* experiments, but this could also be boosted by IL-6 secretion by non-parenchymal liver cells like Kupffer cells. Another possible scenario for the disruption of HNF-4α circuit by tumor environment could be the secretion of exosomes containing miRNA, in particular miR-24, by immune cells. Exosomes are small extracellular vesicles derived from the multivesicular body-sorting pathway, which are produced by various cells like epithelial cells, immune cells and tumor cells. Recently, it has been shown that some miRNAs, particularly those deregulated in cancer, are encapsulated into exosomes and play a role as cell-cell mediators[41]. In particular, miR-24 has been detected in exosomal structures, and a similar mechanism might exist in liver, permitting miR-24 to be delivered to hepatocytes by neighboring cells.

The most promising result of this study was that intravenous delivery of miR-124 mimic encapsulated into liposomes was able to prevent and reduce the growth of liver cancer. To date, few works have demonstrated the potency of an miRNA-based anticancer approach. However, the great advantage of HCC is that mimics or inhibitors against miRNAs are preferentially distributed to the liver[42]. For that reason, the first miRNA-based therapy authorized in clinical trials is the anti-miR-122 compound Miravirsen, indicated for the treatment of hepatitis C virus (HCV), which uses miR-122 in hepatocytes for its replication. This clinical trial follows promising results obtained in primates infected with HCV and treated with an anti-miR-122[43], which was well-tolerated[44]. Concerning the HNF-4α circuit, the authors showed the efficiency of a mimic of miR-124 to perturb HCC development, but we could also conceive the injection of inhibitors against miR-24 and miR-629 to avoid HNF-4α loss. Even if miRNA inhibitor delivery appeared as a promising therapeutic approach, the use of these modulators poses a number of challenges. The question of treatment specificity could be raised. In fact, an miRNA could target multiple mRNAs[45,46], and, thus, many cellular pathways, pro- or anti-tumorigenic. It could be difficult to restrict the effect of one miRNA to only one target of interest. However, this notion could be generalized to many other treatments currently used, such as proteasome[47] or histone deacetylase inhibitors[48], which have demonstrated efficacy. Another major disadvantage could be the inhibition of a non-identified target. Their delivery could also be an important challenge, although many improvements have appeared recently, with chemical modifications limiting their degradation by nucleases[49-51]. The other problem is that these inhibitors preferentially target the liver and other tissues are more difficult to enter[42]. Finally, these inhibitors could also

be toxic[52,53]. As suggested by the data of Hatziapostolou, mimics or inhibitors could be delivered in a free formulation, or protected into liposomes or even into exosomes, as was shown for siRNAs[54]. In conclusion, miR-124-based treatment could be a therapeutic strategy of choice for HCC, as an alternative to the anti-STAT3 approach[55]. This therapeutic option has the great advantage of targeting the inflammatory, as well as the metabolic initiators, of HCC, and its efficiency demonstrated in this work argue for an epigenetic switch as the major event underlying cancer initiation.

REFERENCES

1 **Sladek R**, Giguère V. Orphan nuclear receptors: an emerging family of metabolic regulators. *Adv Pharmacol* 2000; **47**: 23-87 [PMID: 10582084]

2 **Garrison WD**, Battle MA, Yang C, Kaestner KH, Sladek FM, Duncan SA. Hepatocyte nuclear factor 4alpha is essential for embryonic development of the mouse colon. *Gastroenterology* 2006; **130**: 1207-1220 [PMID: 16618389 DOI: 10.1053/j.gastro.2006.01.003]

3 **Gupta RK**, Gao N, Gorski RK, White P, Hardy OT, Rafiq K, Brestelli JE, Chen G, Stoeckert CJ, Kaestner KH. Expansion of adult beta-cell mass in response to increased metabolic demand is dependent on HNF-4alpha. *Genes Dev* 2007; **21**: 756-769 [PMID: 17403778 DOI: 10.1101/gad.1535507]

4 **Hayhurst GP**, Lee YH, Lambert G, Ward JM, Gonzalez FJ. Hepatocyte nuclear factor 4alpha (nuclear receptor 2A1) is essential for maintenance of hepatic gene expression and lipid homeostasis. *Mol Cell Biol* 2001; **21**: 1393-1403 [PMID: 11158324 DOI: 10.1128/MCB.21.4.1393-1403.2001]

5 **Stegmann A**, Hansen M, Wang Y, Larsen JB, Lund LR, Ritié L, Nicholson JK, Quistorff B, Simon-Assmann P, Troelsen JT, Olsen J. Metabolome, transcriptome, and bioinformatic cis-element analyses point to HNF-4 as a central regulator of gene expression during enterocyte differentiation. *Physiol Genomics* 2006; **27**: 141-155 [PMID: 16868071 DOI: 10.1152/physiolgenomics.00314.2005]

6 **Odom DT**, Zizlsperger N, Gordon DB, Bell GW, Rinaldi NJ, Murray HL, Volkert TL, Schreiber J, Rolfe PA, Gifford DK, Fraenkel E, Bell GI, Young RA. Control of pancreas and liver gene expression by HNF transcription factors. *Science* 2004; **303**: 1378-1381 [PMID: 14988562 DOI: 10.1126/science.1089769]

7 **Chen WS**, Manova K, Weinstein DC, Duncan SA, Plump AS, Prezioso VR, Bachvarova RF, Darnell JE. Disruption of the HNF-4 gene, expressed in visceral endoderm, leads to cell death in embryonic ectoderm and impaired gastrulation of mouse embryos. *Genes Dev* 1994; **8**: 2466-2477 [PMID: 7958910]

8 **Parviz F**, Matullo C, Garrison WD, Savatski L, Adamson JW, Ning G, Kaestner KH, Rossi JM, Zaret KS, Duncan SA. Hepatocyte nuclear factor 4alpha controls the development of a hepatic epithelium and liver morphogenesis. *Nat Genet* 2003; **34**: 292-296 [PMID: 12808453 DOI: 10.1038/ng1175]

9 **Kuo CJ**, Conley PB, Chen L, Sladek FM, Darnell JE, Crabtree GR. A transcriptional hierarchy involved in mammalian cell-type specification. *Nature* 1992; **355**: 457-461 [PMID: 1734282 DOI: 10.1038/355457a0]

10 **Xu L**, Hui L, Wang S, Gong J, Jin Y, Wang Y, Ji Y, Wu X, Han Z, Hu G. Expression profiling suggested a regulatory role of liver-enriched transcription factors in human hepatocellular carcinoma. *Cancer Res* 2001; **61**: 3176-3181 [PMID: 11306505]

11 **Lazarevich NL**, Fleishman DI. Tissue-specific transcription factors in progression of epithelial tumors. *Biochemistry (Mosc)* 2008; **73**: 573-591 [PMID: 18605982]

12 **Ning BF**, Ding J, Yin C, Zhong W, Wu K, Zeng X, Yang W, Chen YX, Zhang JP, Zhang X, Wang HY, Xie WF. Hepatocyte nuclear factor 4 alpha suppresses the development of hepatocellular carcinoma. *Cancer Res* 2010; **70**: 7640-7651 [PMID: 20876809 DOI: 10.1158/0008-5472.CAN-10-0824]

13 **Yin C**, Lin Y, Zhang X, Chen YX, Zeng X, Yue HY, Hou JL, Deng X, Zhang JP, Han ZG, Xie WF. Differentiation therapy of hepatocellular carcinoma in mice with recombinant adenovirus carrying hepatocyte nuclear factor-4alpha gene. *Hepatology* 2008; **48**: 1528-1539 [PMID: 18925631 DOI: 10.1002/hep.22510]

14 **Ahn SH**, Shah YM, Inoue J, Morimura K, Kim I, Yim S, Lambert G, Kurotani R, Nagashima K, Gonzalez FJ, Inoue Y. Hepatocyte nuclear factor 4alpha in the intestinal epithelial cells protects against inflammatory bowel disease. *Inflamm Bowel Dis* 2008; **14**: 908-920 [PMID: 18338782 DOI: 10.1002/ibd.20413]

15 **Darsigny M**, Babeu JP, Seidman EG, Gendron FP, Levy E, Carrier J, Perreault N, Boudreau F. Hepatocyte nuclear factor-4alpha promotes gut neoplasia in mice and protects against the production of reactive oxygen species. *Cancer Res* 2010; **70**: 9423-9433 [PMID: 21062980 DOI: 10.1158/0008-5472.CAN-10-1697]

16 **Anson M**, Crain-Denoyelle AM, Baud V, Chereau F, Gougelet A, Terris B, Yamagoe S, Colnot S, Viguier M, Perret C, Couty JP. Oncogenic β-catenin triggers an inflammatory response that determines the aggressiveness of hepatocellular carcinoma in mice. *J Clin Invest* 2012; **122**: 586-599 [PMID: 22251704 DOI: 10.1172/JCI43937]

17 **Luedde T**, Beraza N, Kotsikoris V, van Loo G, Nenci A, De Vos R, Roskams T, Trautwein C, Pasparakis M. Deletion of NEMO/IKKgamma in liver parenchymal cells causes steatohepatitis and hepatocellular carcinoma. *Cancer Cell* 2007; **11**: 119-132 [PMID: 17292824 DOI: 10.1016/j.ccr.2006.12.016]

18 **Maeda S**, Kamata H, Luo JL, Leffert H, Karin M. IKKbeta couples hepatocyte death to cytokine-driven compensatory proliferation that promotes chemical hepatocarcinogenesis. *Cell* 2005; **121**: 977-990 [PMID: 15989949 DOI: 10.1016/j.cell.2005.04.014]

19 **Berasain C**, Castillo J, Perugorria MJ, Latasa MU, Prieto J, Avila MA. Inflammation and liver cancer: new molecular links . *Ann NY Acad Sci* 2009; **1155**: 206-221 [PMID: 19250206 DOI: 10.1111/j.1749-6632.2009.03704.x]

20 **Wang Z**, Bishop EP, Burke PA. Expression profile analysis of the inflammatory response regulated by hepatocyte nuclear factor 4α. *BMC Genomics* 2011; **12**: 128 [PMID: 21352552 DOI: 10.1186/1471-2164-12-128]

21 **Hatziapostolou M**, Polytarchou C, Aggelidou E, Drakaki A, Poultsides GA, Jaeger SA, Ogata H, Karin M, Struhl K, Hadzopoulou-Cladaras M, Iliopoulos D. An HNF4α-miRNA inflammatory feedback circuit regulates hepatocellular oncogenesis. *Cell* 2011; **147**: 1233-1247 [PMID: 22153071 DOI: 10.1016/j.cell.2011.10.043]

22 **Pillai RS**, Bhattacharyya SN, Filipowicz W. Repression of protein synthesis by miRNAs: how many mechanisms? *Trends Cell Biol* 2007; **17**: 118-126 [PMID: 17197185 DOI: 10.1016/j.tcb.2006.12.007]

23 **Lee RC**, Feinbaum RL, Ambros V. The C. elegans heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. *Cell* 1993; **75**: 843-854 [PMID: 8252621]

24 **He L**, Hannon GJ. MicroRNAs: small RNAs with a big role in gene regulation. *Nat Rev Genet* 2004; **5**: 522-531 [PMID: 15211354 DOI: 10.1038/nrg1379]

25 **Bushati N**, Cohen SM. microRNA functions. *Annu Rev Cell Dev Biol* 2007; **23**: 175-205 [PMID: 17506695 DOI: 10.1146/annurev.cellbio.23.090506.123406]

26 **Hwang HW**, Mendell JT. MicroRNAs in cell proliferation, cell death, and tumorigenesis. *Br J Cancer* 2006; **94**: 776-780 [PMID: 16495913 DOI: 10.1038/sj.bjc.6603023]

27 **Stefani G**, Slack FJ. Small non-coding RNAs in animal development. *Nat Rev Mol Cell Biol* 2008; **9**: 219-230 [PMID: 18270516 DOI: 10.1038/nrm2347]

28 **Calin GA**, Croce CM. MicroRNA signatures in human cancers. *Nat Rev Cancer* 2006; **6**: 857-866 [PMID: 17060945 DOI: 10.1038/nrc1997]

29 **Deng S**, Calin GA, Croce CM, Coukos G, Zhang L. Mechanisms of microRNA deregulation in human cancer. *Cell Cycle* 2008; **7**: 2643-2646 [PMID: 18719391]

30 **Lu J**, Getz G, Miska EA, Alvarez-Saavedra E, Lamb J, Peck D, Sweet-Cordero A, Ebert BL, Mak RH, Ferrando AA, Downing JR, Jacks T, Horvitz HR, Golub TR. MicroRNA expression profiles classify human cancers. *Nature* 2005; **435**: 834-838 [PMID: 15944708 DOI: 10.1038/nature03702]

31 **Coulouarn C**, Factor VM, Andersen JB, Durkin ME, Thorgeirsson SS. Loss of miR-122 expression in liver cancer correlates with suppression of the hepatic phenotype and gain of metastatic properties. *Oncogene* 2009; **28**: 3526-3536 [PMID: 19617899 DOI: 10.1038/onc.2009.211]

32 **Pineau P**, Volinia S, McJunkin K, Marchio A, Battiston C, Terris B, Mazzaferro V, Lowe SW, Croce CM, Dejean A. miR-221 overexpression contributes to liver tumorigenesis. *Proc Natl Acad Sci USA* 2010; **107**: 264-269 [PMID: 20018759 DOI: 10.1073/pnas.0907904107]

33 **Huang S**, He X. The role of microRNAs in liver cancer progression. *Br J Cancer* 2011; **104**: 235-240 [PMID: 21102580 DOI: 10.1038/sj.bjc.6606010]

34 **Yu H**, Pardoll D, Jove R. STATs in cancer inflammation and immunity: a leading role for STAT3. *Nat Rev Cancer* 2009; **9**: 798-809 [PMID: 19851315 DOI: 10.1038/nrc2734]

35 **He G**, Karin M. NF-κB and STAT3 - key players in liver inflammation and cancer. *Cell Res* 2011; **21**: 159-168 [PMID: 21187858 DOI: 10.1038/cr.2010.183]

36 **Heinrich PC**, Behrmann I, Haan S, Hermanns HM, Müller-Newen G, Schaper F. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. *Biochem J* 2003; **374**: 1-20 [PMID: 12773095 DOI: 10.1042/BJ20030407]

37 **van Rooij E**, Marshall WS, Olson EN. Toward microRNA-based therapeutics for heart disease: the sense in antisense. *Circ Res* 2008; **103**: 919-928 [PMID: 18948630 DOI: 10.1161/CIRCRESAHA.108.183426]

38 **Singh S**, Narang AS, Mahato RI. Subcellular fate and off-target effects of siRNA, shRNA, and miRNA. *Pharm Res* 2011; **28**: 2996-3015 [PMID: 22033880 DOI: 10.1007/s11095-011-0608-1]

39 **Iliopoulos D**, Hirsch HA, Struhl K. An epigenetic switch involving NF-kappaB, Lin28, Let-7 MicroRNA, and IL6 links inflammation to cell transformation. *Cell* 2009; **139**: 693-706 [PMID: 19878981 DOI: 10.1016/j.cell.2009.10.014]

40 **Takagi S**, Nakajima M, Kida K, Yamaura Y, Fukami T, Yokoi T. MicroRNAs regulate human hepatocyte nuclear factor 4alpha, modulating the expression of metabolic enzymes and cell cycle. *J Biol Chem* 2010; **285**: 4415-4422 [PMID: 20018894 DOI: 10.1074/jbc.M109.085431]

41 **Ha HL**, Shin HJ, Feitelson MA, Yu DY. Oxidative stress and antioxidants in hepatic pathogenesis. *World J Gastroenterol* 2010; **16**: 6035-6043 [PMID: 21182217]

42 **Roberts J**, Palma E, Sazani P, Ørum H, Cho M, Kole R. Efficient and persistent splice switching by systemically delivered LNA oligonucleotides in mice. *Mol Ther* 2006; **14**: 471-475 [PMID: 16854630 DOI: 10.1016/j.ymthe.2006.05.017]

43 **Lanford RE**, Hildebrandt-Eriksen ES, Petri A, Persson R, Lindow M, Munk ME, Kauppinen S, Ørum H. Therapeutic silencing of microRNA-122 in primates with chronic hepatitis C virus infection. *Science* 2010; **327**: 198-201 [PMID: 19965718 DOI: 10.1126/science.1178178]

44 **Hildebrandt-Eriksen ES**, Aarup V, Persson R, Hansen HF, Munk ME, Ørum H. A locked nucleic acid oligonucleotide targeting microRNA 122 is well-tolerated in cynomolgus monkeys. *Nucleic Acid Ther* 2012; **22**: 152-161 [PMID: 22545703 DOI: 10.1089/nat.2011.0332]

45 **Baek D**, Villén J, Shin C, Camargo FD, Gygi SP, Bartel DP. The impact of microRNAs on protein output. *Nature* 2008; **455**: 64-71 [PMID: 18668037 DOI: 10.1038/nature07242]

46 **Selbach M**, Schwanhäusser B, Thierfelder N, Fang Z, Khanin R, Rajewsky N. Widespread changes in protein synthesis induced by microRNAs. *Nature* 2008; **455**: 58-63 [PMID: 18668040 DOI: 10.1038/nature07228]

47 **Kuhn DJ**, Hunsucker SA, Chen Q, Voorhees PM, Orlowski M, Orlowski RZ. Targeted inhibition of the immunoproteasome is a potent strategy against models of multiple myeloma that overcomes resistance to conventional drugs and nonspecific proteasome inhibitors. *Blood* 2009; **113**: 4667-4676 [PMID: 19050304 DOI: 10.1182/blood-2008-07-171637]

48 **Delcuve GP**, Khan DH, Davie JR. Roles of histone deacetylases in epigenetic regulation: emerging paradigms from studies with inhibitors. *Clin Epigenetics* 2012; **4**: 5 [PMID: 22414492 DOI: 10.1186/1868-7083-4-5]

49 **Akinc A**, Zumbuehl A, Goldberg M, Leshchiner ES, Busini V, Hossain N, Bacallado SA, Nguyen DN, Fuller J, Alvarez R, Borodovsky A, Borland T, Constien R, de Fougerolles A, Dorkin JR, Narayanannair Jayaprakash K, Jayaraman M, John M, Koteliansky V, Manoharan M, Nechev L, Qin J, Racie T, Raitcheva D, Rajeev KG, Sah DW, Soutschek J, Toudjarska I, Vornlocher HP, Zimmermann TS, Langer R, Anderson DG. A combinatorial library of lipid-like materials for delivery of RNAi therapeutics. *Nat Biotechnol* 2008; **26**: 561-569 [PMID: 18438401 DOI: 10.1038/nbt1402]

50 **Soutschek J**, Akinc A, Bramlage B, Charisse K, Constien R, Donoghue M, Elbashir S, Geick A, Hadwiger P, Harborth J, John M, Kesavan V, Lavine G, Pandey RK, Racie T, Rajeev KG, Röhl I, Toudjarska I, Wang G, Wuschko S, Bumcrot D, Koteliansky V, Limmer S, Manoharan M, Vornlocher HP. Therapeutic silencing of an endogenous gene by systemic administration of modified siRNAs. *Nature* 2004; **432**: 173-178 [PMID: 15538359 DOI: 10.1038/nature03121]

51 **Stenvang J**, Petri A, Lindow M, Obad S, Kauppinen S. Inhibition of microRNA function by antimiR oligonucleotides. *Silence* 2012; **3**: 1 [PMID: 22230293 DOI: 10.1186/1758-907X-3-1]

52 **Agrawal S**, Zhao Q. Mixed backbone oligonucleotides: improvement in oligonucleotide-induced toxicity in vivo. *Antisense Nucleic Acid Drug Dev* 1998; **8**: 135-139 [PMID: 9593053]

53 **Zhou W**, Agrawal S. Mixed-backbone oligonucleotides as second-generation antisense agents with reduced phosphorothioate-related side effects. *Bioorg Med Chem Lett* 1998; **8**: 3269-3274 [PMID: 9873716]

54 **Alvarez-Erviti L**, Seow Y, Yin H, Betts C, Lakhal S, Wood MJ. Delivery of siRNA to the mouse brain by systemic injection of targeted exosomes. *Nat Biotechnol* 2011; **29**: 341-345 [PMID: 21423189 DOI: 10.1038/nbt.1807]

55 **Li WC**, Ye SL, Sun RX, Liu YK, Tang ZY, Kim Y, Karras JG, Zhang H. Inhibition of growth and metastasis of human hepatocellular carcinoma by antisense oligonucleotide targeting signal transducer and activator of transcription 3. *Clin Cancer Res* 2006; **12**: 7140-7148 [PMID: 17145839 DOI: 10.1158/1078-0432.CCR-06-0484]

P- Reviewer Bonino F **S- Editor** Gou SX
L- Editor Stewart GJ **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i4.445

World J Gastroenterol 2013 January 28; 19(4): 445-456
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

TOPIC HIGHLIGHT

Anca Trifan, Professor, *Series Editor*

Update on adrenal insufficiency in patients with liver cirrhosis

Anca Trifan, Stefan Chiriac, Carol Stanciu

Anca Trifan, Stefan Chiriac, Carol Stanciu, "Gr. T. Popa" University of Medicine and Pharmacy, Institute of Gastroenterology and Hepatology, "St. Spiridon" University Hospital, 700111 Iaşi, Romania

Author contributions: Trifan A prepared the first draft, revised the article critically for important intellectual content, and performed the final edit of the paper; Chiriac S performed the literature research, wrote and edited the paper; Stanciu C contributed in the conception, design and drafting of this paper, revised and gave the final approval for the version to be published.

Correspondence to: Carol Stanciu, MD, PhD, FRCP, Professor, "Gr. T. Popa" University of Medicine and Pharmacy, Institute of Gastroenterology and Hepatology, "St. Spiridon" University Hospital, Independenței 1, 700111 Iași, Romania. stanciucarol@yahoo.com

Telephone: +40-72-2306020 Fax: +40-23-2264411

Received: November 5, 2012 Revised: December 3, 2012
Accepted: December 20, 2012
Published online: January 28, 2013

Abstract

Liver cirrhosis is a major cause of mortality worldwide, often with severe sepsis as the terminal event. Over the last two decades, several studies have reported that in septic patients the adrenal glands respond inappropriately to stimulation, and that the treatment with corticosteroids decreases mortality in such patients. Both cirrhosis and septic shock share many hemodynamic abnormalities such as hyperdynamic circulatory failure, decreased peripheral vascular resistance, increased cardiac output, hypo-responsiveness to vasopressors, increased levels of proinflammatory cytokines [interleukine(IL)-1, IL-6, tumor necrosis factor-alpha] and it has, consequently, been reported that adrenal insufficiency (AI) is common in critically ill cirrhotic patients. AI may also be present in patients with stable cirrhosis without sepsis and in those undergoing liver transplantation. The term hepato-adrenal syndrome defines AI in patients with advanced liver disease with sepsis and/or other complications, and it suggests that it could be a feature of liver disease *per se*, with a different pathogenesis from that of septic shock. Relative AI is the term given to inadequate cortisol response to stress. More recently, another term is used, namely "critical illness related corticosteroid insufficiency" to define "an inadequate cellular corticosteroid activity for the severity of the patient's illness". The mechanisms of AI in liver cirrhosis are not completely understood, although decreased levels of high-density lipoprotein cholesterol and high levels of proinflammatory cytokines and circulatory endotoxin have been suggested. The prevalence of AI in cirrhotic patients varies widely according to the stage of the liver disease (compensated or decompensated, with or without sepsis), the diagnostic criteria defining AI and the methodology used. The effects of corticosteroid therapy on cirrhotic patients with septic shock and AI are controversial. This review aims to summarize the existing published information regarding AI in patients with liver cirrhosis.

© 2013 Baishideng. All rights reserved.

Key words: Liver cirrhosis; Adrenal insufficiency; Septic shock; Corticosteroid therapy

Trifan A, Chiriac S, Stanciu C. Update on adrenal insufficiency in patients with liver cirrhosis. *World J Gastroenterol* 2013; 19(4): 445-456 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i4/445.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i4.445

INTRODUCTION

Adrenocortical dysfunction in patients with liver cirrhosis has been described for over half a century[1], but was ignored until a decade ago when several studies reported that some septic patients had an inappropriately low response of adrenal glands to stimulation, and treatment with corticosteroids decreased mortality[2,3]. Relative adrenal insufficiency (RAI) is the term given to inadequate production of cortisol with respect to the severity of

illness[4,5]. More recently, another term, namely critical illness related corticosteroid insufficiency (CIRCI) defined as "inadequate cellular corticosteroid activity for the severity of the patient's illness"[6], has been used. Despite a large number of published studies during recent years, the concepts of RAI and CIRCI are still under debate.

Liver cirrhosis is a major cause of mortality worldwide[7], often with septic shock as the terminal event[8]. It is a well-established fact that cirrhotic patients have increased susceptibility to bacterial infections[9]. Both cirrhosis and septic shock share many hemodynamic abnormalities such as hyperdynamic circulatory failure, decreased peripheral vascular resistance, decreased mean arterial pressure, increased cardiac output, hyporesponsiveness to vasopressors, increased levels of proinflammatory cytokines [interleukine (IL)-1, IL-6, tumor necrosis factor-α (TNF-α)][5,10,11] and, consequently, several studies reported that adrenal insufficiency (AI) is common in critically ill cirrhotic patients[8,12-14]. Furthermore, AI may occur in compensated and decompensated cirrhosis without sepsis[14-20] or in early and late post-liver transplantation (LT)[12,21-23]. Nowadays, liver cirrhosis is considered to be among the major groups of high-risk diseases with a predisposition to AI[24]. The term hepatoadrenal syndrome is used to define AI in patients with advanced liver disease with sepsis and/or other complications[12,15], suggesting that adrenocortical insufficiency may be a feature of liver disease *per se*, with a different pathogenesis from that occurring in septic shock.

Mechanisms of AI in cirrhotic patients are not entirely known, but they may include impaired synthesis in total cholesterol, high-density lipoprotein (HDL) cholesterol and low-density lipoprotein (LDL) cholesterol, as well as increased levels of proinflammatory cytokines and circulating endotoxin (e.g., lipopolysaccharide)[25-27]. The effects of corticosteroid therapy on cirrhotic patients with septic shock and AI are controversial, some studies reporting favorable results[12-14,28], while a recent randomized control study[29] has shown no benefit.

This review aims to summarize the existing published data regarding all aspects of AI prevalence, diagnosis and treatment in patients with liver cirrhosis.

PHYSIOLOGY OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS: A SHORT REVIEW

Cortisol is the main glucocorticoid secreted by the adrenal cortex under the control of adrenocorticotropic hormone (ACTH) which is released from the pituitary gland. The stimulus for ACTH release is corticotropin-releasing hormone (CRH) secreted by the paraventricular nuclei of the hypothalamus. Among factors influencing cortisol synthesis and production (diurnal rhythm of ACTH secretion, negative feedback by cortisol), stress plays the most important role. During stress and severe illness, activation of the hypothalamic-pituitary-adrenal (HPA) axis by the action of cytokines and other factors results in increased secretion of CRH, which will stimulate the production of ACTH and, consequently, increased release of cortisol into the circulatory system[30]. Cortisol is an essential component of the global adaptation to stress, contributing to the maintenance of cellular and organ homeostasis. Adequate levels of cortisol are absolutely necessary to increase cardiac output and vascular tonus, and to decrease proinflammatory cytokines (IL-1, IL-6, TNF-α) released[31,32] in order to overcome critical illness.

Over 90% of circulating cortisol is bound to corticosteroid-binding-globulin (CBG) (also called transcortin) and albumin, with less than 10% in the free biologically active form[33]. CBG is the predominant binding site (85%), with albumin binding smaller amounts of circulating cortisol. During severe sepsis, CBG levels fall, determining a higher percentage of free cortisol[34]. Hypoalbuminemia, frequently present in cirrhotic patients, has also been suggested to increase the free cortisol fraction[35,36]. Approximately 80% of circulating cortisol is synthesized both at rest and during stress from plasma cholesterol (particularly in the form of HDL cholesterol) and this could be relevant in patients with liver cirrhosis where cholesterol is low and may limit the synthesis of cortisol[26]. In the liver, cortisol is converted to its inactive metabolite cortisone by the enzyme 11β - hidroxysteroid dehydrogenase. After diffusion across the cell membrane, cortisol binds to glucocorticoid receptor and translocates into the nucleus of the cell[37] where its effects are exerted (increased vascular tonus and cardiac output, protein catabolism, lipolysis, hyperglycemia, and decreased cytokine production)[38]. These effects of cortisol are beneficial in critical illness, and several studies have shown that corticosteroid therapy is beneficial in patients with severe sepsis or septic shock[12-14,39,40]. As adrenal glands do not store cortisol, this must urgently be synthesized from its precursor, cholesterol, under any conditions of stress. In cirrhotic patients there is a low substrate (HDL cholesterol) for the synthesis of cortisol, favoring AI in conditions of stress[26].

PATHOGENESIS

Mechanisms leading to AI in liver cirrhosis remain largely unknown, although some hypotheses such as endotoxemia, decreased levels of apolipoprotein A-1, HDL cholesterol and LDL cholesterol, increased levels of proinflammatory mediators, structural damage to the adrenal gland due to infarction or hemorrhage, bacterial translocation of enteric organisms, "exhaustion" of the adrenal cortex, and glucocorticoid resistance have been suggested[12,41-49]. Many (if not all) of these pathophysiologic mechanisms are also involved in the genesis of AI in critically ill patients with sepsis[50-56].

As we have mentioned, cholesterol is the main source of steroidogenic substrate in the adrenal gland[26,57]. Several studies reported a significant decrease in the level

of serum HDL in cirrhotic patients which was related to the severity of the disease[12,26,47]. Furthermore, increased levels of circulating endotoxin (lipopolysaccharide) and TNF-α inhibit cortisol synthesis, limiting the delivery of HDL cholesterol to the adrenal gland[58-60]. In addition to this, TNF-α, IL-1 and IL-6 decrease hepatocyte synthesis of apolipoprotein A-1[58], the major component of HDL cholesterol. The lack of substrate for steroidogenesis will eventually lead to the so-called "adrenal exhaustion syndrome"[42] which contributes to AI in cirrhotic patients.

Besides low levels of serum total cholesterol, HDL-cholesterol and LDL-cholesterol, other factors may play a definite role in the pathogenesis of AI in patients with liver cirrhosis. Thus, coagulopathy (frequent in liver cirrhosis) may cause adrenal hemorrhage and infarction leading to structural damage of the adrenal gland[5], resulting in AI. Systemic inflammation is common in cirrhotic patients[61]. Bacterial translocation of enteric organisms has been demonstrated in patients with advanced liver cirrhosis[41,62].

A high prevalence of AI reported in patients with stable cirrhosis[15-19,63], similar to that reported in cirrhosis complicated by sepsis/septic shock, suggests that AI may be a feature of liver disease *per se*, with a different pathogenesis from that occurring in septic shock. These findings are consistent with the observations of Marik *et al*[12] who put forward the term hepato-adrenal syndrome in order to define AI in patients with advanced liver disease.

DIAGNOSIS

Diagnosis of AI made on clinical grounds in critically ill cirrhotic patients is impossible because of the lack of typical addisonian features[5,13]. Hypotension refractory to vasopressors and fluid resuscitation is the most important clinical sign in such patients[52]. Therefore, the diagnosis of AI in patients with liver cirrhosis is based on the following laboratory tests.

Standard dose

Measurement of serum total cortisol, either at baseline or following stimulation by the standard dose-short synacthen test (SD-SST) or low dose-short synacthen test (LD-SST). Baseline serum total cortisol levels under 414 nmol/L[8,13,20,64-66], < 250 nmol/L[45] or < 138 nmol/L[67] have been used to define AI in different studies. The following thresholds were used to diagnose subnormal response to SD-SST or LD-SST: (1) a peak cortisol level (defined as the highest cortisol concentration after synacthen stimulation) < 690 nmol/L[16], < 552 nmol/L[12], < 500 nmol/L[14,15,18,45], < 442 nmol/L[17]; and (2) a delta cortisol (defined as the difference between peak and basal cortisol) less than 250 nmol/L[8,13,15-20,45,64-67].

As one can easily see, there are differences in the thresholds of serum total cortisol used to define AI in published studies, which may explain significant discrepancies in the prevalence of AI in cirrhotic patients. Moreover, the diagnosis of AI based on serum total cortisol in patients with cirrhosis may be inaccurate due to changes in serum concentrations of CBG and albumin (both synthesized in the liver) which are usually low[68-70]. It has been already shown that low levels of CBG and albumin lead to overestimation of the diagnosis of AI[45,67]. As we have mentioned before, over 90% of serum circulating cortisol is bound to CBG and albumin, with less than 10% in the free form. Standard laboratory assays of serum total cortisol measure the bound plus free fractions. This means that a decrease in the binding protein levels, as it often happens in cirrhosis, will reduce serum total cortisol, affecting the interpretation of SD-SST/LD-SST[35,44], and this may lead to the overestimation of AI in cirrhotic patients[45]. However, most of the studies evaluating adrenal function in critically ill patients with liver cirrhosis still rely on the measurement of serum total cortisol, both at baseline and after stimulation.

Serum free cortisol assays are considered the most reliable method to assess adrenal function in critically ill patients[71]. There are several methods used to measure serum free cortisol (gel filtration, ultrafiltration, equilibrium dialysis)[72], all of them expensive and inconvenient for routine clinical practice[73]. In patients with liver cirrhosis, the serum free cortisol level is not altered by a reduced concentration of CBG and albumin[74] and it therefore appears to be a more appropriate marker for assessing adrenal function in such patients[44,74]. Some studies reported significant differences in diagnosis of AI using serum total cortisol and free cortisol criteria in cirrhotic patients with septic shock[75] or in those with stable cirrhosis[15], while others found that assessing serum free cortisol had limited additive diagnostic value over serum total cortisol[76]. Serum free cortisol levels under 50 nmol/L at baseline or less than 86 nmol/L after synacthen stimulation are suggestive for the diagnosis of AI (in critically ill patients)[35], although the reference range for baseline values in healthy subjects varies from 8-25 nmol/L[71] to 12-70 nmol/L[44,77].

Due to the limitations of available assays to estimate serum free cortisol, surrogate markers may be used, such as Coolens equation "$U^2 \times K (1 + N) + U [1 + N + K (G - T)] - T = 0$", where T is total cortisol, G is CBG, U is unbound cortisol, K is the affinity of CBG for cortisol at 37 ℃ and N is the ratio of albumin-bound to unbound cortisol[68], free cortisol index (FCI) (serum total cortisol concentration divided by CBG level)[78], and salivary cortisol[71,79]. However, Coolens equation and FCI do not take into account the concentration of low serum albumin and CBG frequently present in cirrhotic patients and, therefore, both surrogates may not be suitable to estimate serum free cortisol in such patients[69-71]. By contrast, salivary cortisol, regardless of serum binding protein levels, correlates well with free cortisol levels[71,79]. Basal value of salivary cortisol < 1.8 ng/mL or a concentration after stimulation (SD-SST) < 12.7 ng/mL, an increment < 3 ng/mL[45] or a peak serum free cortisol < 33 nmol/L[15] are suggestive of AI. However, there are

significant variations in normal salivary cortisol values reported by different studies[74]. Other limits of salivary cortisol are represented by oral candidiasis, low salivary flow, and contaminated salivary samples from gingival bleeding, common in cirrhotic patients[44].

SD-SST

SD-SST measures total serum cortisol at baseline and 60 min after an intravenous injection of 250 μg of synthetic ACTH. Currently, there are two corticotropic analogues that can be used, namely tetracosactrin (synacthen, Novartis Pharma AG, Basel, Switzerland) and cosyntropin (Cortrosyn, Amphastar Pharmaceuticals, Rancho Cucamonga, CA, United States). Using a supraphysiological dose of 250 μg of corticotropin (which results in approximately 100 times higher than normal maximal stress ACTH levels)[17], SD-SST is not a "physiological test"[17,80]. In the context of critical illness, AI was defined by the International Task Force[6] as a delta cortisol of < 250 nmol/L (< 9 μg/dL) after SD-SST or a random serum total cortisol of < 276 nmol/L (< 10 μg/dL). There is no consensus on the diagnostic criteria of AI in cirrhotic patients, although a delta cortisol under 250 nmol/L has been used by most studies to define AI in such patients[81].

LD-SST

LD-SST uses 1 μg of synacthen given intravenously, and serum cortisol measured after 20 and 30 min (the latter time-point is mostly used). The normal response is a serum cortisol level > 500 nmol/L (> 18 μg/dL)[49]. In a meta-analysis[82] comprising the diagnostic value of SD-SST and LD-SST for diagnosing AI, LD-SST was found to be superior, contrary to another meta-analysis[83] which reported similar operative characteristics for both tests. LD-SST seems to be a more physiological and sensitive test than SD-SST for the diagnosis of AI, and appropriate for use in non-critically ill cirrhotic patients[49].

Insulin-induced hypoglycemia test

Insulin-induced hypoglycemia test (IIHT) has been considered to be the gold standard to evaluate the HPA axis. The test uses injection of 0.15 IU/kg regular insulin to achieve blood glucose less than 40 mg/dL or until symptoms of hypoglycemia develop. Blood samples are taken before and at 15, 30, 45, 60, 90 min post-stimulation. Peak cortisol cut points between 500 and 550 nmol/L (18-20 μg/dL) are used for the diagnosis of adrenal sufficiency. This test is contraindicated in patients with cardio- or cerebrovascular diseases and convulsive disorders. In addition, the IIHT is unpleasant for the patients and therefore it has been replaced by alternative tests (LS-SST, SD-SST) for evaluating the HPA axis[84].

Corticotrophin-releasing hormone test

Corticotrophin-releasing hormone test (CRHT) evaluates the entirety of the HPA axis. Blood samples for the measurement of ACTH and cortisol are taken at baseline and at 15, 30, 45 and 60 min after an intravenous injection of 1 μg/Kg of CRH. Although CRHT is free of serious side effects, it is both difficult and costly and therefore it has been used in few studies in liver disease.

To conclude, in the absence of a gold standard test, SD-SST remains the most used test to assess the adrenal function in critically ill cirrhotic patients, while LD-SST seems to be more appropriate in those with stable cirrhosis. At present, serum free cortisol and salivary cortisol are the most accurate methods for the diagnosis of AI in cirrhotic patients, but cannot be used in routine clinical practice. The use of salivary cortisol needs to be validated. As diagnosis of AI in cirrhotics is of major clinical importance, there is an urgent need for a consensus as to which is the most appropriate diagnostic test of AI in such category of patients.

PREVALENCE AND EXISTING EVIDENCE

Initial reports on AI in liver cirrhosis were followed by multiple studies (Tables 1 and 2) and, recently, by excellent systematic reviews[43,44,46,49,81]. There are significant discrepancies between studies on the prevalence of AI in patients with liver cirrhosis, mainly because of the different tests used for diagnosis of adrenal dysfunction and the criteria applied to define AI. Thus, the prevalence of AI varies between critically ill cirrhotic patients (10%-87%; Table 1), those with stable cirrhosis (7%-83%; Table 2), and patients with liver transplant (61%-92%; Table 1). Overall, several published studies have reported a high prevalence of AI both in critically and non-critically ill cirrhotic patients[17,29,63,64,69,85] as well as in those who had received liver transplant[12].

Critically ill patients with liver cirrhosis

Almost all studies that included critically ill patients with liver cirrhosis[8,13,20,29,64-66,74,85] used SD-SST for the diagnosis of AI and only two performed LD-SST[12,16]. With SD-SST, the reported prevalence of AI in critically ill cirrhotics varied between 10%[74] and 87%[85], while with LD-SST, the prevalence range was between 33%[12] and 60%[16].

Harry *et al*[14] reported a prevalence of AI (defined as peak cortisol levels less than 500 nmol/L) of 69% in critically ill cirrhotic patients requiring vasopressor support. In a prospective study including 25 cirrhotic patients with severe sepsis, Fernández *et al*[13] reported a very high incidence of AI (68%) using SD-SST and defining AI either as baseline serum total cortisol level less than 414 nmol/L or a delta cortisol lower than 250 nmol/L in those with a baseline concentration below 966 nmol/L. The AI prevalence rate was correlated with the severity of liver disease (76% Child-Pugh C *vs* 25% Child-Pugh B).

SD-SST was also used to evaluate adrenal function in a prospective study which included 101 critically ill patients with cirrhosis and severe sepsis[8]. Authors found that 51% of their patients met the criteria for AI (defined as baseline serum total cortisol values under 414 nmol/L

Table 1 Prevalence of adrenal insufficiency in critically ill patients with liver cirrhosis

Ref.	No. of patients (type of cirrhosis)	Diagnosis and definition of AI	Prevalence of AI
Harry *et al*[14]	20 (ALF/CLD)	SD-SST: Peak cortisol < 500 nmol/L[1]	69%
Marik *et al*[12]	340	LD-SST: Peak cortisol < 552 nmol/L or	72%
	(ALF: 24)	random cortisol level < 414 nmol/L in non-stressed patients or	33%
	(CLD: 146)	random cortisol level < 552 nmol/L in stressed patients	66%
	(recent LT: 119)		92%
	(remote LT: 51)		61%
Tsai *et al*[8]	101 (cirrhosis+ severe sepsis)	SD-SST: Baseline cortisol < 414 nmol/L or delta cortisol < 250 nmol/L if baseline cortisol between 414 and 938 nmol/L	51%
Fernandez *et al*[13]	25 (cirrhosis + septic shock)	SD-SST: Baseline cortisol < 414 nmol/L or delta cortisol < 250 nmol/L if baseline cortisol between 414 and 966 nmol/L	68%
Thierry *et al*[64]	14 (cirrhosis + septic shock)	SD-SST: Baseline cortisol < 414 nmol/L; delta cortisol < 250 nmol/L	77%
du Cheyron *et al*[65]	50 (critically ill cirrhosis)	SD-SST: Baseline cortisol < 414 nmol/L; delta cortisol < 250 nmol/L if baseline cortisol between 414 and 938 nmol/L	82%
Vasu *et al*[86]	24 (critically ill cirrhotics)	SD-SST: Definition of AI was not reported	62%
Arabi *et al*[29]	75 (cirrhosis + septic shock)	SD-SST: Delta cortisol < 250 nmol/L	76%
Mohamed *et al*[85]	15 (cirrhosis+septic shock)	SD-SST: Definition of AI was not reported	87%
Thevenot *et al*[74]	30 (cirrhosis + sepsis)	SD-SST: Peak serum total cortisol < 510 nmol/L	10%
Acevedo *et al*[89]	166 (decompensated cirrhosis)	SD-SST: Delta cortisol < 250 nmol/L	26%
Graupera *et al*[20]	37 (severe acute bleeding)	SD-SST: Baseline cortisol < 414 nmol/L and/or delta cortisol < 250 nmol/L	38%
Triantos *et al*[16]	20 (cirrhosis with variceal bleeding)	SD-SST: Baseline cortisol < 276 nmol/L or delta cortisol < 250 nmol/L	30%
		LD-SST: Peak serum cortisol < 690 nmol/L or a delta cortisol < 250 nmol/L	60%
El Damarawy *et al*[66]	45 (cirrhosis with septic shock or HRS, cirrhosis without septic shock or HRS)	SD-SST: Baseline cortisol < 414 nmol/L or delta cortisol < 250 nmol/L in patients with baseline cortisol < 966 nmol/L	73%

[1]To convert serum total cortisol concentrations from nanomoles per liter to micrograms per deciliter divide by 27.59[79]. ALF: Acute liver failure; CLD: Chronic liver disease; HRS: Hepatorenal syndrome; LT: Liver transplant; AI: Adrenal insufficiency; SD-SST: Standard dose short synacthen test; LD-SST: Low dose short synacthen test.

or delta cortisol lower than 250 nmol/L with a baseline value between 414 and 938 nmol/L) which was related to disease severity [Child-Pugh and model for end-stage liver disease (MELD) scores] and increased mortality. Recently, Arabi *et al*[29], using the same test (SD-SST) and definition for AI (delta cortisol < 250 nmol/L) in a similar group of critically ill patients (cirrhosis with septic shock) reported an even higher AI prevalence rate (76%).

The SD-SST test was also used in several other studies to assess adrenal function in critically ill cirrhotic patients[64-66,74,85,86].

Adrenal function has also been evaluated by SD-SST in cirrhotic patients with variceal bleeding[16,20]. Graupera *et al*[20] reported AI prevalence (defined as baseline serum cortisol < 414 nmol/L or delta cortisol < 250 nmol/L) in 38% of bleeding patients. AI was associated with increased risk of failure to control bleeding and lower survival rate at 6 wk. In a prospective observational study on 20 cirrhotic patients with variceal bleeding and 60 with stable cirrhosis, Triantos *et al*[16] reported an AI prevalence rate (defined as basal cortisol < 276 nmol/L or delta cortisol < 250 nmol/L following SD-SST) of 30% (similar to that in stable cirrhosis); with the use of LD-SST, AI prevalence (defined as a peak cortisol < 690 nmol/L or a delta cortisol < 250 nmol/L) was significantly higher in bleeders (60%) than in stable cirrhotics (48%).

LD-SST was also previously used by Marik *et al*[12] to evaluate adrenal function in 340 critically ill patients with liver disease (24 with fulminant hepatic failure, 146 critically ill cirrhotics, 51 with remote LT, and 119 having recently undergone LT). AI was defined as having a random cortisol level of < 552 nmol/L in highly stressed patients (hypotension, hepatic failure, respiratory failure) and a random cortisol level of < 414 nmol/L or a 30 min post LD-SST level of < 552 nmol/L in all other patients. Out of 340 patients studied, 245 (72%) met the criteria for AI (33% fulminant hepatic failure, 66% critically ill cirrhotics, 61% remote LT, 92% recent LT).

Non-critically ill cirrhotics

AI is also common in patients with stable liver cirrhosis (Table 2). However, as in critically ill cirrhotic patients, AI prevalence rate in those with stable liver cirrhosis varies significantly, depending on the diagnostic test used.

In a prospective study, Tan *et al*[15] evaluated adrenal function in 43 clinically stable cirrhotic patients. All patients underwent SD-SST, and AI was defined by delta cortisol < 250 nmol/L or a peak total cortisol < 500 nmol/L, or a peak serum free cortisol < 33 nmol/L. The prevalence of AI was 47% using delta cortisol < 250 nmol/L, 39% using peak total cortisol < 500 nmol/L, and 12% with serum free cortisol < 33 nmol/L. This study clearly shows that the reported prevalence of AI depends largely on the diagnostic test used and criteria for defining AI.

Galbois *et al*[45] have evaluated adrenal function in 88 patients hospitalized for complications of cirrhosis without bleeding and shock. Salivary and serum total cortisol were assessed 60 min before and after stimulation with SD-SST in all patients. Serum free cortisol was estimated

Table 2 Prevalence of adrenal insufficiency in patients with liver cirrhosis, not critically ill

Ref.	No. of patients (type of cirrhosis)	Diagnosis and definition of AI	Prevalence of AI
McDonald *et al*[69]	38 (stable cirrhosis)	IIHT: Reduction in maximal increments of plasma cortisol	64%
		SD-SST: Reduction in maximal increments of plasma cortisol	39%
Zietz *et al*[112]	52 (stable cirrhosis)	CRHT: Peak cortisol < 550 nmol/L or an increase < 250 nmol/L[1]	58%
		rise of plasma ACTH < twice the baseline	42%
Sigalas *et al*[87]	47 (stable cirrhosis)	SD-SST: Baseline cortisol < 250 nmol/L and delta cortisol < 250 nmol/L	36%
Alessandria *et al*[88]	25 (cirrhosis and ascites)	SD-SST: Delta cortisol < 250 nmol/L	36%
Jang *et al*[63]	18 (stable cirrhosis)	SD-SST: Baseline cortisol < 414 nmol/L delta cortisol < 250 nmol/L	83%
Acevedo *et al*[19]	198 (10 compensated and	SD-SST: Baseline cortisol < 414 nmol/L	64%
	188 decompensated cirrhosis)	delta cortisol < 250 nmol/L	27%
Galbois *et al*[45]	88 (stable cirrhosis)	SD-SST: (1) Serum total cortisol: Baseline cortisol < 250 nmol/L or peak cortisol < 500 nmol/L or delta cortisol < 250 nmol/L	33%
		(2) Salivary cortisol: Basal salivary cortisol < 1.8 ng/mL or post-stimulation values < 12.7 ng/mL or increase values < 3 ng/mL	9%
Tan *et al*[15]	43 (stable cirrhosis)	SD-SST: Peak total cortisol < 500 nmol/L;	39%
		delta cortisol < 250 nmol/L;	47%
		peak plasma free cortisol < 33 nmol/L;	12%
		any set of criteria	58%
Thevenot *et al*[67]	95 (stable cirrhosis)	LD-SST: Baseline cortisol < 138 nmol/L;	7%
		< 440 nmol/L after stimulation;	19%
		⩽ 500 nmol/L after stimulation;	27%
		delta cortisol < 250 nmol/L	49%
Fede *et al*[17]	101 (stable cirrhosis)	LD-SST: Peak serum cortisol < 500 nmol/L;	38%
		peak serum cortisol < 442 nmol/L;	29%
		delta cortisol < 250 nmol/L	60%
Triantos *et al*[16]	60 (stable cirrhosis)	SD-SST: Peak serum cortisol < 500 nmol/L	30%
		LD-SST: Peak serum cortisol < 500 nmol/L	48%
Mohamed *et al*[85]	15 (stable cirrhosis)	SD-SST: Definition of AI was not reported	53%
Risso *et al*[18]	85 (cirrhosis with ascites, without sepsis or shock)	SD-SST: Delta cortisol < 250 nmol/L and/or peak cortisol < 500 nmol/L	39%
Vincent *et al*[73]	26 (liver impairment)	SD-SST: Serum total cortisol < 550 nmol/L;	46%
		free cortisol index < 12	13%

[1]To convert serum total cortisol concentrations from nanomoles per liter to micrograms per deciliter divide by 27.59[79]. AI: Adrenal insufficiency; SD-SST: Standard dose short synacthen test; LD–SST: Low dose short synacthen test; CRHT: Corticotropin-releasing hormone test; IIHT: Insulin-induced hypoglycemia test; ACTH: Adrenocorticotropic hormone.

from serum total cortisol and CBG levels using Coolens' formula[68]. The following definitions of AI were used by the authors: (1) according to serum total cortisol assays: baseline < 250 nmol/L, or a peak total cortisol < 500 nmol/L, or delta cortisol < 250 nmol/L; (2) according to salivary cortisol assays: baseline < 1.8 ng/mL, or an increase < 3 ng/mL or a concentration < 12.7 ng/mL after stimulation. The results indicated a significant difference in AI prevalence depending on the test used: 33% when serum total cortisol was considered *vs* 9.1% using salivary cortisol.

Another study performed by Thevenot *et al*[74] has demonstrated that assessment of adrenal function with measurements of serum total cortisol overestimated AI prevalence in cirrhotic patients. In this study, baseline and post-synacthen serum total cortisol, serum free cortisol and salivary cortisol concentrations were measured in 125 cirrhotic patients (95 non-septic, 30 septic). AI was defined as serum total cortisol < 510.4 nmol/L after SD-SST. AI was found in nine patients (7.2%) (6 non-septic; 3 septic) and restricted to cirrhotics with Child-Pugh C. Serum total cortisol concentrations, CBG and albumin levels significantly decreased in non-septic patients as liver function deteriorated (from Child-Pugh A to C). Cirrhotic patients with or without AI had similar basal serum free cortisol and salivary cortisol levels. As the serum total cortisol level overestimated the prevalence of AI in cirrhotic patients, and serum free cortisol is not suitable for routine laboratory use, authors concluded that measurement of salivary cortisol is a useful approach in such patients. The same group of investigators[67] analyzed only the 95 hemodynamically stable cirrhotic patients from the previously mentioned study, who underwent a LD-SST. The serum total cortisol and serum free cortisol concentrations were measured 30 min before and after LD-SST. AI was defined as: (1) basal serum total cortisol < 138 nmol/L and < 440 nmol/L after stimulation; (2) serum total cortisol < 500 nmol/L after stimulation; and (3) cortisol increment < 250 nmol/L. AI prevalence rates varied significantly according to the threshold used: 7.4 % with basal serum total cortisol, 19% using serum cortisol < 440 nmol/L, 27.4 % with serum cortisol < 500 nmol/L, and 49.4% with delta cortisol. Serum free cortisol levels before and after LD-SST stimulation were higher in the more severe cirrhotic patients regardless of CBG and albumin concentrations, and directly associated with the risk of non-transplant-related mortality in hemodynamically stable patients with cirrhosis.

In opposition to the above mentioned studies, recently, in a prospective study, Molenaar *et al*[76], using SD-SST, assessed the value of free *vs* total cortisol levels while evaluating AI in 49 septic and 63 non-septic patients with treatment-insensitive hypotension and found that total cortisol correlated with free cortisol during critical illness. Moreover, in sepsis, hypoalbuminemia did not affect total and free cortisol, contrary to the findings of other published studies[45,67].

Others, using SD-SST or LD-SST to diagnose adrenal dysfunction in patients with stable liver cirrhosis reported high AI prevalence rates[16-19,63,69,73,85,87,88]. Fede *et al*[17] reported an AI prevalence of 38% in 101 patients with stable cirrhosis (absence of infections or hemodynamic instability). AI, defined as a peak serum total cortisol level < 500 nmol/L after LD-SST, was correlated with the severity of liver disease graded according to Child-Pugh or MELD scores.

Using SD-SST in 85 cirrhotics with ascites but without sepsis, Risso *et al*[18] reported AI (delta cortisol < 250 nmol/L and/or peak cortisol < 500 nmol/L) in 39% of patients.

Vincent *et al*[73] evaluated adrenal function by SD-SST in 26 patients with liver impairment. Authors defined AI as serum total cortisol < 550 nmol/L or FCI < 12. Three patients (13%) met both criteria, 12 patients (46%) had a serum total cortisol < 550 nmol/L but an FCI > 12. When serum total cortisol was used, 46% of patients had AI, while when using FCI only 13% fulfilled the criteria for AI. Authors suggested that FCI is better suited for the evaluation of AI in patients with liver impairment.

Acevedo *et al*[19], using SD-SST, evaluated the prevalence of AI in 198 patients with liver cirrhosis [10 with compensated, 188 with decompensated cirrhosis and complications (hepatic encephalopathy, spontaneous bacterial peritonitis, ascites, gastrointestinal bleeding, hepatorenal syndrome)]. AI defined as basal serum total cortisol < 414 nmol/L was found in 64% of patients, and only in 27% when delta cortisol < 250 nmol/L was used, with no differences between compensated and decompensated cirrhosis. The same group of researchers evaluated the prevalence and prognostic value of AI in 166 patients with advanced cirrhosis (no severe sepsis or septic shock)[89]. AI, defined as delta cortisol < 250 nmol/L after SD-SST, was found in 26% of patients. Those with AI had a higher degree of circulatory dysfunction, greater prevalence of systemic inflammatory response syndrome, increased probability to develop severe infections, and higher hospital mortality rates than patients without AI.

AI after LT

AI has been reported both early as well as late after LT[12,21-23,90].

With LD-SST, Marik *et al*[12] found that 92% of 119 patients undergoing recent LT and maintained on steroid-free immunosuppressive regimens had AI. The steroid-free immunosuppressive regimen may expose patients undergoing LT to an increased risk for AI, while the use of steroids intra and postoperatively in LT may reduce such a risk or mask an AI[46]. Furthermore, LD-SST is not recommended for the diagnosis of AI in high-stress conditions like LT[6] as it may lead to an overestimated AI prevalence in such patients.

Toniutto *et al*[21], using SD-SST, reported an AI prevalence rate of 26% in 87 patients having received LT for end-stage liver disease and maintained on prolonged immunosuppressive treatment.

Patel *et al*[90] reported significantly reduced requirements for fluid, vasopressors, invasive ventilation, and renal replacement therapy, and intensive care unit stay for patients undergoing LT who received 1000 mg methylprednisolone prior to the liver graft reperfusion.

TREATMENT

Cortisol has several beneficial effects such as an increase of the vascular tonus and cardiac output, enhancement of catecholamine responsiveness, inhibition of the production of nitric oxide, modulation of cytokine production in septic shock[32,91-97], but the effects of corticosteroid therapy in sepsis, severe sepsis and septic shock remain, however, controversial. Thus, a significant reduction in mortality rate with hydrocortisone therapy in patients with septic shock has been reported in several studies and meta-analyses[6,28,39,98-101], while others have shown no effect on the 28-d mortality rate[14,29,102]. Both doses and duration of corticosteroid therapy vary significantly in published studies[6,28,39,40,102,103]. Thus, some used a daily dose of hydrocortisone (or equivalent) of 200-300 mg ("low-dose", also called "physiologic-dose" or "stress-dose")[3,28,39,98,100-105] while others used a "supra-physiologic" dose (> 300 mg)[98,106-108].

None of the early studies using high doses of corticosteroids for short courses reported any benefit[98,106-108], while more recent studies using a "physiologic-dose" for longer durations have shown a significant reduction in vasopressor agents requirement and in intensive care unit length of stay, greater shock resolution, and decreased mortality[6,28,39,98,100,104,105,109-111]. A randomized, double-blind placebo controlled trial, CORTICUS (Corticosteroid Therapy of Septic Shock)[102] including 499 patients with septic shock randomized to hydrocortisone (50 mg intravenously every 6 h for 5 d, followed by 50 mg intravenously every 12 h for 3 d, and then by 50 mg daily for 3 d) or placebo, concluded that there was no benefit in terms of mortality, although steroid administration was associated with a greater shock reversal, but also with a higher incidence of episodes of new infections. On the other hand, Annane *et al*[28] in a randomized, double-blind controlled trial have found that the administration of hydrocortisone (50 mg intravenously every 6 h) and oral fludrocortisone (50 μg once daily) in patients with refractory septic shock and AI (delta cortisol < 250 nmol/L) resulted in a 30% decrease in 28-d mortality. It should be mentioned that consensus statements from an international task force[6] recommended corticosteroid therapy

Table 3 Published studies on corticosteroid therapy in patients with liver cirrhosis

Ref.	No. of patients (type of cirrhosis)	Study design	Steroid dose	Outcomes
Harry *et al*[14]	20 (ALF or ACLF)	Retrospective	Hydrocortisone 300 mg/d	Reduction in vasopressor doses, but higher incidence of infection and no survival benefit
Marik *et al*[12]	140 (ALF or CLD)	Not RCT	Hydrocortisone 300 mg/d	Reduction in the dose of norepinephrine at 24 h, and lower mortality rate increased survival
Fernandez *et al*[13]	17 (cirrhosis and septic shock)	Prospective but not RCT	Hydrocortisone 200 mg/d	Significant increase in shock resolution and high hospital survival rate
Arabi *et al*[29]	39 (cirrhosis and septic shock)	RCT	Hydrocortisone 200 mg/d	Reduction in vasopressor doses and higher rates of shock reversal, but no benefit in 28 d mortality, increase in gastrointestinal bleeding and shock relapse

ALF: Acute liver failure; ACLF: Acute-on-chronic liver failure; CLD: Chronic liver disease; RCT: Randomized controlled trial.

(intravenous hydrocortisone 200-300 mg/d in four divided doses for a week before tapering slowly) in patients with vasopressor-dependant septic shock.

Like in patients with severe sepsis/septic shock with other causes than liver cirrhosis, as mentioned above, the effects of steroid therapy in cirrhotic patients with AI remain controversial, some studies reporting beneficial results[12-14] while a recent randomized control study[29] has shown no benefit (Table 3).

Harry *et al*[14] evaluated the effects of stress doses of hydrocortisone in a retrospective comparative study including 40 patients. Twenty patients received hydrocortisone (300 mg/d) for 4-5 d. In patients with acute-on-chronic liver failure requiring norepinephrine support, the results showed a reduction in vasopressor doses, but no survival benefit; moreover, corticosteroid therapy was associated with a significant increase in infections.

Another study, carried out by Marik *et al*[12] evaluated the effect of 300 mg/d hydrocortisone given intravenously in vasopressor-dependant patients with acute or chronic liver disease. In patients with AI, treatment with hydrocortisone was associated with a significant reduction of the norepinephrine dosage at 24 h and with a lower mortality ($P = 0.02$), whereas in those patients without AI hydrocortisone did not affect the norepinephrine dose.

Fernández *et al*[13], in a prospective but non-randomized study have evaluated adrenal function by SD-SST and the effects of low-dose hydrocortisone in 25 patients with advanced cirrhosis and septic shock. Patients with AI received intravenous hydrocortisone (50 mg every 6 h) and results were compared with those obtained from a retrospective 50 cirrhotic patients with septic shock in whom adrenal function was not investigated and who did not receive corticosteroid therapy. Results showed that hydrocortisone therapy was associated with a significant increase in shock resolution and hospital survival rate. Authors suggested that all cirrhotic patients with AI should be treated with hydrocortisone.

Recently, Arabi *et al*[29] in a randomized controlled trial, have shown that low dose hydrocortisone therapy in cirrhotic patients with septic shock had a significant reduction in vasopressor doses and higher rates of shock reversal, but it did not reduce mortality and was associated with an increase in adverse effects (gastrointestinal bleeding) and shock relapse.

Based on the above mentioned studies, there are still several unsolved problems and questions awaiting answers. Thus, re-evaluation of both doses and duration of corticosteroid therapy is necessary. Obviously, further prospective randomized clinical studies are needed to assess the effect of corticosteroid therapy in critically ill cirrhotic patients with AI.

CONCLUSION

AI occurs frequently in patients with liver cirrhosis both during critical illness and in stable disease. Studies, however, do not agree on the prevalence of AI in cirrhotic patients, mostly because of the different criteria and the methodology used to define AI. Diagnosis of AI in patients with liver cirrhosis remains controversial (particularly in those critically ill) as all diagnostic tests proved their limitations. Pathogenesis of AI in liver cirrhosis is still unknown, although decreased levels of cholesterol (mainly HDL cholesterol) and increased levels of pro-inflammatory cytokines and circulating endotoxin have been put forward. Some data suggest that AI may be a feature of cirrhosis *per se*, with a pathogenesis subtly different from that occurring in septic shock from other causes. Yet, there is still controversy in what concerns treatment with corticosteroids, although some cirrhotic patients with vasopressor resistant shock may benefit. However, further prospective, randomized clinical trials are necessary to assess the effect of corticosteroid therapy in critically ill patients with cirrhosis.

REFERENCES

1 **Peterson RE**. Adrenocortical steroid metabolism and adrenal cortical function in liver disease. *J Clin Invest* 1960; **39**: 320-331 [PMID: 14432260 DOI: 10.1172/JCI104043]

2 **Annane D**, Sébille V, Troché G, Raphaël JC, Gajdos P, Bellissant E. A 3-level prognostic classification in septic shock based on cortisol levels and cortisol response to corticotropin. *JAMA* 2000; **283**: 1038-1045 [PMID: 10697064 DOI: 10.1001/jama.283.8.1038]

3 **Annane D**, Bellissant E, Bollaert PE, Briegel J, Keh D, Kupfer Y. Corticosteroids for severe sepsis and septic shock: a systematic review and meta-analysis. *BMJ* 2004; **329**: 480

[PMID: 15289273 DOI: 10.1136/bmj.38181.482222.55]
4 **Rothwell PM**, Udwadia ZF, Lawler PG. Cortisol response to corticotropin and survival in septic shock. *Lancet* 1991; **337**: 582-583 [PMID: 1671944 DOI: 10.1016/0140-6736(91)91641-7]
5 **Cooper MS**, Stewart PM. Corticosteroid insufficiency in acutely ill patients. *N Engl J Med* 2003; **348**: 727-734 [PMID: 12594318 DOI: 10.1056/NEJMra020529]
6 **Marik PE**, Pastores SM, Annane D, Meduri GU, Sprung CL, Arlt W, Keh D, Briegel J, Beishuizen A, Dimopoulou I, Tsagarakis S, Singer M, Chrousos GP, Zaloga G, Bokhari F, Vogeser M, American College of Critical Care Medicine. Recommendations for the diagnosis and management of corticosteroid insufficiency in critically ill adult patients: consensus statements from an international task force by the American College of Critical Care Medicine. *Crit Care Med* 2008; **36**: 1937-1949 [PMID: 18496365 DOI: 10.1097/CCM.0b013e31817603ba]
7 **Méndez-Sánchez N**, Villa AR, Zamora-Valdés D, Morales-Espinosa D, Uribe M. Worldwide mortality from cirrhosis. *Ann Hepatol* 2007; **6**: 194-195 [PMID: 17786150]
8 **Tsai MH**, Peng YS, Chen YC, Liu NJ, Ho YP, Fang JT, Lien JM, Yang C, Chen PC, Wu CS. Adrenal insufficiency in patients with cirrhosis, severe sepsis and septic shock. *Hepatology* 2006; **43**: 673-681 [PMID: 16557538 DOI: 10.1002/hep.21101]
9 **Gustot T**, Durand F, Lebrec D, Vincent JL, Moreau R. Severe sepsis in cirrhosis. *Hepatology* 2009; **50**: 2022-2033 [PMID: 19885876 DOI: 10.1002/hep.23264]
10 **Bouachour G**, Tirot P, Varache N, Gouello JP, Harry P, Alquier P. Hemodynamic changes in acute adrenal insufficiency. *Intensive Care Med* 1994; **20**: 138-141 [PMID: 8201094 DOI: 10.1007/BF01707669]
11 **Blendis L**, Wong F. The hyperdynamic circulation in cirrhosis: an overview. *Pharmacol Ther* 2001; **89**: 221-231 [PMID: 11516477 DOI: 10.1016/S0163-7258(01)00124-3]
12 **Marik PE**, Gayowski T, Starzl TE, Hepatic Cortisol Research and Adrenal Pathophysiology Study Group. The hepatoadrenal syndrome: a common yet unrecognized clinical condition. *Crit Care Med* 2005; **33**: 1254-1259 [PMID: 15942340 DOI: 10.1097/01.CCM.0000164541.12106.57]
13 **Fernández J**, Escorsell A, Zabalza M, Felipe V, Navasa M, Mas A, Lacy AM, Ginès P, Arroyo V. Adrenal insufficiency in patients with cirrhosis and septic shock: Effect of treatment with hydrocortisone on survival. *Hepatology* 2006; **44**: 1288-1295 [PMID: 17058239 DOI: 10.1002/hep.21352]
14 **Harry R**, Auzinger G, Wendon J. The effects of supraphysiological doses of corticosteroids in hypotensive liver failure. *Liver Int* 2003; **23**: 71-77 [PMID: 12654129 DOI: 10.1034/j.1600-0676.2003.00813.x]
15 **Tan T**, Chang L, Woodward A, McWhinney B, Galligan J, Macdonald GA, Cohen J, Venkatesh B. Characterising adrenal function using directly measured plasma free cortisol in stable severe liver disease. *J Hepatol* 2010; **53**: 841-848 [PMID: 20739086 DOI: 10.1016/j.jhep.2010.05.020]
16 **Triantos CK**, Marzigie M, Fede G, Michalaki M, Giannakopoulou D, Thomopoulos K, Garcovich M, Kalafateli M, Chronis A, Kyriazopoulou V, Jelastopoulou E, Nikolopoulou V, O'Beirne J, Burroughs AK. Critical illness-related corticosteroid insufficiency in patients with cirrhosis and variceal bleeding. *Clin Gastroenterol Hepatol* 2011; **9**: 595-601 [PMID: 21545846 DOI: 10.1016/j.cgh.2011.03.033]
17 **Fede G**, Spadaro L, Tomaselli T, Privitera G, Piro S, Rabuazzo AM, Sigalas A, Xirouchakis E, O'Beirne J, Garcovich M, Tsochatzis E, Purrello F, Burroughs AK. Assessment of adrenocortical reserve in stable patients with cirrhosis. *J Hepatol* 2011; **54**: 243-250 [PMID: 21056503 DOI: 10.1016/j.jhep.2010.06.034]
18 **Risso A**, Alessandria C, Elia C, Mezzabotta L, Andrealli A, Spandre M, Morgando A, Marzano A, Rizzetto M. Adrenal dysfunction in nonseptic cirrhotic patients with ascites: Impact on survival. *Dig Liv Dis* 2011; **43** Suppl 2: S74-75 [DOI: 10.1016/S1590-8658(11)60031-5]
19 **Acevedo J**, Fernandez J, Castro M, Roca D, Gines P, Arroyo V. Prognostic value of relative adrenal insufficiency in decompensated cirrhosis. *J Hepatol* 2010; **52** Suppl 1: S65 [DOI: 10.1016/S0168-8278(10)60148-1]
20 **Graupera I**, Hernandez-Gea V, Rodriguez J, Colomo A, Poca M, Llao J, Rigla M, Aracil C, Guarner C, Villanueva C. Incidence and prognostic significance of relative adrenal insufficiency in cirrhotic patients with severe variceal bleeding (abstract). The Liver Meeting® 2010 (AASLD). *Hepatology* 2010; **52** Suppl 1: 267A
21 **Toniutto P**, Fabris C, Fumolo E, Bitetto D, Fornasiere E, Falleti E, Rapetti R, Minisini R, Pirisi M. Prevalence and risk factors for delayed adrenal insufficiency after liver transplantation. *Liver Transpl* 2008; **14**: 1014-1019 [PMID: 18581463 DOI: 10.1002/lt.21465]
22 **Iwasaki T**, Tominaga M, Fukumoto T, Kusunoki N, Sugimoto T, Kido M, Ogata S, Takebe A, Tanaka M, Ku Y. Relative adrenal insufficiency manifested with multiple organ dysfunction in a liver transplant patient. *Liver Transpl* 2006; **12**: 1896-1899 [PMID: 17133575 DOI: 10.1002/lt.21006]
23 **Singh N**, Gayowski T, Marino IR, Schlichtig R. Acute adrenal insufficiency in critically ill liver transplant recipients. Implications for diagnosis. *Transplantation* 1995; **59**: 1744-1745 [PMID: 7604447 DOI: 10.1097/00007890-199506270-00020]
24 **Bornstein SR**. Predisposing factors for adrenal insufficiency. *N Engl J Med* 2009; **360**: 2328-2339 [PMID: 19474430 DOI: 10.1056/NEJMra0804635]
25 **Gaillard RC**, Turnill D, Sappino P, Muller AF. Tumor necrosis factor alpha inhibits the hormonal response of the pituitary gland to hypothalamic releasing factors. *Endocrinology* 1990; **127**: 101-106 [PMID: 2163305 DOI: 10.1210/endo-127-1-101]
26 **Cicognani C**, Malavolti M, Morselli-Labate AM, Zamboni L, Sama C, Barbara L. Serum lipid and lipoprotein patterns in patients with liver cirrhosis and chronic active hepatitis. *Arch Intern Med* 1997; **157**: 792-796 [PMID: 9125012 DOI: 10.1001/archinte.1997.00440280120012]
27 **Albillos A**, de la Hera A, González M, Moya JL, Calleja JL, Monserrat J, Ruiz-del-Arbol L, Alvarez-Mon M. Increased lipopolysaccharide binding protein in cirrhotic patients with marked immune and hemodynamic derangement. *Hepatology* 2003; **37**: 208-217 [PMID: 12500206 DOI: 10.1053/jhep.2003.50038]
28 **Annane D**, Sébille V, Charpentier C, Bollaert PE, François B, Korach JM, Capellier G, Cohen Y, Azoulay E, Troché G, Chaumet-Riffaud P, Bellissant E. Effect of treatment with low doses of hydrocortisone and fludrocortisone on mortality in patients with septic shock. *JAMA* 2002; **288**: 862-871 [PMID: 12186604 DOI: 10.1001/jama.288.7.862]
29 **Arabi YM**, Aljumah A, Dabbagh O, Tamim HM, Rishu AH, Al-Abdulkareem A, Knawy BA, Hajeer AH, Tamimi W, Cherfan A. Low-dose hydrocortisone in patients with cirrhosis and septic shock: a randomized controlled trial. *CMAJ* 2010; **182**: 1971-1977 [PMID: 21059778 DOI: 10.1503/cmaj.090707]
30 **Arlt W**, Stewart PM. Adrenal corticosteroid biosynthesis, metabolism, and action. *Endocrinol Metab Clin North Am* 2005; **34**: 293-313, viii [PMID: 15850843 DOI: 10.1016/j.ecl.2005.01.002]
31 **Snijdewint FG**, Kapsenberg ML, Wauben-Penris PJ, Bos JD. Corticosteroids class-dependently inhibit in vitro Th1- and Th2-type cytokine production. *Immunopharmacology* 1995; **29**: 93-101 [PMID: 7775161 DOI: 10.1016/0162-3109(94)00048-K]
32 **Yang S**, Zhang L. Glucocorticoids and vascular reactivity. *Curr Vasc Pharmacol* 2004; **2**: 1-12 [PMID: 15320828 DOI: 10.2174/1570161043476483]

33 **Stewart PM.** The adrenal cortex. In: Kronenberg HM, Melmed S, Polonsky KS, Larsen PR, editors. Williams textbook of endocrinology. 11th ed. Philadelphia: Saunders, 2008: 445-503

34 **Marik PE**. Mechanisms and clinical consequences of critical illness associated adrenal insufficiency. *Curr Opin Crit Care* 2007; **13**: 363-369 [PMID: 17599004 DOI: 10.1097/MCC.0b013e32818a6d74]

35 **Hamrahian AH**, Oseni TS, Arafah BM. Measurements of serum free cortisol in critically ill patients. *N Engl J Med* 2004; **350**: 1629-1638 [PMID: 15084695 DOI: 10.1056/NEJMoa020266]

36 **de Jong MF**, Beishuizen A, Spijkstra JJ, Groeneveld AB. Relative adrenal insufficiency as a predictor of disease severity, mortality, and beneficial effects of corticosteroid treatment in septic shock. *Crit Care Med* 2007; **35**: 1896-1903 [PMID: 17568326 DOI: 10.1097/01.CCM.0000275387.51629.ED]

37 **Duma D**, Jewell CM, Cidlowski JA. Multiple glucocorticoid receptor isoforms and mechanisms of post-translational modification. *J Steroid Biochem Mol Biol* 2006; **102**: 11-21 [PMID: 17070034 DOI: 10.1016/j.jsbmb.2006.09.009]

38 **Prigent H**, Maxime V, Annane D. Clinical review: corticotherapy in sepsis. *Crit Care* 2004; **8**: 122-129 [PMID: 15025773 DOI: 10.1186/cc2374]

39 **Briegel J**, Forst H, Haller M, Schelling G, Kilger E, Kuprat G, Hemmer B, Hummel T, Lenhart A, Heyduck M, Stoll C, Peter K. Stress doses of hydrocortisone reverse hyperdynamic septic shock: a prospective, randomized, double-blind, single-center study. *Crit Care Med* 1999; **27**: 723-732 [PMID: 10321661 DOI: 10.1097/00003246-199904000-00025]

40 **Oppert M**, Schindler R, Husung C, Offermann K, Gräf KJ, Boenisch O, Barckow D, Frei U, Eckardt KU. Low-dose hydrocortisone improves shock reversal and reduces cytokine levels in early hyperdynamic septic shock. *Crit Care Med* 2005; **33**: 2457-2464 [PMID: 16276166 DOI: 10.1097/01.CCM.0000186370.78639.23]

41 **Cirera I**, Bauer TM, Navasa M, Vila J, Grande L, Taurá P, Fuster J, García-Valdecasas JC, Lacy A, Suárez MJ, Rimola A, Rodés J. Bacterial translocation of enteric organisms in patients with cirrhosis. *J Hepatol* 2001; **34**: 32-37 [PMID: 11211904 DOI: 10.1016/S0168-8278(00)00013-1]

42 **Marik PE**. Adrenal-exhaustion syndrome in patients with liver disease. *Intensive Care Med* 2006; **32**: 275-280 [PMID: 16432671 DOI: 10.1007/s00134-005-0005-5]

43 **O'Beirne J**, Holmes M, Agarwal B, Bouloux P, Shaw S, Patch D, Burroughs A. Adrenal insufficiency in liver disease - what is the evidence? *J Hepatol* 2007; **47**: 418-423 [PMID: 17629587 DOI: 10.1016/j.jhep.2007.06.008]

44 **Thevenot T**, Borot S, Remy-Martin A, Sapin R, Penfornis A, Di Martino V, Monnet E. Assessing adrenal function in cirrhotic patients: is there a reliable test? *Gastroenterol Clin Biol* 2009; **33**: 584-588 [PMID: 19473798 DOI: 10.1016/j.gcb.2009.03.011]

45 **Galbois A**, Rudler M, Massard J, Fulla Y, Bennani A, Bonnefont-Rousselot D, Thibault V, Reignier S, Bourrier A, Poynard T, Thabut D. Assessment of adrenal function in cirrhotic patients: salivary cortisol should be preferred. *J Hepatol* 2010; **52**: 839-845 [PMID: 20385427 DOI: 10.1016/j.jhep.2010.01.026]

46 **O'Beirne J.** Adrenal function in liver disease. In: Gines P, Forns X, Abraldes JG, Fernndez J, Bataller R, Rodes J, Arroyo V, editors. Therapy in Liver Diseases. Barcelona: Elsevier, 2011: 217-225

47 **Etogo-Asse FE**, Vincent RP, Hughes SA, Auzinger G, Le Roux CW, Wendon J, Bernal W. High density lipoprotein in patients with liver failure; relation to sepsis, adrenal function and outcome of illness. *Liver Int* 2012; **32**: 128-136 [PMID: 22098564 DOI: 10.1111/j.1478-3231.2011.02657.x]

48 **Amarapurkar DN**. Adrenal function in cirrhosis: the pendulum swings. *J Gastroenterol Hepatol* 2012; **27**: 1543-1544 [PMID: 22994431 DOI: 10.1111/j.1440-1746.2012.07235.x]

49 **Fede G**, Spadaro L, Tomaselli T, Privitera G, Germani G, Tsochatzis E, Thomas M, Bouloux PM, Burroughs AK, Purrello F. Adrenocortical dysfunction in liver disease: a systematic review. *Hepatology* 2012; **55**: 1282-1291 [PMID: 22234976 DOI: 10.1002/hep.25573]

50 **Beishuizen A**, Thijs LG, Vermes I. Decreased levels of dehydroepiandrosterone sulphate in severe critical illness: a sign of exhausted adrenal reserve? *Crit Care* 2002; **6**: 434-438 [PMID: 12398784 DOI: 10.1186/cc1530]

51 **Marik PE**, Zaloga GP. Adrenal insufficiency in the critically ill: a new look at an old problem. *Chest* 2002; **122**: 1784-1796 [PMID: 12426284 DOI: 10.1378/chest.122.5.1784]

52 **Marik PE**, Zaloga GP. Adrenal insufficiency during septic shock. *Crit Care Med* 2003; **31**: 141-145 [PMID: 12545007 DOI: 10.1097/00003246-200301000-00022]

53 **van Leeuwen HJ**, Heezius EC, Dallinga GM, van Strijp JA, Verhoef J, van Kessel KP. Lipoprotein metabolism in patients with severe sepsis. *Crit Care Med* 2003; **31**: 1359-1366 [PMID: 12771603 DOI: 10.1097/01.CCM.0000059724.08290.51]

54 **van der Voort PH**, Gerritsen RT, Bakker AJ, Boerma EC, Kuiper MA, de Heide L. HDL-cholesterol level and cortisol response to synacthen in critically ill patients. *Intensive Care Med* 2003; **29**: 2199-2203 [PMID: 14566456 DOI: 10.1007/s00134-003-2021-7]

55 **Marik PE**. Adrenal insufficiency: the link between low apolipoprotein A-I levels and poor outcome in the critically ill? *Crit Care Med* 2004; **32**: 1977-1978; author reply 1977-1978 [PMID: 15343040 DOI: 10.1097/01.CCM.0000132895.89019.32]

56 **Chien JY**, Jerng JS, Yu CJ, Yang PC. Low serum level of high-density lipoprotein cholesterol is a poor prognostic factor for severe sepsis. *Crit Care Med* 2005; **33**: 1688-1693 [PMID: 16096442 DOI: 10.1097/01.CCM.0000171183.79525.6B]

57 **Yaguchi H**, Tsutsumi K, Shimono K, Omura M, Sasano H, Nishikawa T. Involvement of high density lipoprotein as substrate cholesterol for steroidogenesis by bovine adrenal fasciculo-reticularis cells. *Life Sci* 1998; **62**: 1387-1395 [PMID: 9585166 DOI: 10.1016/S0024-3205(98)00077-0]

58 **Ettinger WH**, Varma VK, Sorci-Thomas M, Parks JS, Sigmon RC, Smith TK, Verdery RB. Cytokines decrease apolipoprotein accumulation in medium from Hep G2 cells. *Arterioscler Thromb* 1994; **14**: 8-13 [PMID: 8274481 DOI: 10.1161/01.ATV.14.1.8]

59 **Baranova I**, Vishnyakova T, Bocharov A, Chen Z, Remaley AT, Stonik J, Eggerman TL, Patterson AP. Lipopolysaccharide down regulates both scavenger receptor B1 and ATP binding cassette transporter A1 in RAW cells. *Infect Immun* 2002; **70**: 2995-3003 [PMID: 12010990 DOI: 10.1128/IAI.70.6.2995-3003.2002]

60 **Vishnyakova TG**, Bocharov AV, Baranova IN, Chen Z, Remaley AT, Csako G, Eggerman TL, Patterson AP. Binding and internalization of lipopolysaccharide by Cla-1, a human orthologue of rodent scavenger receptor B1. *J Biol Chem* 2003; **278**: 22771-22780 [PMID: 12651854 DOI: 10.1074/jbc.M211032200]

61 **Cervoni JP**, Thévenot T, Weil D, Muel E, Barbot O, Sheppard F, Monnet E, Di Martino V. C-reactive protein predicts short-term mortality in patients with cirrhosis. *J Hepatol* 2012; **56**: 1299-1304 [PMID: 22314431 DOI: 10.1016/j.jhep.2011.12.030]

62 **Zapater P**, Francés R, González-Navajas JM, de la Hoz MA, Moreu R, Pascual S, Monfort D, Montoliu S, Vila C, Escudero A, Torras X, Cirera I, Llanos L, Guarner-Argente C, Palazón JM, Carnicer F, Bellot P, Guarner C, Planas R, Solá R, Serra MA, Muñoz C, Pérez-Mateo M, Such J. Serum and ascitic fluid bacterial DNA: a new independent prognostic factor in noninfected patients with cirrhosis. *Hepatology* 2008; **48**: 1924-1931 [PMID: 19003911 DOI: 10.1002/hep.22564]

63 **Jang JY,** Cho WY, Jeong SW, Kim SG, Cheon YK, Kim YS, Cho YD, Kim H, Lee JS, Shim CS, Kim BS. Relative adrenal insufficiency in patients with chronic liver disease. *Hepatology* 2008; **48:** 1088A

64 **Thierry S**, Giroux Leprieur E, Lecuyer L, Brocas E, Van de Louw A. Echocardiographic features, mortality, and adrenal function in patients with cirrhosis and septic shock. *Acta Anaesthesiol Scand* 2008; **52**: 45-51 [PMID: 17996000 DOI: 10.1111/j.1399-6576.2007.01491.x]

65 **du Cheyron D**, Bouchet B, Cauquelin B, Guillotin D, Ramakers M, Daubin C, Ballet JJ, Charbonneau P. Hyperreninemic hypoaldosteronism syndrome, plasma concentrations of interleukin-6 and outcome in critically ill patients with liver cirrhosis. *Intensive Care Med* 2008; **34**: 116-124 [PMID: 17906854 DOI: 10.1007/s00134-007-0864-z]

66 **El Damarawy M,** Hamed G, Heikal A, Darwish H,Badr M. Meld Score as a Predictor for Hepato Adrenal Syndrome. *J Am Sci* 2012; **8:** 208-211

67 **Thevenot T**, Dorin R, Monnet E, Qualls CR, Sapin R, Grandclement E, Borot S, Sheppard F, Weil D, Degand T, Di Martino V, Kazlauskaite R. High serum levels of free cortisol indicate severity of cirrhosis in hemodynamically stable patients. *J Gastroenterol Hepatol* 2012; **27**: 1596-1601 [PMID: 22647073 DOI: 10.1111/j.1440-1746.2012.07188.x]

68 **Coolens JL**, Van Baelen H, Heyns W. Clinical use of unbound plasma cortisol as calculated from total cortisol and corticosteroid-binding globulin. *J Steroid Biochem* 1987; **26**: 197-202 [PMID: 3560936 DOI: 10.1016/0022-4731(87)90071-9]

69 **McDonald JA**, Handelsman DJ, Dilworth P, Conway AJ, McCaughan GW. Hypothalamic-pituitary adrenal function in end-stage non-alcoholic liver disease. *J Gastroenterol Hepatol* 1993; **8**: 247-253 [PMID: 8390870 DOI: 10.1111/j.1440-1746.1993.tb01195.x]

70 **Wiest R**, Moleda L, Zietz B, Hellerbrand C, Schölmerich J, Straub R. Uncoupling of sympathetic nervous system and hypothalamic-pituitary-adrenal axis in cirrhosis. *J Gastroenterol Hepatol* 2008; **23**: 1901-1908 [PMID: 18554237 DOI: 10.1111/j.1440-1746.2008.05456.x]

71 **Arafah BM**. Hypothalamic pituitary adrenal function during critical illness: limitations of current assessment methods. *J Clin Endocrinol Metab* 2006; **91**: 3725-3745 [PMID: 16882746 DOI: 10.1210/jc.2006-0674]

72 **Vogeser M**, Groetzner J, Küpper C, Briegel J. Free serum cortisol during the postoperative acute phase response determined by equilibrium dialysis liquid chromatography-tandem mass spectrometry. *Clin Chem Lab Med* 2003; **41**: 146-151 [PMID: 12666999 DOI: 10.1515/CCLM.2003.024]

73 **Vincent RP,** Etogo-Asse FE, Dew T, Bernal W, Alaghband-Zadeh J, le Roux CW. Serum total cortisol and free cortisol index give different information regarding the hypothalamus-pituitary-adrenal axis reserve in patients with liver impairment. *Ann Clin Biochem* 2009; **46:** 505-507 [DOI: 10.1258/acb.2009.009030]

74 **Thevenot T**, Borot S, Remy-Martin A, Sapin R, Cervoni JP, Richou C, Vanlemmens C, Cleau D, Muel E, Minello A, Tirziu S, Penfornis A, Di Martino V, Monnet E. Assessment of adrenal function in cirrhotic patients using concentration of serum-free and salivary cortisol. *Liver Int* 2011; **31**: 425-433 [PMID: 21281437 DOI: 10.1111/j.1478-3231.2010.02431.x]

75 **Cohen J**, Smith ML, Deans RV, Pretorius CJ, Ungerer JP, Tan T, Jones M, Venkatesh B. Serial changes in plasma total cortisol, plasma free cortisol, and tissue cortisol activity in patients with septic shock: an observational study. *Shock* 2012; **37**: 28-33 [PMID: 21993448 DOI: 10.1097/SHK.0b013e318239b809]

76 **Molenaar N**, Johan Groeneveld AB, Dijstelbloem HM, de Jong MF, Girbes AR, Heijboer AC, Beishuizen A. Assessing adrenal insufficiency of corticosteroid secretion using free versus total cortisol levels in critical illness. *Intensive Care Med* 2011; **37**: 1986-1993 [PMID: 21850531 DOI: 10.1007/s00134-011-2342-x]

77 **Schlienger JL,** Sapin R, Gasser F, Briche-Prouveur S, Dreyfuss M. Intérêt cortisol libre plasmatique en pratique Clinique. *Sem Hop Paris* 1989; **65:** 2067-2070

78 **le Roux CW**, Chapman GA, Kong WM, Dhillo WS, Jones J, Alaghband-Zadeh J. Free cortisol index is better than serum total cortisol in determining hypothalamic-pituitary-adrenal status in patients undergoing surgery. *J Clin Endocrinol Metab* 2003; **88**: 2045-2048 [PMID: 12727952 DOI: 10.1210/jc.2002-021532]

79 **Arafah BM**, Nishiyama FJ, Tlaygeh H, Hejal R. Measurement of salivary cortisol concentration in the assessment of adrenal function in critically ill subjects: a surrogate marker of the circulating free cortisol. *J Clin Endocrinol Metab* 2007; **92**: 2965-2971 [PMID: 17535998 DOI: 10.1210/jc.2007-0181]

80 **Soni A**, Pepper GM, Wyrwinski PM, Ramirez NE, Simon R, Pina T, Gruenspan H, Vaca CE. Adrenal insufficiency occurring during septic shock: incidence, outcome, and relationship to peripheral cytokine levels. *Am J Med* 1995; **98**: 266-271 [PMID: 7872343 DOI: 10.1016/S0002-9343(99)80373-8]

81 **Fernández J,** Acevedo J. Adrenal Function in Chronic Liver Failure. In: Ginès P, Kamath PS, Arroyo V, editors. Chronic Liver Failure: Mechanisms and Management. New York: Humana Press Inc., 2011: 377-391

82 **Kazlauskaite R**, Evans AT, Villabona CV, Abdu TA, Ambrosi B, Atkinson AB, Choi CH, Clayton RN, Courtney CH, Gonc EN, Maghnie M, Rose SR, Soule SG, Tordjman K, Consortium for Evaluation of Corticotropin Test in Hypothalamic-Pituitary Adrenal Insufficiency. Corticotropin tests for hypothalamic-pituitary- adrenal insufficiency: a meta-analysis. *J Clin Endocrinol Metab* 2008; **93**: 4245-4253 [PMID: 18697868 DOI: 10.1210/jc.2008-0710]

83 **Dorin RI**, Qualls CR, Crapo LM. Diagnosis of adrenal insufficiency. *Ann Intern Med* 2003; **139**: 194-204 [PMID: 12899587]

84 **Agha A**, Tomlinson JW, Clark PM, Holder G, Stewart PM. The long-term predictive accuracy of the short synacthen (corticotropin) stimulation test for assessment of the hypothalamic-pituitary-adrenal axis. *J Clin Endocrinol Metab* 2006; **91**: 43-47 [PMID: 16249286 DOI: 10.1210/jc.2005-1131]

85 **Mohamed MB,** Hamed G Heikal A, Darwish H. Prevalence of adenocortical insufficiency in patients with liver cirrhosis, liver cirrhosis with septic shock and in patients with hepatorenal syndrome. *J Am Sci* 2011; **7:** 391-400

86 **Vasu TS,** Stewart J, Cavallazzi RS, Hirani A, Marik PE. Hepatoadrenal syndrome: prevalence and factors predicting adrenal insufficiency in critically ill patients with liver disease. *Am J Respir Crit Care Med* 2009; **179:** A1588

87 **Sigalas A,** Xirouchakis E, Manousou P, Corbani A, Calvaruso V, Patch D, Burroughs AK, O'Beirne J. Adrenal impairment is frequent finding in stable cirrhosis and is related to disease severity. *Hepatology* 2007; **46:** 573A

88 **Alessandria C,** Mezzabotta L, Carello M, Debernardi-Venon W, Martini S, Rizzetto M, Marzano A. Relative adrenal insufficiency in cirrhosis: relevance in patients with ascites and treatment with hydrocortisone in refractory ascites. *Digest Liver Dis* 2009; **41:** A13 [DOI: 10.1016/j.dld.2008.12.029]

89 **Acevedo J,** Fernández J, Castro M, Roca D, Ginès P, Arroyo V. Impact of relative adrenal insufficiency on circulatory function and mortality in advanced cirrhosis. *J Hepatol* 2011; **54** Suppl 1: S61 [DOI: 10.1016/S0168-8278(11)60141-4]

90 **Patel SBR,** Butt T, O'Beirne J, Mallett S. Steroid administration during liver transplantation reduces the need for physiological support post operatively - More evidence for relative adrenal insufficiency in liver failure. *Eur J Anaesthesiol* 2010; **27:** 176 [DOI: 10.1097/00003643-201006121-00563]

91 **Ullian ME**. The role of corticosteriods in the regulation of vascular tone. *Cardiovasc Res* 1999; **41**: 55-64 [PMID: 10325953 DOI: 10.1016/S0008-6363(98)00230-2]

92 **Beishuizen A**, Thijs LG. Relative adrenal failure in intensive care: an identifiable problem requiring treatment? *Best Pract*

Res Clin Endocrinol Metab 2001; **15**: 513-531 [PMID: 11800521 DOI: 10.1053/beem.2001.0167]
93 **Keh D**, Boehnke T, Weber-Cartens S, Schulz C, Ahlers O, Bercker S, Volk HD, Doecke WD, Falke KJ, Gerlach H. Immunologic and hemodynamic effects of "low-dose" hydrocortisone in septic shock: a double-blind, randomized, placebo-controlled, crossover study. *Am J Respir Crit Care Med* 2003; **167**: 512-520 [PMID: 12426230 DOI: 10.1164/rccm.200205-446OC]
94 **Radomski MW**, Palmer RM, Moncada S. Glucocorticoids inhibit the expression of an inducible, but not the constitutive, nitric oxide synthase in vascular endothelial cells. *Proc Natl Acad Sci USA* 1990; **87**: 10043-10047 [PMID: 1702214 DOI: 10.1073/pnas.87.24.10043]
95 **Annane D**, Bellissant E, Sebille V, Lesieur O, Mathieu B, Raphael JC, Gajdos P. Impaired pressor sensitivity to noradrenaline in septic shock patients with and without impaired adrenal function reserve. *Br J Clin Pharmacol* 1998; **46**: 589-597 [PMID: 9862249 DOI: 10.1046/j.1365-2125.1998.00833.x]
96 **Chrousos GP**. The hypothalamic-pituitary-adrenal axis and immune-mediated inflammation. *N Engl J Med* 1995; **332**: 1351-1362 [PMID: 7715646 DOI: 10.1056/NEJM199505183322008]
97 **Turnbull AV**, Rivier CL. Regulation of the hypothalamic-pituitary-adrenal axis by cytokines: actions and mechanisms of action. *Physiol Rev* 1999; **79**: 1-71 [PMID: 9922367]
98 **Minneci PC**, Deans KJ, Banks SM, Eichacker PQ, Natanson C. Meta-analysis: the effect of steroids on survival and shock during sepsis depends on the dose. *Ann Intern Med* 2004; **141**: 47-56 [PMID: 15238370]
99 **Bellissant E**, Annane D. Effect of hydrocortisone on phenylephrine-mean arterial pressure dose-response relationship in septic shock. *Clin Pharmacol Ther* 2000; **68**: 293-303 [PMID: 11014411 DOI: 10.1067/mcp.2000.109354]
100 **Bollaert PE**, Charpentier C, Levy B, Debouverie M, Audibert G, Larcan A. Reversal of late septic shock with supraphysiologic doses of hydrocortisone. *Crit Care Med* 1998; **26**: 645-650 [PMID: 9559600 DOI: 10.1097/00003246-199804000-00010]
101 **Meduri GU**, Marik PE, Chrousos GP, Pastores SM, Arlt W, Beishuizen A, Bokhari F, Zaloga G, Annane D. Steroid treatment in ARDS: a critical appraisal of the ARDS network trial and the recent literature. *Intensive Care Med* 2008; **34**: 61-69 [PMID: 18000649 DOI: 10.1007/s00134-007-0933-3]
102 **Sprung CL**, Annane D, Keh D, Moreno R, Singer M, Freivogel K, Weiss YG, Benbenishty J, Kalenka A, Forst H, Laterre PF, Reinhart K, Cuthbertson BH, Payen D, Briegel J, CORTICUS Study Groupf. Hydrocortisone therapy for patients with septic shock. *N Engl J Med* 2008; **358**: 111-124 [PMID: 18184957 DOI: 10.1056/NEJMoa071366]
103 **Marik PE**. Critical illness-related corticosteroid insufficiency. *Chest* 2009; **135**: 181-193 [PMID: 19136406 DOI: 10.1378/chest.08-1149]
104 **Confalonieri M**, Urbino R, Potena A, Piattella M, Parigi P, Puccio G, Della Porta R, Giorgio C, Blasi F, Umberger R, Meduri GU. Hydrocortisone infusion for severe community-acquired pneumonia: a preliminary randomized study. *Am J Respir Crit Care Med* 2005; **171**: 242-248 [PMID: 15557131 DOI: 10.1164/rccm.200406-808OC]
105 **Cicarelli DD**, Vieira JE, Benseñor FE. Early dexamethasone treatment for septic shock patients: a prospective randomized clinical trial. *Sao Paulo Med J* 2007; **125**: 237-241 [PMID: 17992396 DOI: 10.1590/S1516-31802007000400009]
106 **Cronin L**, Cook DJ, Carlet J, Heyland DK, King D, Lansang MA, Fisher CJ. Corticosteroid treatment for sepsis: a critical appraisal and meta-analysis of the literature. *Crit Care Med* 1995; **23**: 1430-1439 [PMID: 7634816 DOI: 10.1097/00003246-199508000-00019]
107 **The Veterans Administration Systemic Sepsis Cooperative Study Group.** Effect of high-dose glucocorticoid therapy on mortality in patients with clinical signs of systemic sepsis. *N Engl J Med* 1987; **317**: 659-665 [PMID: 2888017]
108 **Lefering R**, Neugebauer EA. Steroid controversy in sepsis and septic shock: a meta-analysis. *Crit Care Med* 1995; **23**: 1294-1303 [PMID: 7600840 DOI: 10.1097/00003246-199507000-00021]
109 **Schneider AJ**, Voerman HJ. Abrupt hemodynamic improvement in late septic shock with physiological doses of glucocorticoids. *Intensive Care Med* 1991; **17**: 436-437 [PMID: 1774404 DOI: 10.1007/BF01720688]
110 **Chawla K,** Kupfer Y, Goldman I. Hydrocorticsone reverses refractory septic shock. *Crit Care Med* 1999; **27** Suppl 1: A33 [DOI: 10.1097/00003246-199901001-00022]
111 **Yildiz O**, Doganay M, Aygen B, Güven M, Keleştimur F, Tutuû A. Physiological-dose steroid therapy in sepsis [ISRCTN36253388]. *Crit Care* 2002; **6**: 251-259 [PMID: 12133187]
112 **Zietz B**, Lock G, Plach B, Drobnik W, Grossmann J, Schölmerich J, Straub RH. Dysfunction of the hypothalamic-pituitary-glandular axes and relation to Child-Pugh classification in male patients with alcoholic and virus-related cirrhosis. *Eur J Gastroenterol Hepatol* 2003; **15**: 495-501 [PMID: 12702906 DOI: 10.1097/00042737-200305000-00007]

P- Reviewers LesmanaLA, Llompart-Pou J
S- Editor Song XX **L- Editor** A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i4.457

World J Gastroenterol 2013 January 28; 19(4): 457-462
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

REVIEW

Obesity and cholangiocarcinoma

Mansour A Parsi

Mansour A Parsi, Center for Endoscopy and Pancreatobiliary Disorders, Digestive Disease Institute, Cleveland Clinic, Cleveland, OH 44195, United States
Author contributions: Parsi MA is the sole contributor to this manuscript.
Correspondence to: Mansour A Parsi, MD, MPH, Center for Endoscopy and Pancreatobiliary Disorders, Digestive Disease Institute, Cleveland Clinic, Cleveland, OH 44195, United States. parsim@ccf.org
Telephone: +1-216-4454880 Fax: +1-216-4446284
Received: June 21, 2012 Revised: August 3, 2012
Accepted: August 14, 2012
Published online: January 28, 2013

Abstract

It is estimated that about half of the population in developed countries are either overweight or obese. In some developing nations obesity rates have increased to surpass those seen in Western countries. This rate increase in obesity has many implications as obesity has been associated with numerous negative health effects including increased risks of hypertension, diabetes, cardiovascular disease, stroke, liver disease, apnea, and some cancer types. Obesity is now considered to be one of the major public health concerns facing the society. Cholangiocarcinomas (bile duct cancers) are malignant tumors arising from cholangiocytes inside or outside of the liver. Although cholangiocarcinomas are relatively rare, they are highly lethal. The low survival rate associated with cholangiocarcinoma is due to the advanced stage of the disease at the time of diagnosis. Prevention is therefore especially important in this cancer type. Some data suggest that the incidence of cholangiocarcinoma in the western world is on the rise. Increasing rate of obesity may be one of the factors responsible for this increase. Determining whether obesity is a risk factor for cholangiocarcinoma has significant clinical and societal implications as obesity is both prevalent and modifiable. This paper seeks to provide a summary of the current knowledge linking obesity and cholangiocarcinoma, and encourage further research on this topic.

© 2013 Baishideng. All rights reserved.

Key words: Cholangiocarcinoma; Obesity; Epidemiology; Bile ducts; Cancer

Parsi MA. Obesity and cholangiocarcinoma. *World J Gastroenterol* 2013; 19(4): 457-462 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i4/457.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i4.457

INTRODUCTION

Obesity is increasing worldwide and affects a large portion of the population in developed[1-5] and some developing nations[6-13]. In many countries obesity and its comorbidities have become a major public health concern[1,14-16]. Recently, obesity-associated cancer has attracted great attention. Epidemiological studies have provided strong evidence that obesity can increase the incidence of many cancer types[17] including colon[18,19], breast[20,21], liver[22], endometrium[23,24], kidney[25], esophagus[26], stomach[27] and pancreas[28].

Cholangiocarcinomas (bile duct cancers) are malignant tumors arising from cholangiocytes inside or outside of the liver. Traditionally cancers arising from the cholangiocytes inside the liver were grouped together with hepatic cancers and classified as primary liver tumors, while those arising from the cholangiocytes outside the liver were grouped together with cancers of the gallbladder and the ampulla of Vater and classified as biliary cancers[29]. More recently, the term cholangiocarcinoma has been used to refer to cancers arising from cholangiocytes irrespective of their location[29].

Intrahepatic cholangiocarcinomas originate from cholangiocytes lining the small intrahepatic ductules while extrahepatic cholangiocarcinomas arise from cholangiocytes lining the larger bile ducts (right and left hepatic ducts, common hepatic duct, and common bile duct)[30]. The distinction between intrahepatic and extrahepatic cholangiocarcinomas is important, as the incidence and risk factors associated with each may be different[31-33].

In Western countries established risk factors for cholangiocarcinoma are age, sex, primary sclerosing cholangitis, and bile duct cysts. Less-established potential risk factors include cirrhosis, diabetes, obesity, alcohol, and smoking. Some data suggest that the incidence of cholangiocarcinoma in the Western world is on the rise. Increasing rate of obesity may be one of the factors responsible for this increase.

Although cholangiocarcinomas are rare in Western countries, they are highly lethal[34]. Annual mortality associated with cholangiocarcinoma is virtually identical to its incidence throughout the world, underscoring the high case fatality rate of this cancer type[35]. The low survival rate associated with cholangiocarcinoma is due to the advanced stage of the disease at the time of diagnosis[36]. Prevention is therefore especially important in this cancer type. Determining whether obesity is a risk factor for cholangiocarcinoma has significant clinical and societal implications as obesity is both prevalent and modifiable.

EPIDEMIOLOGY

Obesity prevalence

Obesity is considered to be among the most alarming health concerns in the United States. It has been estimated that each year, more than 300 000 Americans die from illnesses related to overweight and obesity[37]. The Centers for Disease Control and Prevention (CDC) defines obesity based on the body mass index (BMI). BMI is the ratio of weight in kilograms (kg) to height in meters squared (m^2). Per CDC criteria, a BMI of 18.5-25 kg/m^2 is normal, 25-30 kg/m^2 is overweight and $\geqslant$ 30 kg/m^2 is obese[38]. The most recent statistics from the National Health and Nutrition Examination Surveys using BMI as a measure of overweight and obesity indicate that 35.7% of adults 20 years or older were obese in 2009-2010[39]. Compared with the statistics for 1976-1980 this reflects a 2-fold increase in the prevalence of adult obesity[40,41]. This rate increase in obesity has many implications as obesity has been associated with numerous negative health effects including increased risks of hypertension, diabetes, cardiovascular disease, stroke, liver disease, apnea, and some cancer types possibly also cholangiocarcinoma. Therefore, as a potentially modifiable risk factor, a reduction in the prevalence of obesity among adults could have a substantial impact on morbidity, mortality, and incidence of various cancers.

Cholangiocarcinoma incidence

The available epidemiologic data on incidence and prevalence of cholangiocarcinoma are difficult to interpret because of differing classifications and diagnostic criteria across studies[29,32,42]. For instance, some studies group intrahepatic cholangiocarcinomas with liver cancers while others group extrahepatic cholangiocarcinomas with gallbladder cancers and cancers of the ampulla of Vater. Furthermore, some studies misclassify types of cholangiocarcinomas. For example, Klatskin tumors (a type of extrahepatic cholangiocarcinoma), at times, have been misclassified as intrahepatic cholangiocarcinoma[29,30]. Finally, some studies use histology as the only diagnostic criterion while others accept other forms of diagnosis such as clinical evaluation or imaging studies.

Despite these shortcomings, the available data suggest a highly variable incidence in different parts of the world, with the highest incidence in Southeast Asia and lowest in Australia[43,44]. The incidence of cholangiocarcinoma has been reported to be as high as 96 cases per 100 000 in men and 38 per 100 000 in women in northeast Thailand[43]. In Australia, the reported incidence is 0.2 per 100 000 among Australian men and 0.1 per 100 000 among Australian women[43]. In other Western countries including the Unites States the incidence is close to 1 case per 100 000 population[43]. In Western countries, approximately 80% of cholangiocarcinomas are extrahepatic[45]. Differing exposure to risk factors is thought to account for the varying geographic incidences, with parasitic infections and hepatolithiasis being more prevalent in Asia[46-48].

Recent data show that the incidence of intrahepatic cholangiocarcinoma is increasing around the world, while the incidence of extrahepatic cholangiocarcinoma has been largely stable or even decreasing[32,43,49]. Although the exact underlying mechanism for this discrepancy is not known, it suggests that intrahepatic and extrahepatic cholangiocarcinomas may be biologically different[32,49,50].

Finally, there is evidence suggesting under-diagnosis of cholangiocarcinoma. For example among patients transplanted for liver cirrhosis of various etiologies, an incidental cholangiocarcinoma was found in approximately 12% of explanted livers[51]. This suggests that the true incidence of cholangiocarcinoma may be higher than previously reported.

Obesity as a risk factor for cholangiocarcinoma

Data assessing the association of obesity and cholangiocarcinoma are limited. So far, four epidemiologic studies have tried to assess the link between obesity and cholangiocarcinoma (Table 1). Three of these studies were specifically designed to identify risk factors for cholangiocarcinoma[52-54].

One of those studies using the Surveillance, Epidemiology, and End Results database that links cancer registry data and Medicare enrollment and claims files, reported a significant association between obesity and intrahepatic cholangiocarcinoma, but not between obesity and extrahepatic cholangiocarcinoma[52]. That study however, has several limitations. The study only included patients 65 years of age and older, therefore the findings might not be generalizable to a younger population. Furthermore, because Medicare data are collected for billing rather than research purposes, completeness and accuracy of the Medicare data on prevalence of obesity and cholangiocarcinoma can be questioned. Finally, the possibility of diagnostic bias cannot be excluded because obese people with different medical conditions are more likely to undergo testing and thus have more diagnoses than other people.

Another population-based study conducted in Denmark did not find any significant association between

Table 1 Epidemiologic studies assessing obesity as a potential risk factor for cholangiocarcinoma

Ref.	Study design	Type of CC	Study findings	Study limitations
Welzel *et al*[52]	Case-control	ICC and ECC	Positive association between obesity and ICC; no association between obesity and ECC	Only included age ⩾ 65 yr; database limitations; possible diagnostic bias
Welzel *et al*[53]	Case-control	ICC	No association between obesity and ICC	ECC not included
Grange *et al*[54]	Case-control	NS	Positive association between obesity and cholangiocarcinoma	Type of CC not specified; diagnosis subject to misclassification
Oh *et al*[55]	Cohort	NS	Positive association between obesity and cholangiocarcinoma	Does not distinguish ECC and ICC; included only males; database limitations

CC: Cholangiocarcinoma; ECC: Extrahepatic cholangiocarcinoma; ICC: Intrahepatic cholangiocarcinoma; NS: Not stated.

obesity and intrahepatic cholangiocarcinoma[53]. That study used a fairly accurate and complete national registry. Another strength of that study was histological confirmation of cholangiocarcinoma diagnosis in all cases. Extrahepatic cholangiocarcinoma, however, was not studied.

The third study, conducted in the United Kingdom by Grainge *et al*[54], found that obese patients (BMI ⩾ 30 kg/m^2) had 1.5 times the risk of cholangiocarcinoma compared with those with BMI < 25 kg/m^2. In that study the type of cholangiocarcinoma (intrahepatic versus extrahepatic) was not specified. Another limitation of that study was that the diagnosis was not based on histology and therefore subject to misclassification.

Aside from studies looking specifically to identify risk factors for cholangiocarcinoma, a South Korean population-based study tried to assess the effect of excess weight on incidence of various cancers including cholangiocarcinoma[55]. The study found that compared to the reference group (BMI 18.5-23 kg/m^2), increasing BMI was associated with an increase in the relative risk for development of cholangiocarcinoma[55]. That study, however, did not distinguish between intrahepatic and extrahepatic cholangiocarcinomas, included only males, and the database used was not inclusive of the entire population.

In summary, the epidemiologic data available on obesity as a risk factor for cholangiocarcinoma show conflicting results and are too limited to make any conclusions.

BIOLOGIC MECHANISMS FOR POSITIVE ASSOCIATION BETWEEN OBESITY AND CHOLANGIOCARCINOMA

Obesity is an excess of adipose tissue. Adipose tissue is a biologically active organ which in addition to adipocytes contains multiple other cell types such as pre-adipocytes, endothelial cells, macrophages and other immune cells[56]. Adipose tissue secretes molecules into the bloodstream, which signal to other metabolic organs or to the brain to coordinate responses to altered metabolic demands[57]. Some of these molecules, known as adipokines, have a role in modulating the risk of cancer development. Among adipokines, some of the most studied are leptin, adiponectin and pro-inflammatory molecules.

Leptin is a hormone that is primarily secreted by the adipose tissue[58]. It acts on the hypothalamus to regulate food consumption and caloric homeostasis[58,59]. Although the major physiological site of leptin action is in the central nervous system, leptin receptors are also expressed at lower levels in peripheral tissues[60]. Two studies have shown presence of leptin receptors on cholangiocytes[61,62]. Circulating serum leptin is increased in obesity, and has been suggested as a risk factor for cholangiocarcinoma[62]. A recent study showed that normal and malignant intrahepatic cholangiocytes express leptin and leptin receptors[62]. The investigators of that study also showed that leptin stimulates growth and migration, and prevents apoptosis of intrahepatic cholangiocarcinoma cells *in vitro*[62]. They also showed that genetic ablation of leptin-mediated signaling inhibits cancer development and growth in an animal model of cholangiocarcinoma[62].

Adiponectin is another adipokine that may be involved in cancer development[63]. It acts on a number of tissues to regulate glucose and lipid metabolism. Adiponectin levels are reduced in obesity. Several basic and epidemiological studies have suggested that adiponectin has antitumor effects[63,64]. Although the link between adiponectin and cholangiocarcinoma has not been studied, there is convincing evidence for an inverse association between adiponectin levels and cancer risk for several cancer types[59,63,65-67].

Several pro-inflammatory cytokines are secreted by monocytes and other immune cells that infiltrate adipose tissues in obesity[68,69]. Among these cytokines, interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) have been found to have a role in development of cholangiocarcinoma[70]. A growing body of evidence suggests that the inflammatory environment in the obese state is linked to the development of cancer through various mechanisms[71]. IL-6 seems to have an important role in the pathogenesis and growth of cholangiocarcinoma and has been shown to act by both an autocrine and paracrine manner stimulating several intracellular pathways involved in survival and growth of malignant cholangiocytes[36,72-74]. TNF-α is a mediator of inflammation with actions directed towards both tissue destruction and recovery[70]. Evidence suggests that TNF-α may act as an endogenous tumor promoter in addition to its role in immune responses[75,76]. Overexpression of TNF-α receptor genes has been observed in cholangiocarcinoma associated with hepatolithiasis[75-78].

Although not specifically linked to cholangiocarcino-

ma, there are multiple other substances that have directly or indirectly been linked to cancer in general[57]. Research on the role of different substances on development and progression of different cancers including cholangiocarcinoma is evolving.

DISCUSSION

It is estimated that about half of the population in developed countries are either overweight or obese[79]. In some regions of the world overweight and obesity affects more than two-thirds of the population[80]. By 2008, an estimated 1.5 billion adults globally were overweight and 500 million adults were obese[81]. Because of such high prevalence, establishing a clear link between excess body weight and cholangiocarcinoma can have significant effect on prevention of cholangiocarcinoma. Establishing a link may also encourage further research on identification of underlying pathophysiologic mechanisms that can potentially lead to discovery of better treatment options.

Research on obesity and cholangiocarcinoma risk should be encouraged through a variety of activities, including large cooperative initiatives, database resources, epidemiologic studies, basic science studies, and increase in funding resources.

If a link between excess weight and cholangiocarcinoma is established, multiple new questions will emerge that will need to be answered. Does reversal of obesity affect the risk for cholangiocarcinoma? Are there any differences between different modes of weight reduction (calorie restriction, exercise, drugs, bariatric surgery) in terms of cholangiocarcinoma risk reduction? Can the link between obesity and cholangiocarcinoma be somehow disrupted in the absence of weight loss (which for many individuals is a difficult task)?

Answers to these and other questions that will surely follow require well-designed studies that incorporate molecular, genetic, metabolic and nutritional factors.

REFERENCES

1 **Nguyen DM**, El-Serag HB. The epidemiology of obesity. *Gastroenterol Clin North Am* 2010; **39**: 1-7 [PMID: 20202574 DOI: 10.1016/j.gtc.2009.12.014]

2 **Centers for Disease Control and Prevention (CDC).** Vital signs: state-specific obesity prevalence among adults --- United States, 2009. *MMWR Morb Mortal Wkly Rep* 2010; **59**: 951-955 [PMID: 20689500]

3 **von Ruesten A**, Steffen A, Floegel A, van der A DL, Masala G, Tjønneland A, Halkjaer J, Palli D, Wareham NJ, Loos RJ, Sørensen TI, Boeing H. Trend in obesity prevalence in European adult cohort populations during follow-up since 1996 and their predictions to 2015. *PLoS One* 2011; **6**: e27455 [PMID: 22102897 DOI: 10.1371/journal.pone.0027455]

4 **Daigre JL**, Atallah A, Boissin JL, Jean-Baptiste G, Kangambega P, Chevalier H, Balkau B, Smadja D, Inamo J. The prevalence of overweight and obesity, and distribution of waist circumference, in adults and children in the French Overseas Territories: the PODIUM survey. *Diabetes Metab* 2012; **38**: 404-411 [PMID: 22626474 DOI: 10.1016/j.diabet.2012.03.008]

5 **Sundborn G**, Metcalf PA, Gentles D, Scragg R, Dyall L, Black P, Jackson R. Overweight and obesity prevalence among adult Pacific peoples and Europeans in the Diabetes Heart and Health Study (DHAHS) 2002-2003, Auckland New Zealand. *N Z Med J* 2010; **123**: 30-42 [PMID: 20360794]

6 **Ayatollahi SM**, Ghoreshizadeh Z. Prevalence of obesity and overweight among adults in Iran. *Obes Rev* 2010; **11**: 335-337 [PMID: 20202133 DOI: 10.1111/j.1467-789X.2010.00725.x]

7 **Gigante DP**, Moura EC, Sardinha LM. Prevalence of overweight and obesity and associated factors, Brazil, 2006. *Rev Saude Publica* 2009; **43** Suppl 2: 83-89 [PMID: 19936502 DOI: 10.1590/S0034-89102009000900011]

8 **Gültekin T**, Ozer BK, Akin G, Bektaş Y, Sağir M, Güleç E. Prevalence of overweight and obesity in Turkish adults. *Anthropol Anz* 2009; **67**: 205-212 [PMID: 19739469]

9 **Gupta N**, Goel K, Shah P, Misra A. Childhood obesity in developing countries: epidemiology, determinants, and prevention. *Endocr Rev* 2012; **33**: 48-70 [PMID: 22240243 DOI: 10.1210/er.2010-0028]

10 **Koukoulis GN**, Sakka C, Katsaros F, Goutou M, Tsirona S, Tsiapali E, Piterou A, Stefanidis I, Stathakis N. High rates of obesity prevalence in adults living in central Greece: data from the ARGOS study. *Hormones* (Athens) 2010; **9**: 253-262 [PMID: 20688623]

11 **Mohamud WN**, Musa KI, Khir AS, Ismail AA, Ismail IS, Kadir KA, Kamaruddin NA, Yaacob NA, Mustafa N, Ali O, Isa SH, Bebakar WM. Prevalence of overweight and obesity among adult Malaysians: an update. *Asia Pac J Clin Nutr* 2011; **20**: 35-41 [PMID: 21393108]

12 **Xi B**, Liang Y, He T, Reilly KH, Hu Y, Wang Q, Yan Y, Mi J. Secular trends in the prevalence of general and abdominal obesity among Chinese adults, 1993-2009. *Obes Rev* 2012; **13**: 287-296 [PMID: 22034908 DOI: 10.1111/j.1467-789X.2011.00944.x]

13 **Gill T**. Epidemiology and health impact of obesity: an Asia Pacific perspective. *Asia Pac J Clin Nutr* 2006; **15** Suppl: 3-14 [PMID: 16928656]

14 **World Health Organization.** Controlling the global obesity epidemic. Available from: URL: http://www.who.int/nutrition/topics/obesity/en/

15 **Orsi CM**, Hale DE, Lynch JL. Pediatric obesity epidemiology. *Curr Opin Endocrinol Diabetes Obes* 2011; **18**: 14-22 [PMID: 21157323 DOI: 10.1097/MED.0b013e3283423de1]

16 **Selassie M**, Sinha AC. The epidemiology and aetiology of obesity: a global challenge. *Best Pract Res Clin Anaesthesiol* 2011; **25**: 1-9 [PMID: 21516909]

17 **Renehan AG**, Tyson M, Egger M, Heller RF, Zwahlen M. Body-mass index and incidence of cancer: a systematic review and meta-analysis of prospective observational studies. *Lancet* 2008; **371**: 569-578 [PMID: 18280327 DOI: 10.1016/S0140-6736(08)60269-X]

18 **Frezza EE**, Wachtel MS, Chiriva-Internati M. Influence of obesity on the risk of developing colon cancer. *Gut* 2006; **55**: 285-291 [PMID: 16239255 DOI: 10.1136/gut.2005.073163]

19 **Larsson SC**, Wolk A. Obesity and colon and rectal cancer risk: a meta-analysis of prospective studies. *Am J Clin Nutr* 2007; **86**: 556-565 [PMID: 17823417]

20 **Ligibel J**. Obesity and breast cancer. *Oncology* (Williston Park) 2011; **25**: 994-1000 [PMID: 22106549]

21 **Yang XR**, Chang-Claude J, Goode EL, Couch FJ, Nevanlinna H, Milne RL, Gaudet M, Schmidt MK, Broeks A, Cox A, Fasching PA, Hein R, Spurdle AB, Blows F, Driver K, Flesch-Janys D, Heinz J, Sinn P, Vrieling A, Heikkinen T, Aittomäki K, Heikkilä P, Blomqvist C, Lissowska J, Peplonska B, Chanock S, Figueroa J, Brinton L, Hall P, Czene K, Humphreys K, Darabi H, Liu J, Van 't Veer LJ, van Leeuwen FE, Andrulis IL, Glendon G, Knight JA, Mulligan AM, O'Malley FP, Weerasooriya N, John EM, Beckmann MW, Hartmann A, Weihbrecht SB, Wachter DL, Jud SM, Loehberg CR, Baglietto L, English DR, Giles GG, McLean CA, Severi G, Lambrechts D, Vandorpe T, Weltens C, Paridaens R, Smeets A, Neven P, Wildiers H, Wang X, Olson JE, Cafourek V, Fredericksen Z, Kosel M, Vachon C, Cramp HE, Connley D, Cross SS, Balasubramanian SP, Reed MW, Dörk T, Bremer M, Meyer

A, Karstens JH, Ay A, Park-Simon TW, Hillemanns P, Arias Pérez JI, Menéndez Rodríguez P, Zamora P, Benítez J, Ko YD, Fischer HP, Hamann U, Pesch B, Brüning T, Justenhoven C, Brauch H, Eccles DM, Tapper WJ, Gerty SM, Sawyer EJ, Tomlinson IP, Jones A, Kerin M, Miller N, McInerney N, Anton-Culver H, Ziogas A, Shen CY, Hsiung CN, Wu PE, Yang SL, Yu JC, Chen ST, Hsu GC, Haiman CA, Henderson BE, Le Marchand L, Kolonel LN, Lindblom A, Margolin S, Jakubowska A, Lubiński J, Huzarski T, Byrski T, Górski B, Gronwald J, Hooning MJ, Hollestelle A, van den Ouweland AM, Jager A, Kriege M, Tilanus-Linthorst MM, Collée M, Wang-Gohrke S, Pylkäs K, Jukkola-Vuorinen A, Mononen K, Grip M, Hirvikoski P, Winqvist R, Mannermaa A, Kosma VM, Kauppinen J, Kataja V, Auvinen P, Soini Y, Sironen R, Bojesen SE, Ørsted DD, Kaur-Knudsen D, Flyger H, Nordestgaard BG, Holland H, Chenevix-Trench G, Manoukian S, Barile M, Radice P, Hankinson SE, Hunter DJ, Tamimi R, Sangrajrang S, Brennan P, McKay J, Odefrey F, Gaborieau V, Devilee P, Huijts PE, Tollenaar RA, Seynaeve C, Dite GS, Apicella C, Hopper JL, Hammet F, Tsimiklis H, Smith LD, Southey MC, Humphreys MK, Easton D, Pharoah P, Sherman ME, Garcia-Closas M. Associations of breast cancer risk factors with tumor subtypes: a pooled analysis from the Breast Cancer Association Consortium studies. *J Natl Cancer Inst* 2011; **103**: 250-263 [PMID: 21191117 DOI: 10.1093/jnci/djq526]

22 **Larsson SC**, Wolk A. Overweight, obesity and risk of liver cancer: a meta-analysis of cohort studies. *Br J Cancer* 2007; **97**: 1005-1008 [PMID: 17700568]

23 **Kaaks R**, Lukanova A, Kurzer MS. Obesity, endogenous hormones, and endometrial cancer risk: a synthetic review. *Cancer Epidemiol Biomarkers Prev* 2002; **11**: 1531-1543 [PMID: 12496040]

24 **Lane G**. Obesity and gynaecological cancer. *Menopause Int* 2008; **14**: 33-37 [PMID: 18380959 DOI: 10.1258/mi.2007.007036]

25 **Mathew A**, George PS, Ildaphonse G. Obesity and kidney cancer risk in women: a meta-analysis (1992-2008). *Asian Pac J Cancer Prev* 2009; **10**: 471-478 [PMID: 19640194]

26 **Murray L**, Romero Y. Role of obesity in Barrett's esophagus and cancer. *Surg Oncol Clin N Am* 2009; **18**: 439-452 [PMID: 19500735 DOI: 10.1016/j.soc.2009.03.010]

27 **Yang P**, Zhou Y, Chen B, Wan HW, Jia GQ, Bai HL, Wu XT. Overweight, obesity and gastric cancer risk: results from a meta-analysis of cohort studies. *Eur J Cancer* 2009; **45**: 2867-2873 [PMID: 19427197 DOI: 10.1016/j.ejca.2009.04.019]

28 **Zyromski NJ**, White PB. Pancreatic cancer in obesity: epidemiology, clinical observations, and basic mechanisms. *Anticancer Agents Med Chem* 2011; **11**: 470-478 [PMID: 21492072]

29 **Hammill CW**, Wong LL. Intrahepatic cholangiocarcinoma: a malignancy of increasing importance. *J Am Coll Surg* 2008; **207**: 594-603 [PMID: 18926465 DOI: 10.1016/j.jamcollsurg.2008.04.031]

30 **Welzel TM**, McGlynn KA, Hsing AW, O'Brien TR, Pfeiffer RM. Impact of classification of hilar cholangiocarcinomas (Klatskin tumors) on the incidence of intra- and extrahepatic cholangiocarcinoma in the United States. *J Natl Cancer Inst* 2006; **98**: 873-875 [PMID: 16788161 DOI: 10.1093/jnci/djj234]

31 **Gatto M**, Bragazzi MC, Semeraro R, Napoli C, Gentile R, Torrice A, Gaudio E, Alvaro D. Cholangiocarcinoma: update and future perspectives. *Dig Liver Dis* 2010; **42**: 253-260 [PMID: 20097142 DOI: 10.1016/j.dld.2009.12.008]

32 **Cardinale V**, Semeraro R, Torrice A, Gatto M, Napoli C, Bragazzi MC, Gentile R, Alvaro D. Intra-hepatic and extrahepatic cholangiocarcinoma: New insight into epidemiology and risk factors. *World J Gastrointest Oncol* 2010; **2**: 407-416 [PMID: 21160904]

33 **Patel T**. Cholangiocarcinoma. *Nat Clin Pract Gastroenterol Hepatol* 2006; **3**: 33-42 [PMID: 16397610]

34 **Mihalache F**, Tantau M, Diaconu B, Acalovschi M. Survival and quality of life of cholangiocarcinoma patients: a prospective study over a 4 year period. *J Gastrointestin Liver Dis* 2010; **19**: 285-290 [PMID: 20922193]

35 **Khan SA**, Toledano MB, Taylor-Robinson SD. Epidemiology, risk factors, and pathogenesis of cholangiocarcinoma. *HPB (Oxford)* 2008; **10**: 77-82 [PMID: 18773060 DOI: 10.1080/13651820801992641]

36 **Fava G**, Lorenzini I. Molecular pathogenesis of cholangiocarcinoma. *Int J Hepatol* 2012; **2012**: 630543 [PMID: 21994887]

37 Office of the Surgeon General (Released July 2003). Available from: URL: http: //www.surgeongeneral.gov/news/testimony/obesity07162003.html

38 **Centers for Disease Control and Prevention.** Defining Overweight and Obesity. Available from: URL: http: //www.cdc.gov/obesity/defining.html

39 **Ogden CL,** Carroll ME, Kit BK, Flegal KM. Prevalence of obesity in the United States, 2009-2010. *NCHS Dta Bief* 2012; (82): 1-8

40 **Flegal KM**, Carroll MD, Kit BK, Ogden CL. Prevalence of obesity and trends in the distribution of body mass index among US adults, 1999-2010. *JAMA* 2012; **307**: 491-497 [PMID: 22253363 DOI: 10.1001/jama.2012.39]

41 **Flegal KM**, Carroll MD, Ogden CL, Curtin LR. Prevalence and trends in obesity among US adults, 1999-2008. *JAMA* 2010; **303**: 235-241 [PMID: 20071471 DOI: 10.1001/jama.2009.2014]

42 **Charbel H**, Al-Kawas FH. Cholangiocarcinoma: epidemiology, risk factors, pathogenesis, and diagnosis. *Curr Gastroenterol Rep* 2011; **13**: 182-187 [PMID: 21271364 DOI: 10.1007/s11894-011-0178-8]

43 **Shaib Y**, El-Serag HB. The epidemiology of cholangiocarcinoma. *Semin Liver Dis* 2004; **24**: 115-125 [PMID: 15192785 DOI: 10.1055/s-2004-828889]

44 **Sripa B**, Pairojkul C. Cholangiocarcinoma: lessons from Thailand. *Curr Opin Gastroenterol* 2008; **24**: 349-356 [PMID: 18408464 DOI: 10.1097/MOG.0b013e3282fbf9b3]

45 **Khan SA**, Emadossadaty S, Ladep NG, Thomas HC, Elliott P, Taylor-Robinson SD, Toledano MB. Rising trends in cholangiocarcinoma: is the ICD classification system misleading us? *J Hepatol* 2012; **56**: 848-854 [PMID: 22173164 DOI: 10.1016/j.jhep.2011.11.015]

46 **Sripa B**, Bethony JM, Sithithaworn P, Kaewkes S, Mairiang E, Loukas A, Mulvenna J, Laha T, Hotez PJ, Brindley PJ. Opisthorchiasis and Opisthorchis-associated cholangiocarcinoma in Thailand and Laos. *Acta Trop* 2011; **120** Suppl 1: S158-S168 [PMID: 20655862 DOI: 10.1016/j.actatropica.2010.07.006]

47 **Mulvenna J**, Yonglitthipagon P, Sripa B, Brindley PJ, Loukas A, Bethony JM. Banking on the future: biobanking for "omics" approaches to biomarker discovery for Opisthorchis-induced cholangiocarcinoma in Thailand. *Parasitol Int* 2012; **61**: 173-177 [PMID: 21855650 DOI: 10.1016/j.parint.2011.06.005]

48 **Shin HR**, Oh JK, Masuyer E, Curado MP, Bouvard V, Fang YY, Wiangnon S, Sripa B, Hong ST. Epidemiology of cholangiocarcinoma: an update focusing on risk factors. *Cancer Sci* 2010; **101**: 579-585 [PMID: 20085587 DOI: 10.1111/j.1349-7006.2009.01458.x]

49 **Patel T**. Increasing incidence and mortality of primary intrahepatic cholangiocarcinoma in the United States. *Hepatology* 2001; **33**: 1353-1357 [PMID: 11391522]

50 **Tyson GL**, El-Serag HB. Risk factors for cholangiocarcinoma. *Hepatology* 2011; **54**: 173-184 [PMID: 21488076]

51 Colmenero J, Castro-Narro G, Fleck A, Miquel R, Garcia-Valdecasas J, Navasa M. Unexpected etiologies of cryptogenic liver disease and incidental tumors in adult liver transplantation: a clinical-pathological study in 815 patients. *Hepatology* 2009; **50** Suppl 4: S580

52 **Welzel TM**, Graubard BI, El-Serag HB, Shaib YH, Hsing AW, Davila JA, McGlynn KA. Risk factors for intrahepatic and extrahepatic cholangiocarcinoma in the United States: a population-based case-control study. *Clin Gastroenterol Hepatol* 2007; **5**: 1221-1228 [PMID: 17689296 DOI: 10.1016/j.cgh.2007.05.020]

53 **Welzel TM**, Mellemkjaer L, Gloria G, Sakoda LC, Hsing

AW, El Ghormli L, Olsen JH, McGlynn KA. Risk factors for intrahepatic cholangiocarcinoma in a low-risk population: a nationwide case-control study. *Int J Cancer* 2007; **120**: 638-641 [PMID: 17109384 DOI: 10.1002/ijc.22283]
54 **Grainge MJ**, West J, Solaymani-Dodaran M, Aithal GP, Card TR. The antecedents of biliary cancer: a primary care case-control study in the United Kingdom. *Br J Cancer* 2009; **100**: 178-180 [PMID: 19018260 DOI: 10.1038/sj.bjc.6604765]
55 **Oh SW**, Yoon YS, Shin SA. Effects of excess weight on cancer incidences depending on cancer sites and histologic findings among men: Korea National Health Insurance Corporation Study. *J Clin Oncol* 2005; **23**: 4742-4754 [PMID: 16034050 DOI: 10.1200/JCO.2005.11.726]
56 **van Kruijsdijk RC**, van der Wall E, Visseren FL. Obesity and cancer: the role of dysfunctional adipose tissue. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 2569-2578 [PMID: 19755644 DOI: 10.1158/1055-9965.EPI-09-0372]
57 **Khandekar MJ**, Cohen P, Spiegelman BM. Molecular mechanisms of cancer development in obesity. *Nat Rev Cancer* 2011; **11**: 886-895 [PMID: 22113164]
58 **Mantzoros CS**, Magkos F, Brinkoetter M, Sienkiewicz E, Dardeno TA, Kim SY, Hamnvik OP, Koniaris A. Leptin in human physiology and pathophysiology. *Am J Physiol Endocrinol Metab* 2011; **301**: E567-E584 [PMID: 21791620]
59 **Nock NL,** Berger NA. Obesity and Cancer: Overview of Mechanisms. In: Berger NA, editor. Cancer and Energy Balance, Epidemiology and Overview (Energy Balance and Cancer). New York: Springer, 2010: 129-179 [DOI: 10.1007/978-1-4419-5515-9_5]
60 **Cohen P**, Zhao C, Cai X, Montez JM, Rohani SC, Feinstein P, Mombaerts P, Friedman JM. Selective deletion of leptin receptor in neurons leads to obesity. *J Clin Invest* 2001; **108**: 1113-1121 [PMID: 11602618]
61 **Sayed-Ahmed A**, Rudas P, Bartha T. Partial cloning and localisation of leptin and its receptor in the one-humped camel (Camelus dromedarius). *Vet J* 2005; **170**: 264-267 [PMID: 16129347 DOI: 10.1016/j.tvjl.2004.04.019]
62 **Fava G**, Alpini G, Rychlicki C, Saccomanno S, DeMorrow S, Trozzi L, Candelaresi C, Venter J, Di Sario A, Marzioni M, Bearzi I, Glaser S, Alvaro D, Marucci L, Francis H, Svegliati-Baroni G, Benedetti A. Leptin enhances cholangiocarcinoma cell growth. *Cancer Res* 2008; **68**: 6752-6761 [PMID: 18701500 DOI: 10.1158/0008-5472.CAN-07-6682]
63 **Barb D**, Pazaitou-Panayiotou K, Mantzoros CS. Adiponectin: a link between obesity and cancer. *Expert Opin Investig Drugs* 2006; **15**: 917-931 [PMID: 16859394 DOI: 10.1517/13543784.15.8.917]
64 **Grossmann ME**, Ray A, Nkhata KJ, Malakhov DA, Rogozina OP, Dogan S, Cleary MP. Obesity and breast cancer: status of leptin and adiponectin in pathological processes. *Cancer Metastasis Rev* 2010; **29**: 641-653 [PMID: 20821253 DOI: 10.1007/s10555-010-9252-1]
65 **An W**, Bai Y, Deng SX, Gao J, Ben QW, Cai QC, Zhang HG, Li ZS. Adiponectin levels in patients with colorectal cancer and adenoma: a meta-analysis. *Eur J Cancer Prev* 2012; **21**: 126-133 [PMID: 21960184 DOI: 10.1097/CEJ.0b013e32834c9b55]
66 **Chen X**, Wang Y. Adiponectin and breast cancer. *Med Oncol* 2011; **28**: 1288-1295 [PMID: 20625941 DOI: 10.1007/s12032010-9617-x]
67 **Tian YF**, Chu CH, Wu MH, Chang CL, Yang T, Chou YC, Hsu GC, Yu CP, Yu JC, Sun CA. Anthropometric measures, plasma adiponectin, and breast cancer risk. *Endocr Relat Cancer* 2007; **14**: 669-677 [PMID: 17914097 DOI: 10.1677/ERC-06-0089]
68 **Weisberg SP**, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 2003; **112**: 1796-1808 [PMID: 14679176]
69 **Ruan H**, Zarnowski MJ, Cushman SW, Lodish HF. Standard isolation of primary adipose cells from mouse epididymal fat pads induces inflammatory mediators and down-regulates adipocyte genes. *J Biol Chem* 2003; **278**: 47585-47593 [PMID: 12975378 DOI: 10.1074/jbc.M305257200]
70 **Braicu C,** Burz C, Berindan-Neagoe I, Balacescu O, Tantau M, Cristea V, Irimie A. Molecular markers in the pathogenesis of cholangiocarcinoma: Potential for early detection and selection of appropriate treatment. *GR* 2009; **2:** 132-140 [DOI:10.4021/gr2009.06.1299]
71 **Grivennikov SI**, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell* 2010; **140**: 883-899 [PMID: 20303878 DOI: 10.1016/j.cell.2010.01.025]
72 **Meng F**, Yamagiwa Y, Ueno Y, Patel T. Over-expression of interleukin-6 enhances cell survival and transformed cell growth in human malignant cholangiocytes. *J Hepatol* 2006; **44**: 1055-1065 [PMID: 16469407 DOI: 10.1016/j.jhep.2005.10.030]
73 **Akiyama T**, Hasegawa T, Sejima T, Sahara H, Seto K, Saito H, Takashima S. Serum and bile interleukin 6 after percutaneous transhepatic cholangio-drainage. *Hepatogastroenterology* 1998; **45**: 665-671 [PMID: 9684114]
74 **Okada K**, Shimizu Y, Nambu S, Higuchi K, Watanabe A. Interleukin-6 functions as an autocrine growth factor in a cholangiocarcinoma cell line. *J Gastroenterol Hepatol* 1994; **9**: 462-467 [PMID: 7827297 DOI: 10.1111/j.1440-1746.1994.tb01275.x]
75 **Ohira S**, Sasaki M, Harada K, Sato Y, Zen Y, Isse K, Kozaka K, Ishikawa A, Oda K, Nimura Y, Nakanuma Y. Possible regulation of migration of intrahepatic cholangiocarcinoma cells by interaction of CXCR4 expressed in carcinoma cells with tumor necrosis factor-alpha and stromal-derived factor-1 released in stroma. *Am J Pathol* 2006; **168**: 1155-1168 [PMID: 16565491 DOI: 10.2353/ajpath.2006.050204]
76 **Tanimura Y**, Kokuryo T, Tsunoda N, Yamazaki Y, Oda K, Nimura Y, Naing Mon N, Huang P, Nakanuma Y, Chen MF, Jan YY, Yeh TS, Chiu CT, Hsieh LL, Hamaguchi M. Tumor necrosis factor alpha promotes invasiveness of cholangiocarcinoma cells via its receptor, TNFR2. *Cancer Lett* 2005; **219**: 205-213 [PMID: 15723721 DOI: 10.1016/j.canlet.2004.07.027]
77 **Blechacz B**, Gores GJ. Cholangiocarcinoma: advances in pathogenesis, diagnosis, and treatment. *Hepatology* 2008; **48**: 308-321 [PMID: 18536057]
78 **Sirica AE**. Cholangiocarcinoma: molecular targeting strategies for chemoprevention and therapy. *Hepatology* 2005; **41**: 5-15 [PMID: 15690474]
79 **Larsson SC**, Wolk A. Excess body fatness: an important cause of most cancers. *Lancet* 2008; **371**: 536-537 [PMID: 18280312 DOI: 10.1016/S0140-6736(08)60247-0]
80 **Swinburn BA**, Sacks G, Hall KD, McPherson K, Finegood DT, Moodie ML, Gortmaker SL. The global obesity pandemic: shaped by global drivers and local environments. *Lancet* 2011; **378**: 804-814 [PMID: 21872749 DOI: 10.1016/S0140-6736(11)60813-1]
81 **Finucane MM**, Stevens GA, Cowan MJ, Danaei G, Lin JK, Paciorek CJ, Singh GM, Gutierrez HR, Lu Y, Bahalim AN, Farzadfar F, Riley LM, Ezzati M. National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9·1 million participants. *Lancet* 2011; **377**: 557-567 [PMID: 21295846 DOI: 10.1016/S0140-6736(10)62037-5]

P- Reviewers Hanazaki K, Quiros RM
S- Editor Gou SX **L- Editor** A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i4.463

World J Gastroenterol 2013 January 28; 19(4): 463-481
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

REVIEW

Sedation in gastrointestinal endoscopy: Current issues

John K Triantafillidis, Emmanuel Merikas, Dimitrios Nikolakis, Apostolos E Papalois

John K Triantafillidis, Emmanuel Merikas, Dimitrios Nikolakis, Department of Gastroenterology and Center for Inflammatory Bowel Disease, "Saint Panteleimon" General Hospital, Nikaia, 12461 Haidari, Greece
Apostolos E Papalois, Experimental Research Center, ELPEN Pharmaceuticals, 19009 Athens, Greece
Author contributions: All authors have made substantial and equal contributions to the study.
Correspondence to: John K Triantafillidis, MD, PhD, Professor, Director, Department of Gastroenterology and Center for Inflammatory Bowel Disease, "Saint Panteleimon" General Hospital, Nikaia, Iera odos 354, 12461 Haidari, Greece. jktrian@gmail.com
Telephone: +30-210-5819481 Fax: +30-210-5810970
Received: August 16, 2012 Revised: November 11, 2012
Accepted: December 25, 2012
Published online: January 28, 2013

Abstract

Diagnostic and therapeutic endoscopy can successfully be performed by applying moderate (conscious) sedation. Moderate sedation, using midazolam and an opioid, is the standard method of sedation, although propofol is increasingly being used in many countries because the satisfaction of endoscopists with propofol sedation is greater compared with their satisfaction with conventional sedation. Moreover, the use of propofol is currently preferred for the endoscopic sedation of patients with advanced liver disease due to its short biologic half-life and, consequently, its low risk of inducing hepatic encephalopathy. In the future, propofol could become the preferred sedation agent, especially for routine colonoscopy. Midazolam is the benzodiazepine of choice because of its shorter duration of action and better pharmacokinetic profile compared with diazepam. Among opioids, pethidine and fentanyl are the most popular. A number of other substances have been tested in several clinical trials with promising results. Among them, newer opioids, such as remifentanil, enable a faster recovery. The controversy regarding the administration of sedation by an endoscopist or an experienced nurse, as well as the optimal staffing of endoscopy units, continues to be a matter of discussion. Safe sedation in special clinical circumstances, such as in the cases of obese, pregnant, and elderly individuals, as well as patients with chronic lung, renal or liver disease, requires modification of the dose of the drugs used for sedation. In the great majority of patients, sedation under the supervision of a properly trained endoscopist remains the standard practice worldwide. In this review, an overview of the current knowledge concerning sedation during digestive endoscopy will be provided based on the data in the current literature.

© 2013 Baishideng. All rights reserved.

Key words: Gastrointestinal endoscopy; Endoscopy; Sedation; Analgesia; Digestive system

Triantafillidis JK, Merikas E, Nikolakis D, Papalois AE. Sedation in gastrointestinal endoscopy: Current issues. *World J Gastroenterol* 2013; 19(4): 463-481 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i4/463.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i4.463

INTRODUCTION

Today, both the number and complexity of endoscopic procedures have increased considerably due to the wide availability and application of sedation, which facilitates successful endoscopic procedures because it can relieve patients' anxiety and discomfort, concurrently allowing them to experience a rapid recovery with the use of antidotes. Consequently, the willingness of patients to undergo endoscopy, irrespective of the severity of their situation, is increasing.

The best methods for analgesia and sedation during digestive endoscopy are still debated. Intravenous sedation can be administered by the endoscopist who concurrently performs the procedure while a qualified nurse monitors the patient's state of consciousness and vital signs. Providing an adequate regimen of sedation/analgesia may be considered a form of art, which influences,

for example, the quality of the examination and the patient's and physician's satisfaction with the sedation[1]. It must be argued that the optimal level of sedation differs according to the procedure being performed. Deep sedation or even general anaesthesia may be preferred for therapeutic procedures in which it is important for a patient to remain immobile. It is obvious that endoscopists commencing sedation/analgesia should be able to rescue patients whose level of sedation has become deeper than initially intended.

However, even today, many significant issues, such as the benefits, risks, and costs of sedation; the selection of the most suitable drug and combination of drugs for use; and the person responsible for the administration of sedation and monitoring of the patient, especially during time-consuming procedures such as colonoscopies and endoscopic retrograde cholangiopancreatography (ERCPs), remain unanswered[2].

The aim of this review is to provide the reader with an overview of the current knowledge concerning sedation during digestive endoscopy (drugs currently used and drugs under investigation, sedation during upper and lower gastrointestinal (GI) endoscopy and ERCP, and endoscopy in special situations) based on the data available in the current literature.

SEDATION PRACTICES

Sedation practices vary in different countries depending on health system regulations and local circumstances. On the other hand, differences in the setting in which the practice of gastroenterology and endoscopy takes place, e.g., at university hospitals versus community hospitals or private endoscopy units, as well as other systematic practice differences, could influence the attitude of endoscopists concerning sedation practices.

Data concerning the incidence of sedation application in routine practice are rather limited. Among the members of the Canadian Association of Gastroenterology, more than 90% use sedation during colonoscopy[3]. The use of sedation has become a standard practice during GI endoscopy in Italy[4]. Among the members of the Hellenic Society of Gastroenterology, 64% use sedation regularly in cases of upper GI endoscopy, 78% use sedation in colonoscopies, and 100% use sedation during ERCP and endoscopic ultrasound (EUS)[5]. In the United States, more than 98% of colonoscopies are performed with intravenous sedation[6]. In Switzerland, the use of sedation in GI endoscopy has markedly increased, and the use of electronic monitoring has become a standard practice. In this country and during 2003, sedation was used in 78% of upper and lower GI endoscopic procedures, compared with 60% in 1990. In Germany, the majority of esophagogastroduodenoscopy (EGDs; 74%) and colonoscopies (87%) are carried out under sedation[7]. In Spain, sedation is used in 20% of EGDs and 20% of colonoscopies, while ERCP is almost always performed under sedation[8].

With regard to the most common sedation regimen used in different countries, it was reported that most Canadian endoscopists use a combination of midazolam and fentanyl for colonoscopy, while propofol, either alone or in combination with other drugs, is used in a small proportion of patients[3]. Interestingly, a significantly higher proportion of adult gastroenterologists who routinely used propofol are highly satisfied compared with those using other drugs. According to a recent survey among the members of the Italian Society of Digestive Endoscopy, the most commonly employed sedation patterns are benzodiazepines for upper GI endoscopy (50.8%), benzodiazepines plus opioids for colonoscopy (39.5%) and enteroscopy (35.3%), and propofol for ERCP (42.3%) and EUS (35.6%). Concerning the use of propofol, 66% of endoscopists stated that the drug was administered exclusively by anaesthesiologists[4]. In Greece, 62.1% of the endoscopists use synergistic sedation (benzodiazepines plus opioids), 35.3% use benzodiazepines alone and 33.8% use propofol-based sedation in selected cases. Propofol administration is directed by an anaesthesiologist in most cases[5]. In the United States, more than 75% of endoscopists use a benzodiazepine plus narcotic combination, with the combination of midazolam and fentanyl being the most common[6]. In Switzerland, midazolam is the most commonly used medication. The drug is administered by the endoscopy nurse *via* an intravenous cannula. A significant percentage of endoscopists (43%) also use propofol regularly, mainly in a hospital setting. Endoscopists reporting the use of propofol without the assistance of an anaesthesiologist had performed a total of 82 620 procedures. The morbidity in this group of patients was 0.19%, with no cases of mortality[9]. The doses of midazolam and fentanyl used by the Canadian endoscopists are similar to those recommended in the United States (< 6 mg of midazolam and < 200 μg of fentanyl) and in the United Kingdom (< 5 mg of midazolam and < 100 μg of fentanyl)[10]. In Spain, the most common drugs were midazolam for gastroscopy and midazolam and pethidine for colonoscopy and ERCP, while propofol is most frequently used by anaesthesiologists[8]. In Germany, the most frequently used agents for sedation are midazolam (82%) and propofol (74%), and the most common sedation regimens used are propofol plus benzodiazepines (38%) and benzodiazepines plus an opioid (35%)[7].

In Italy, pulse oximetry is the most common system for patient monitoring during endoscopy, while supplemental O_2 is routinely administered by 39.3% of endoscopists[4]. In Greece, pulse oximetry is used in 96% of endoscopic procedures[5]. Major endoscopy societies, including those of Canada and the United States, recommend the use of pulse oximetry, continuous electrocardiogram and blood pressure, and heart rate monitoring in patients receiving propofol[11,12]. In Switzerland, pulse oximetry monitoring is currently used in more than 95% of examinations, compared with 2.5% of examinations in 1990[9]. In Germany, all patients are routinely moni-

Table 1 Percentage of sedation use in different countries

Country	Sedation	Propofol use	Benzodia-zepines alone use	Benzodiaze-pines plus opioids use	No. of nurses present during endoscopy	Pulse oximetry use	Supplemental oxygen use
Canada	90%	12%		always	1		
Italy		42.30% for ERCP (by anaesthesiologists)	50.80%	39.50%		100%	By 39.3% of endoscopists
Greece	EGD: 64%; Colonoscopy: 78%; ERCP: 100%; EUS: 100%	33.80% (in selected cases and only by anaesthesiologists)	35.30%	62.10%		96%	
United States	98%	25.70%		74.30%		98.60%	By 72.7% of endoscopists (in all EGDs)
Switzerland	78%	43% (regular use with or without the help of an anaesthesiologist)	Midazolam for the majority of endoscopies		1	95%	
Spain	EGD: 20%; Colonoscopy: 20%; ERCP: 100%; EGD: 74%	Only by anaesthesiologists	Only for EFD	Only for colonoscopies	1	77%	
Germany	Colonoscopy: 87%	74%	82%	35%		97%	34%

ERCP: Endoscopic retrograde cholangiopancreatography; EGD: Esophagogastroduodenoscopy; EUS: Endoscopic ultrasound; EFD: Energy flux density.

tored by pulse oximetry, while automated blood pressure monitoring and/or electrocardiography are applied in 29 and 13% of cases, respectively. Supplemental oxygen is routinely administered to 34% of patients[7].

Data concerning endoscopy practices in developing nations are scarce. In a study comparing endoscopy practices between endoscopists practising in 46 developed and developing countries, no significant differences in the use of a benzodiazepine and opioid combination, propofol alone, or unsedated endoscopy were found. Sedation is used for most endoscopic procedures, leading to the conclusion that sedation practices do not significantly differ between developing and developed countries[13].

Table 1 provides a summary of sedation practices in some parts of the world.

DRUGS CURRENTLY USED FOR SEDATION IN GI ENDOSCOPY

Various types of sedation and analgesia techniques are used during GI endoscopy procedures. Currently, there is no standard sedation regimen, and even within individual institutions, the choice of sedation may depend on endoscopist preference and the procedure being performed. Benzodiazepines, such as alprazolam, bromazepam, brotizolam, clotiazepam, diazepam, etizolam, flunitrazepam, lorazepam, midazolam, oxazepam and triazolam, are among the most frequently prescribed drugs. These drugs act as anxiolytics, sedatives, hypnotics, amnesics, antiepileptics and muscle relaxants. Among them, midazolam is an important drug and is widely used in everyday endoscopy work. It is now considered to be the benzodiazepine of choice, as it has a shorter duration of action and a better pharmacokinetic profile than diazepam. Other drugs used for sedation include opioids (pethidine and fentanyl), propofol, ketamine and droperidol. Adequate knowledge of the pharmacokinetic properties of these agents is crucial when commencing sedation (Table 2)[14].

The special characteristics of the currently available drugs for digestive endoscopy, as well as the drugs under investigation, are shown in Table 3 and are subsequently summarised in the following sections.

Midazolam

Midazolam is most likely the most widely used drug for sedation in everyday endoscopic work. The action of midazolam is due to the potentiation of the neural inhibition mediated by gamma-aminobutyric acid. In addition to its action on the central nervous system, midazolam exhibits a dose-dependent ventilatory depressant effect and causes a reduction in arterial blood pressure and an increase in heart rate. Midazolam is metabolised by cytochrome P450 enzymes and glucuronide conjugation. CYP3A4 is important in the biotransformation of midazolam.

The duration of action of midazolam is dependent on the duration of its administration. It also has synergistic interactions with other hypnotics and opioids. Various factors, including age, compromised renal function, and liver dysfunction, affect the pharmacokinetics of the drug[15].

Clinical studies: A large number of prospective, randomised, placebo-controlled and non-controlled trials have been published in recent years concerning the efficacy and safety of midazolam alone or in combination with analgesics. The most recent of these trials are mentioned below.

In a study conducted to evaluate the prevalence of hypoxia related to midazolam sedation during upper GI endoscopy, 180 patients referred for selective endoscopy were randomised to either midazolam sedation or pla-

Table 2 Characteristics of the pharmacological agents used to achieve a moderate level of sedation in gastrointestinal endoscopy (*i.v.* administration)[1]

Agent	Chemical structure	Molecular weight (g/moL)	Onset of action (min)	Duration of action	Elimination half-life	Metabolism/excretion
Midazolam	$C_{18}H_{13}ClFN_3$	325.78	1.0-2.5	2-6 h	1.8-6.4 h	Hepatic and intestinal; excreted in urine
Propofol	$C_{12}H_{18}O$	178.27	< 1	3-10 min	Triphasic: 2.2 min, 20 min, 8 h	Hepatic; excreted in urine
Fentanyl	$C_{22}H_{28}N_2O$	336.471	⩽ 1.5	1-2 h	2-7 h	Hepatic; excreted in urine
Meperidine	$C_{15}H_{21}NO_2$	247.33	5	2-4 h	2-7 h	Hepatic; excreted in urine

[1]Modified from Manolaraki *et al*[14].

Table 3 Currently used drugs for sedation and drugs under investigation

Drugs currently used for sedation	Drugs and other practices under investigation
Midazolam	Nitrous oxide gas (N_2O)
Fentanyl	Remimazolam
Propofol	Fospropofol
	Dexmedetomidine
	Alfentanyl
	Remifentanil
	Music

cebo. The results revealed that no patients developed any serious episodes of hypoxia and that the incidence of mild hypoxia was not significantly different between the two groups. There was no significant difference in arterial oxygen saturation as recorded by the endoscopist staff[16].

In another study, haemodynamic responses during gastroscopy in healthy subjects were studied in two groups: midazolam alone *vs* midazolam in combination with meperidine. It was found that blood pressure and oxygen saturation significantly decreased with sedation in both groups during endoscopy, but no significant differences were found between the two groups. Heart rate increased significantly, whereas systolic arterial pressure, diastolic arterial pressure and O_2 saturation (SpO_2) decreased significantly, with both regimes. Patient compliance was significantly better with combined sedation[17].

Midazolam has been tested in combination with a variety of other drugs. In one study, the efficacy and safety of midazolam in combination were tested in 74 patients. The midazolam group received only midazolam, and the midazolam/meperidine group received midazolam plus meperidine. The results showed that there was no significant difference between the two groups with regard to the recovery time, procedure time and mean visual analogue scale scores[18].

In a randomised trial comparing the efficacy and recovery time of two sedation regimens consisting of midazolam in combination with either meperidine or fentanyl in patients submitted for colonoscopy, it was found that the use of fentanyl in combination with low-dose midazolam resulted in a significantly faster recovery from sedation compared with meperidine, without any apparent loss of analgesic effect[19].

The synergistic sedation with low-dose midazolam plus propofol *vs* the standard regimen of midazolam and pethidine for conscious sedation in colonoscopy was investigated in a group of patients that included a large number of elderly patients with comorbidities. The synergistic sedation with low-dose midazolam plus propofol was superior to a standard combination of midazolam and the opioid pethidine for colonoscopy in terms of patient comfort and recovery time[20].

In a prospective, randomised study, the standard regime of midazolam and pethidine *vs* a propofol-fentanyl mixture was tested. It was found that patient-controlled sedation/analgesia with propofol and fentanyl was a safe and effective combination, resulting in a high level of satisfaction for both the patient undergoing upper GI tract endoscopic ultrasonography and the endoscopist[21].

Barriga *et al*[22] evaluated the adequacy of conscious sedation during upper endoscopy using midazolam alone compared with midazolam plus fentanyl. Although, from the endoscopist's perspective, patients in the combination group had better tolerance, no significant differences were found in the patient assessments. These results suggest that an adequate level of sedation can be obtained safely by either midazolam or midazolam plus fentanyl.

Midazolam was also tested as an orally administered premedication in patients undergoing upper GI endoscopy. In a double-blind, placebo-controlled, randomised trial, 130 patients were randomised to receive either 7.5 mg of midazolam orally or a placebo as the premedication. The results showed that the median anxiety score during the procedure in the midazolam group was significantly lower than that in the control group. Moreover, a significantly greater number of patients in the midazolam group graded overall tolerance as "excellent or good" and reported partial or complete amnesia in greater degree compared with the control group. Finally, patients in the midazolam group were more willing to repeat the procedure if necessary. However, the median recovery time was significantly longer in the midazolam group than in the control group. No significant differences in the satisfaction score and haemodynamic changes between the two groups were observed[23].

In conclusion, midazolam must be considered as an excellent drug for achieving safe and effective sedation during upper and lower GI endoscopy, whether used alone or in combination with analgesics.

Propofol

The sedative-hypnotic drug propofol (2,6-diisopropylphenol) is a phenolic derivative with satisfactory sedative,

hypnotic, antiemetic and amnesic properties. Additionally, propofol the advantage of a rapid onset of action and a short recovery profile. The depth of sedation increases in a dose-dependent manner. Propofol is highly lipophilic and, therefore, can rapidly cross the blood-brain barrier, resulting in an early onset of action. Consequently, emergence from sedation is also quite rapid because of its fast redistribution into peripheral tissues. Sedation with propofol can be achieved both by bolus administration and continuous infusion. The drug can cause unconsciousness within 30 s. As an additional advantage, regardless of the length of the sedation period, recovery from propofol will occur within 10-20 min after discontinuation. Propofol also has an excellent amnesic effect and short half-life (4 min *vs* 30 min for midazolam). Currently, there is no dispute regarding propofol's superiority over benzodiazepines (with or without opioids) in terms of the abovementioned physiological effects. However, it must be strongly emphasised that titrating propofol to achieve conscious sedation without inducing general anaesthesia requires significant clinical expertise.

This drug is being increasingly used for sedation during painful diagnostic and therapeutic procedures because it increases the quality of upper GI endoscopy by increasing patients' acceptance of the procedure and improving the diagnostic accuracy of endoscopy[24].

With regard to side effects, propofol is generally associated with good haemodynamic stability, although it can induce a dose-dependent decrease in blood pressure and heart rate. Transient decreases in blood pressure are more prominent during bolus administration. Thus, slow initial infusions are recommended in most patients. Moreover, strict aseptic technique must be used during the handling of the product to prevent accidental extrinsic microbial contamination. There are also some other disadvantages of propofol, including the lack of a pharmacological antagonist. The combination of propofol and midazolam has synergistic effects and may have advantages over the use of propofol as a single agent[20]. Thus, a combined sedation regimen with a benzodiazepine retains the possibility for partial pharmacological reversibility using flumazenil.

However, data from a recent meta-analysis suggest that propofol sedation is not associated with an increased risk of complications. In fact, propofol sedation for colonoscopy was associated with lower complication rates than sedation with traditional agents[25]. Several prospective studies confirmed that lower doses were needed for combined sedation with midazolam/propofol compared with propofol alone during diagnostic or therapeutic endoscopy[26,27].

A large number of clinical trials and meta-analyses of these trials have been published, the main results of which are discussed below.

Clinical studies of propofol in upper GI tract endoscopy: A number of studies revealed that propofol offers significant advantages over benzodiazepines and opioids for sedation during endoscopic procedures. Other prospective studies indicated that propofol was safer and more effective than midazolam and meperidine for reaching and maintaining an adequate level of sedation during endoscopy, resulting in better titration of the level of sedation and a shorter recovery time[28].

A prospective study evaluated the safety and efficacy of nurse-administered, low-dose propofol sedation in 8431 adults submitted to upper GI endoscopy. Propofol was administered by bolus injection at a dose of 40 mg for patients < 70 years old, 30 mg for patients 70-89 years old, and 20 mg for patients 90 years old or older. Only 0.26% of the patients required a transient supplemental oxygen supply, and full recovery occurred in 99.9% of patients 60 min after the procedure. However, men and younger patients required significantly higher doses of propofol than women and older patients. A total of 99% of patients were willing to repeat the same procedure. This study showed that the use of only a low dose of nurse-administered propofol sedation is safe and effective for diagnostic esophagogastro-duodenoscopy[29].

Levitzky *et al*[30] showed that balanced propofol sedation targeted to induce moderate sedation in patients undergoing upper GI endoscopy results in better patient satisfaction and a shorter recovery time than standard sedation alone.

Propofol in lower GI tract endoscopy: The optimal regimen of propofol for colonoscopy sedation is still controversial. Both propofol alone and propofol in combination with opiates (meperidine) or benzodiazepines (midazolam) are frequently used during colonoscopy to achieve moderate levels of sedation. Hsieh *et al*[31] noticed that for sedated colonoscopy, propofol in combination with meperidine is better than propofol alone for improving patients' tolerance and recovery.

The combination of 1.0-2.0 mg of midazolam with either 50-100 mg of fentanyl or *i.v.* propofol of 0.5-2.5 mg/kg allowed patients to undergo colonoscopy under comparable sedative and analgesic conditions. The combination with fentanyl had a significantly smaller effect on pulse rate and blood pressure, while the combination with propofol produced more favourable results, especially in terms of superior amnestic effects[32].

In a study of 300 adults undergoing colonoscopy, the use of fentanyl in combination with low-dose midazolam was found to result in a faster recovery from sedation compared with meperidine without decreasing the analgesic effect[19].

Recently, patient-controlled sedation with propofol has been advocated as a method for dealing with the narrow therapeutic window for moderate sedation. In a relevant study, 50 patients undergoing elective colonoscopy were randomised to receive midazolam/fentanyl or propofol/remifentanil administered *via* patient-controlled sedation. Patients in the propofol/remifentanil group were sedated and recovered significantly more rapidly than patients in the midazolam/fentanyl group[33].

Propofor in therapeutic GI endoscopic procedures: In a prospective, randomised, single-blinded study of 222 patients, Lee *et al*[34] compared the safety and efficacy of balanced propofol sedation with conventional sedation (midazolam and meperidine) in patients undergoing therapeutic endoscopic procedures. They found no significant differences between the balanced propofol sedation and conventional groups with regard to the rates of cardiopulmonary complications and transient interruption of procedures, although balanced propofol sedation provided a significantly higher level of endoscopist satisfaction and better patient cooperation.

Meta-analyses of the use of propofol for sedation: To date, two meta-analyses have been published concerning the safety and efficacy of propofol for GI endoscopy.

The first meta-analysis, published in 2005, included 12 original studies with 1161 patients. Of these patients, 634 received propofol and 527 received midazolam, meperidine, and/or fentanyl. Most of the studies were randomised trials of moderate quality. It was found that the pooled odds ratio for developing hypoxia or hypotension for all of the procedures combined was 0.74 in patients using propofol. The pooled odds ratios were 0.85 for upper GI endoscopy, 0.4 for colonoscopy, and 1.07 for ERCP/EUS. Compared with traditional agents, it was noted that sedation with propofol during colonoscopy appears to result in a lower incidence of cardiopulmonary complications, although the risk of complications associated with upper GI endoscopy seems to be similar[25].

The second meta-analysis included 20 studies on the use of propofol for colonoscopy[35]. The analysis showed that recovery and discharge times were shorter with the use of propofol. There was also higher patient satisfaction with the use of propofol, although no significant differences in the procedure time, cecal intubation rate and number of complications were noticed. Finally, no difference in pain control with non-patient-controlled sedation with propofol compared with the traditional agents was noted. The only disadvantage was that pain control with propofol was inferior compared with the use of traditional agents. The general conclusion from these meta-analyses is that propofol for sedation during colonoscopy results in a faster recovery and discharge time, as well as increased patient satisfaction and acceptance rates of side effects.

In conclusion, propofol provides a faster onset of action and deeper sedation compared with standard doses of benzodiazepines and narcotics. More rapid cognitive and functional recovery should be expected when propofol is used as a single agent compared with benzodiazepines. The drug appears to have more advantages when used for prolonged and therapeutic endoscopic procedures, including ERCP and EUS. Although it can be safely and effectively used by a physician-supervised nurse, patients must be under continuous care, consistent with the care required for patients undergoing deep sedation. Personnel in the endoscopy room should be able to rescue the patient from severe respiratory depression.

Fentanyl

Fentanyl, a μ-opioid receptor agonist, is a synthetic narcotic analgesic characterised by a rapid onset and short duration of action. The action of the drug is related to its agonism of the opioid receptors. It is 100 times more potent than morphine, with 100 μg equivalent to 10 mg of morphine and 75 mg of meperidine (pethidine) in terms of analgesic activity. Its strong potency is largely due to its high lipophilicity, which also explains the rapid penetration of the drug into the central nervous system. Fentanyl binds μ-opioid G-protein-coupled receptors, which inhibits the release of pain neurotransmitters by decreasing intracellular Ca^{2+} levels. It has been used in combination with midazolam, mainly in patients undergoing lower GI endoscopy.

In a trial comparing meperidine with fentanyl, the authors noted that the total procedure time was shorter for those receiving fentanyl than for those receiving meperidine. Based on post-procedure pain scores, examinations performed using meperidine were less painful compared with those performed with fentanyl[36].

A meta-analysis compared the efficacy, safety, and efficiency of agents used for moderate sedation in upper GI endoscopy or colonoscopy in 36 studies involving a total of 3918 patients. Sedation improved patient satisfaction and the willingness of patients to repeat upper GI endoscopy compared with these measures in patients who received no sedation. Midazolam provided superior patient satisfaction and resulted in a less frequent memory of the upper GI endoscopy procedure compared with diazepam. Adverse events and patient/physician assessments were not different between midazolam (with or without narcotics) and propofol. The procedure time was similar, but sedation and recovery times were shorter with propofol than midazolam-based regimens. The results confirmed that moderate sedation provides a higher level of physician and patient satisfaction and a lower risk of serious adverse events compared with other currently available agents. Midazolam-based regimens have longer sedation and recovery times than propofol[37].

DRUGS UNDER INVESTIGATION FOR GI ENDOSCOPY

Various other drugs are also under investigation for GI endoscopy. Among them, prodrug formulations of propofol have been developed to overcome the disadvantages of the lipid-based formulations. So far, the results of the relevant studies appear promising. The most important clinical data are presented below.

Nitrous oxide gas

Nitrous oxide gas (N_2O) (molecular mass 44.013) has been proposed as an alternative to *i.v.* analgesia in patients undergoing lower GI endoscopy. N_2O/O_2 mixtures have a satisfactory analgesic effect and short half-lives, thus providing an alternative method of sedation for colonoscopy procedures.

A systematic review of 11 randomised studies including 623 patients was published in 2010[38]. In these studies, N_2O was compared with a lack of sedation in patients undergoing either flexible sigmoidoscopy or colonoscopy. The results revealed that patient-reported pain was similar for N_2O when undergoing flexible sigmoidoscopy *vs* no sedation and when undergoing colonoscopy *vs i.v.* sedation. No differences in duration, procedure difficulty or complications were identified. N_2O was associated with a more rapid recovery than *i.v.* sedation. This systematic review supports the assumption that N_2O provides comparable analgesia to *i.v.* sedation in patients undergoing colonoscopy. Rapid recovery enables quicker patient discharge and removes the need for a patient to be chaperoned.

In a more recent Cochrane review, 257 patients were randomised to receive a N_2O/O_2 mixture (7 studies), while 225 patients received some form of sedation with or without additional analgesia (6 studies) and 65 patients received placebo (3 studies). Four studies showed that N_2O/O_2 reduced pain/discomfort compared with conventional methods, whereas one study showed that sedation was better and another study showed that N_2O/O_2 was better. Six studies showed that N_2O/O_2 groups had a quicker recovery time and shorter length of hospital stay, whereas one study showed no difference between the two groups. Two studies showed that N_2O/O_2 was safer, whereas one showed that sedation was safer. The conclusion was that N_2O is as efficient as and safer than other pain relief methods used during colonoscopy[39].

A randomised clinical trial compared the efficacy of Entonox (50% N_2O and 50% O_2) with midazolam-fentanyl sedation in 131 patients undergoing elective colonoscopy. Sixty-five patients received Entonox, and 66 patients received midazolam-fentanyl. Patients receiving Entonox had a shorter time to discharge. They also reported significantly less pain and better recovery of psychomotor function immediately after the procedure and at discharge. Patients who received Entonox also reported a higher level of satisfaction. Again, this study concluded that Entonox provides better pain relief and faster recovery than midazolam-fentanyl in patients undergoing elective colonoscopy[40].

A double-blind, randomised, placebo-controlled trial showed that N_2O inhaled intermittently is not an effective substitution for *i.v.* on-demand sedation in the setting of colonoscopy without sedation. In this study, patients inhaled N_2O or placebo on demand. The median patient-reported pain level was 2 in both the N_2O and control groups. Additional sedatives and analgesics were given equally often and at similar doses in both groups. No side effects related to the administration of N_2O were noted[41].

In conclusion, the available data suggest that N_2O is an effective analgesic and sedative agent that must be further investigated in larger studies.

Remimazolam

Remimazolam ($C_{21}H_{19}B_rN_4O_2$; molecular weight 439.304) is a short-acting GABA(A) receptor agonist that exhibits organ-independent metabolism, which was developed as an *i.v.* sedative agent for use in day-case procedures and the induction and maintenance of anaesthesia.

Preclinical studies in animals demonstrated that remimazolam caused a more rapid onset and a shorter duration of action compared with midazolam.

In a phase Ⅱ a clinical trial evaluating remimazolam as a procedural sedative for upper GI endoscopy, the time to recovery from sedation was shorter and more consistent with remimazolam compared with midazolam. Because of its organ-independent metabolism and rapid and predictable onset and recovery profile, remimazolam appears to have potential advantages over other currently available short-acting benzodiazepines. However, its respiratory depressant effect has been reported in numerous studies[42].

Fospropofol

Various prodrug formulations of propofol have been developed to overcome the disadvantages of the lipid-based formulations, the complications of lipid infusion, and the risk of fluctuations in propofol plasma levels due to bolus injection.

Fospropofol ($C_{13}H_{19}Na_2O_5P$, molecular weight: 332.24) is a water-soluble prodrug of propofol, metabolised *in vivo* to produce liberated propofol (producing the sedative effect), phosphate and formaldehyde. After *i.v.* injection, propofol is released from fospropofol by tissue alkaline phosphatases with a pattern of plasma concentrations, resulting in lower peak concentrations and a more gradual decline in drug concentrations compared with standard propofol administration protocols. As a result, the drug has pharmacokinetic and pharmacodynamic properties that differ from those of propofol emulsion. The time of the peak sedative effect after a bolus injection fluctuates between 3 and 7.5 min, compared with 1 min 36 s for propofol[43]. Fospropofol was generally well tolerated in clinical trials. Adverse events are mostly of mild-to-moderate severity and are transient and self-limiting[44].

The 6.5-mg/kg dose of fospropofol provides the ideal balance of efficacy and safety for patients undergoing colonoscopy. In a double-blind trial evaluating 127 patients who received fospropofol (2, 5, 6.5 or 8 mg/kg) or midazolam 0.02 mg/kg following pre-treatment with fentanyl, fospropofol yielded a significant dose-dependent increase in sedation success from 24% (2 mg/kg), 35% (5 mg/kg) and 69% (6.5 mg/kg) to 96% (8 mg/kg; $P < 0.001$)[45].

The same group of authors evaluated the efficacy and safety of *i.v.* fospropofol administration in patients undergoing colonoscopy for moderate sedation. Patients were randomised to receive fospropofol 2 mg/kg, fospropofol 6.5 mg/kg, or midazolam 0.02 mg/kg after pretreatment with *i.v.* fentanyl 50 μg. The results showed that sedation success was higher in the fospropofol 6.5 mg/kg group compared with the 2 mg/kg group (87% *vs* 26%; $P < 0.001$) and was 69% in the midazolam group.

Most adverse events were mild to moderate in intensity, the most common being paraesthesias (68% *vs* 60%) and pruritus (16% *vs* 26%) in the fospropofol 6.5 and 2 mg/kg groups, respectively. Fospropofol 6.5 mg/kg was associated with higher rates of sedation success, memory retention, and physician satisfaction than fospropofol 2 mg/kg[46].

Together, these results suggest that fospropofol is a promising drug requiring further investigation.

Dexmedetomidine

Dexmedetomidine ($C_{13}H_{16}N_2$, molecular mass: 200.28), a pharmacologically active dextroisomer of medetomidine, is a selective α(2)-adrenergic receptor agonist. It is indicated for the sedation of mechanically ventilated adult patients in an intensive care setting and in non-intubated adult patients prior to and/or during surgical and other procedures[47]. The drug should be administered *i.v.* only by experienced individuals, and the patient must be continuously monitored. Additionally, the dose must be adjusted in patients with liver and renal failure, as well as in elderly patients.

Dexmedetomidine can be safely used as a sedoanalgesic agent in colonoscopies because it provides efficient haemodynamic stability, higher satisfaction scores and lower Numeric Rating Scale scores. A study comparing dexmedetomidine (1 μg/kg and as a continuous infusion dose of 0.5 μg/kg per hour) with midazolam (0.05 mg/kg) plus fentanyl citrate (1 μg/kg) with regard to perioperative haemodynamics, sedation, pain, satisfaction and recovery scores during colonoscopy showed that, although statistically significant differences in mean arterial pressure were not detected between the two groups, heart rates were higher and SpO_2 scores were lower in dexmedetomidine group. When the groups were compared using the Ramsay sedation scale, the scores of group I at the 10th and 15th minute were significantly lower than those of group II[48].

In a recent study, Takimoto *et al*[49] showed that sedation with dexmedetomidine is a safe and effective practice in patients with gastric tumours undergoing endoscopic mucosal resection. In their study, 90 patients with gastric tumours were sedated with either dexmedetomidine [*i.v.* infusion of 3.0 μg/kg per hour over 5 min, followed by continuous infusion at 0.4 μg/kg per hour (n = 30), propofol (n = 30), or midazolam (n = 30)]. In all groups, 1 mg of dexmedetomidine was added *i.v.* as needed. The results showed that none of the dexmedetomidine-sedated patients exhibited a significant reduction in oxygen saturation level. Fewer patients in the dexmedetomidine group showed body movement during endoscopy compared with the other groups. The rate of effective sedation was significantly higher in the dexmedetomidine group compared with the midazolam and propofol groups. The mean duration of endoscopic submucosal dissection in the dexmedetomidine group was significantly shorter than that in the other two groups. However, dexmedetomidine alone is most likely not as effective as propofol combined with fentanyl for providing conscious sedation during ERCP, exhibiting concurrently greater haemodynamic instability and prolonged recovery[50].

Alfentanyl

Alfentanyl ($C_{21}H_{32}N_6O_3$, molecular weight: 452.98) is a narcotic analgesic with a rapid onset of action, a very short duration of action, and a potency of approximately one-third that of fentanyl. Recently, it was shown that patient-controlled analgesia pumps and sedation with alfentanyl and fentanyl for colonoscopy are safe, feasible, and acceptable to most patients, although a shorter sedation time makes alfentanyl more attractive, as it reduces the postprocedural workload[51].

Remifentanil

Remifentanil ($C_{20}H_{28}N_2O_5$, molecular weight: 376.447) is a μ-opioid receptor agonist that has important neuroanaesthesia characteristics. It has been used in a small number of clinical trials in patients undergoing GI endoscopic procedures. There are many reports of the use of remifentanil in different settings, including GI endoscopy, with or without background infusion, and the quality of analgesia and patient satisfaction seem to be the same as with standard sedation/analgesia. It seems that remifentanil patient-controlled analgesia is safe and effective for inducing sedoanalgesia during colonoscopy.

In a randomised, double-blind clinical trial, 60 patients undergoing colonoscopy were randomly assigned to either the remifentanil or meperidine group. All of the patients received premedication with midazolam 0.03 mg/kg *i.v.* In the remifentanil group, a bolus dose of remifentanil was given, and a patient-controlled sedation/analgesia pump was set to inject further bolus doses, while patients in the meperidine group received a bolus of meperidine and a sham, patient-controlled sedation analgesia pump. The degree of pain, level of satisfaction with sedoanalgesia of patients and gastroenterologists, and degree of difficulty experienced by the endoscopist, as well as the discharge time and duration of colonoscopy, were not different between the two groups[52].

In another study, the safety and efficacy of remifentanil during colonoscopy compared with the standard combination of midazolam and pethidine were tested in 116 patients who received either midazolam and pethidine or remifentanil only. Recovery was found to be faster in the remifentanil group. There was also a significant difference with regard to the time of hospital discharge. In this study, remifentanil during colonoscopy provided sufficient pain relief with better haemodynamic stability, less respiratory depression, and significantly faster recovery and hospital discharge times than moderate sedation with midazolam and pethidine[53]. However, further studies are needed to confirm these interesting results.

Music

Among methods reported to minimise patient discomfort during GI endoscopy (especially colonoscopy), mu-

sic has been utilised as an important therapeutic tool for effectively relieving stress and inducing analgesia. During recent years, several papers investigating the efficacy of music on patients' stress and pain relief were published. The most important of these publications are discussed below.

Clinical trials concerning the role of music in sedation: More recent clinical trials not included in the abovementioned meta-analyses have revealed rather conflicting results.

In a single-blind, randomised, controlled trial, the authors showed that music significantly reduces discomfort and, consequently, should be routinely provided to patients undergoing colonoscopy. In this study, 109 patients were randomised to receive music-delivering or non-sound-emitting headphones before and during endoscopy. The results revealed that the mean pain score was significantly lower in the music group compared with the control group, while overall satisfaction and willingness to repeat the procedure were significantly improved and the difficulty perceived by physicians was significantly reduced. Interestingly, the total amount of midazolam and pethidine was significantly lower in the music group compared with the control group[54].

Music in the endoscopy room was also found to reduce the anxiety levels in patients undergoing endoscopic procedures. In a controlled trial of 180 patients, the effect of age and type of endoscopic procedure on anxiety levels upon arrival in the unit and immediately before the endoscopy procedure after listening to music or no music (control group) for the same period was investigated. Although anxiety levels were not influenced by age or procedure, it was found that listening to music resulted in a significant reduction in anxiety scores, which was maintained for all age groups, irrespective of the type of endoscopic procedure performed. The authors suggest that providing music in the endoscopy unit is a simple strategy that can improve the well-being of patients[55].

Another study, which was specifically designed to investigate whether listening to music reduced the pain experienced by patients during sigmoidoscopy without sedation or analgesia, concluded that listening to music did not reduce pain intensity. In this study, it was found that the mean pain intensity in the music group was not different from that in the control group, and the proportion of patients with at least moderate pain during sigmoidoscopy did not differ between the two groups[56].

Meta-analyses concerning the role of music in sedation: Three meta-analyses regarding the role of music in sedation have been published to date. At least two of them suggested that music can effectively relieve stress and improve the level of analgesia during GI endoscopy. In the first of these meta-analyses, the authors included six randomised, controlled trials with a total of 641 patients. They found that in studies that did not use pharmacotherapy, patients receiving music therapy exhibited significantly lower anxiety levels compared with controls. Additionally, in studies in which pharmacotherapy was used, patients receiving music therapy exhibited significant reductions in analgesia requirements and an almost significant reduction in sedation requirements compared with controls. Furthermore, the procedure time was significantly reduced in the music therapy group compared with the control group. The authors' conclusion was that music therapy is an effective tool for stress relief and analgesia in patients undergoing GI endoscopic procedures[57].

In the second meta-analysis, which was published in 2008 and focused on colonoscopy, the authors included 8 studies with a total of 722 patients. In four studies, music was transmitted through headphones/earphones (as background music in three studies, and one study did not specify the media method). The results showed that the combined mean time taken for the colonoscopy procedure was shorter in the music group compared with the control group. There was weak evidence of benefit regarding the pain score, blood pressure, and mean recovery time in the music group compared with the control group. No harmful effects from listening to music were reported in any of the studies in this meta-analysis. The only disadvantage found in allowing patients to listen to music through headphones/earphones was the isolation of patients from the medical staff during the procedure. The authors concluded that "listening to music is effective in reducing procedure time and amount of sedation during colonoscopy and should be promoted"[58].

Finally, in the third meta-analysis, which was published in 2009 and included 8 studies with a total of 712 patients, it was found that patients' overall experience scores were significantly improved when they were allowed to listen to music. However, no significant differences were noted in patients' pain scores, mean doses of midazolam and meperidine, procedure time, and willingness to repeat the same procedure in the future, indicating that music improves only patients' overall experience with colonoscopy[59].

One possible explanation for the reduction in the doses used for sedation sedation is that patients in the music group are more relaxed and have less anxiety, resulting in a faster completion of the procedure and the use of less sedation[60]. The reduction in procedure time implies a reduction in the time during which patients feel anxious, frightened, and uncomfortable while undergoing the procedure and may be useful in enhancing the compliance rate. The avoidance of sedation may obviously result in a quicker patient discharge, less need for monitoring, and overall cost savings. Two other advantages of music are its inexpensiveness and ease of implementation[61].

In conclusion, it seems that listening to music, especially during colonoscopy, could reduce the procedure time, anxiety, and amount of sedation needed, without producing any harmful events. As a result, music should be promoted because of its beneficial effect and negligi-

ble cost. However, several aspects of this method, such as the choice of music and the mode of transmission, are worth further investigation.

SEDATION FOR GI ENDOSCOPY IN SPECIAL CLINICAL SITUATIONS

A large number of situations require special attention not only at the beginning of the endoscopic procedure but also during the procedure and recovery. The drugs that must be used, along with the precautions that must be followed, are analysed below.

Obesity

Obesity is a significant health problem that has assumed epidemic proportions. As a result, the number of obese patients requiring endoscopy is increasing. Morbid obesity can result in pulmonary hypertension, obstructive sleep apnoea, and restrictive lung disease. It is relatively unknown how safe the current practices of sedation for endoscopic procedures are in bariatric patients[62]. Therefore, special consideration should be given to these patients, and endoscopists need to be aware of challenges that may be present while performing endoscopic procedures in obese patients[63].

There are limited data on the use of sedation in obese patients. Studies published to date refer mainly to the use of sedation in obese subjects undergoing advanced endoscopic procedures or upper GI endoscopy before bariatric surgery.

In a study involving 69 subjects with morbid obesity submitted to upper GI endoscopy before bariatric surgery, the authors administered sedation with propofol at a mean dose of 380 ± 150 mg (range 80-900 mg). Two patients developed severe hypoxemia, which required bronchoscopic intratracheal O_2 insufflation. Thus, although upper GI endoscopy can be performed safely in obese patients, careful monitoring and anesthesiological support are required, especially in patients with concomitant diseases[64].

In a study investigating the safety of anaesthesia-assisted endoscopy using propofol-mediated sedation in subjects undergoing advanced endoscopic procedures, the authors found that an increased body mass index was associated with an increased frequency of airway manoeuvres and hypoxemia. A multivariate analysis revealed that body mass index was an independent predictor of the appearance of sedation-related complications. Interestingly, in obese individuals, there was no difference in the frequency of sedation-related complications in patients receiving propofol alone or in combination with other drugs. Propofol sedation can be safely used in obese patients undergoing advanced endoscopic procedures when administered by trained professionals, despite the increased frequency of sedation-related complications[65].

Finally, it was found that patients who undergo upper GI endoscopy with either anaesthesiologist- or surgeon-monitored sedation seem to tolerate the procedure equally well. However, significantly fewer patients in the anaesthesiologist-monitored sedation group complained of throat pain after the procedure and/or remembered gagging during the procedure, thus leading to the conclusion that anaesthesiologist-monitored sedation should be considered in patients undergoing preoperative upper endoscopy before bariatric surgery[66].

In conclusion, although quite safe, moderate sedation during endoscopy may pose some risks to obese patients. In particular, the presence of obstructive sleep apnoea may identify a subset of patients at higher risk of complications. Further studies are required in this field, as the number of subjects undergoing bariatric surgery is constantly increasing worldwide.

Chronic liver disease

Endoscopy, either diagnostic and/or therapeutic, is often necessary in patients with chronic liver disease, sometimes on an emergency basis. It is well established that liver disease may impair the metabolism of drugs usually administered for sedation. The evaluation of patients with chronic liver disease before endoscopy should include a full assessment of hepatic function, as well as a complete physical examination to exclude the possibility of the presence of hepatic encephalopathy. As a general rule, sedation should be used especially in patients undergoing ligation of acutely bleeding varices, although in some cases sedation is not necessary[67].

Liver dysfunction could reduce both the clearance of the drugs eliminated by hepatic metabolism or biliary excretion and plasma protein binding. Chronic liver disease is also associated with a reduction in drug-metabolising activities, such as the activity of the CYP450 enzymes. In patients with advanced cirrhosis, it is necessary to adjust the dose of those drugs eliminated by renal excretion[68].

Concerning the drugs used in the sedation of cirrhotic patients, most authors prefer to use propofol instead of benzodiazepines and opioids because of its short biological half-life and lower risk of provoking hepatic encephalopathy.

In a recently performed study, the authors compared sedation with combinations of propofol plus fentanyl and midazolam plus fentanyl in 210 cirrhotic patients undergoing upper GI endoscopy. The doses of midazolam and propofol were 0.05 and 0.25 mg/kg, respectively, while the dose of fentanyl was 50 μg *i.v.* in both groups. It was found that sedation with propofol was more effective and yielded a shorter recovery time than sedation with midazolam, indicating that it is a safe and effective regimen[69].

Another study reported that the use of propofol in patients with cirrhosis does not precipitate minimal or overt hepatic encephalopathy during upper GI endoscopy[70]. The results of this study were recently confirmed in a study showing that sedation with propofol in patients with compensated liver cirrhosis resulted in a shorter time to both recovery and discharge than midazolam, thus not exacerbating sub-clinical hepatic encephalopathy[71].

These results are in accordance with those described in a previous study that demonstrated that propofol sedation does not cause deterioration of minimal hepatic encephalopathy concurrently associated with improved recovery in patients with liver cirrhosis[72].

In conclusion, propofol represents a safe and effective sedation drug that could be used as an alternative to midazolam in patients with liver cirrhosis[73].

Pregnancy

Despite the fact that endoscopy is rarely required during pregnancy and is generally considered to be safe, endoscopists must be aware of the potential risks concerning both the mother and the foetus. Before endoscopy, the endoscopist must calculate the potential foetal risks, mainly due to sedation, and try to correct any possible maternal pathological situation, including hypoxia and hypotension.

During endoscopy, pregnant women should be carefully monitored by continuous electrocardiography, pulse oximetry, and intermittent blood pressure estimation[74].

Sedative drugs comprise a significant foetal risk during endoscopy in pregnant women because of the risk of hypoxia. Additionally, the exposure of pregnant women to radiation during ERCP represents another important risk that should be reduced as much as possible.

Currently, there is no evidence that endoscopy precipitates premature labour. If possible, endoscopic procedures must be performed without any sedation or, alternatively, by administering the lowest effective dose of sedative medication.

Regarding the types of drugs used for sedation, the available literature suggests that midazolam appears to be safe if used carefully[75].

ERCP is rarely necessary during pregnancy, although it cannot be avoided in pregnant women with recurrent biliary colic, abnormal liver function tests, and a dilated bile duct. A relevant study showed that ERCP can be safely performed during pregnancy, leading to successful treatment in almost all patients. Sedation is uneventful for all pregnant women and their foetuses. However, it must be recognised that pregnancy may be associated with a higher rate of post-ERCP pancreatitis compared with the general population[76]. The same conclusion was reached in another study, the authors of which noted that ERCP is a safe procedure during pregnancy, even if the placement of a biliary stent is necessary[77].

In conclusion, upper GI endoscopy, including therapeutic interventions such as the banding of oesophageal varices, seems to be relatively safe for the foetus, although it should be performed only when strongly indicated. Similarly, flexible sigmoidoscopy also appears to be safe for the foetus and, again, should only be performed when strongly indicated. Colonoscopy data suggest that this procedure should be performed only during the second trimester and only if there is a strong indication. Finally, ERCP seems to be relatively safe but should only be performed if there is a strong indication for its use. Sedation is rather safe in ERCP, and midazolam is the preferred pharmaceutical agent by most endoscopists. As a general rule, it may be suggested that endoscopy in pregnant women should always be performed in a hospital by an expert endoscopist and only when strongly indicated[78].

Sedation in celiac disease

It has been suggested that patients with celiac disease exhibit increased rates of neuropsychiatric disturbances and visceral hypersensitivity. In a retrospective cohort study, Lebwohl *et al*[79] noted that 26% of patients with celiac disease required higher amounts of both opioids and midazolam compared with age- and gender-matched controls, possibly due to increased visceral hypersensitivity, chronic opioid/anxiolytic use, and/or underlying neuropsychiatric illness.

Sedation in the elderly

Although GI endoscopy with sedation is increasingly performed in elderly patients, data on the outcomes and side effects of sedation are limited. Age-related pharmacokinetic changes and the presence of comorbidities and polypharmacy complicate drug therapy. Aging results in impairment in the function of multiple organs, including the liver, which may also affect drug metabolism and pharmacokinetics. In addition, older people often have to consume a variety of drugs, the bioavailability of which could be increased. Additionally, lipophilic drugs may have a prolonged half-life. Combined with reduced hepatic and renal clearance mechanisms, this prolonged half-life can prolong the recovery of elderly patients after sedation. In the elderly, hepatic drug clearance of some drugs can be reduced by up to 30%. Midazolam is indicated because there are no major differences in CYP3A4 activity between young and old people. Finally, renal excretion is decreased in most elderly individuals because of the presence of hypertension and coronary heart disease[80].

In the geriatric population, conscious sedation practices are modified by the administration of fewer agents at a slower rate and lower cumulative dose. Midazolam has been widely used in elderly patients[81]. Under certain circumstances, it seems that the benefits, in terms of tolerance of low-dose midazolam for upper GI endoscopic sedation, outweigh the risks in older people. Christe *et al*[82], in a randomised, double-blind, placebo-controlled study, administered either midazolam (30 μg/kg *i.v.*) or saline (placebo) to 65 geriatric inpatients undergoing upper GI endoscopy. The results revealed that midazolam increased the probability of good tolerance. Midazolam resulted in a 10-mmHg reduction in the mean arterial pressure without inducing clinically significant hypotension. Finally, midazolam was associated with a higher risk of hypoxemia after endoscopy, but not of confusion.

In a recent study, it was found that elderly patients submitted for endoscopy required lower mean propofol doses for sedation compared with patients aged < 70

years. No major complications and no difference in the number of minor complications were noted. A favourable safety profile for combined sedation with midazolam/propofol and a higher sensitivity to propofol must be expected in patients older than 70 years of age who have various co-morbidities[83].

A study evaluating the safety of sedation with propofol in patients > 90 years of age showed that for upper GI endoscopy, percutaneous endoscopic gastrostomy, colonoscopy, and ERCP, the mean propofol doses used were 22, 24, 46 and 42 mg, respectively. In upper GI endoscopy, the level of sedation and propofol blood concentrations after administration of the drug in the group of patients > 90 years of age corresponded to those resulting from propofol use in middle-aged patients[84].

Finally, Martínez *et al*[85] found that continuous propofol sedation in patients > 80 years of age is generally as safe as in younger patients, although patients > 80 years showed a greater tendency to develop severe oxygen desaturation during the colonoscopy and endoscopic ultrasonography procedures. In general, there were no significant differences in sedation-related complications between the two groups.

Sedation in time-consuming endoscopic procedures

Time-consuming endoscopic procedures, such as ERCP, endoscopic ultrasonography and endoscopic mucosal resection, require sedation for a significantly longer period of time compared with routine upper and lower GI tract endoscopy. Moreover, endoscopic submucosal dissection for early gastric cancer generally lasts much longer than conventional endoscopy and usually requires moderate-to-deep sedation with close surveillance to ensure patient safety, thus increasing the risks related to sedation and analgesia. Therefore, the administration of safe sedation of a satisfactory degree for a longer period of time is necessary.

During recent years, a significant number of papers have been published examining the most suitable and effective drug or combination of drugs for these complex procedures. The data from these clinical studies are discussed below.

Concerning ERCP, a study comparing satisfaction, recovery scores, and safety profiles for ERCP sedation between continuous infusion of propofol and conventional sedation revealed that the continuous infusion of propofol for ERCP under the direction of a gastroenterologist yields no differences in the procedure completion rate and adverse profiles compared with intermittent meperidine and midazolam injection. However, the infusion of propofol does provide a better recovery profile[86].

It is well known that the dose requirements and complications of propofol are less when used in the diluted form than when used in the undiluted form. In a study investigating diluted and undiluted propofol requirements and recovery time in patients undergoing ERCP, it was shown that the requirements in both groups were comparable, although the incidence of sedation-related hypotension was lower in the diluted group[87].

An interesting study showed that patient-controlled sedation with propofol/remifentanil seems to be a well-accepted sedation regimen for ERCP. Additionally, the study showed that anaesthesiologist-managed propofol sedation using constant propofol infusion is associated with deep sedation without any impact on the degree of patient or gastroenterologist satisfaction[88].

EUS and ERCP can be safely performed under conscious sedation on the same day with minimal adverse events. However, combined procedures are associated with higher doses of sedatives and a slightly longer recovery time[89].

Finally, another interesting study suggested that synergistic sedation with an oral dose (7.5 mg) of midazolam 30 min before *i.v.* propofol is given combined with *i.v.* propofol could result in a significant reduction in the dosage of propofol required and in patient anxiety levels before ERCP[27].

With regard to endoscopic ultrasonography, a prospective, randomised study demonstrated that patient-controlled sedation/analgesia with propofol and fentanyl is a more effective and safe technique compared with midazolam and pethidine, resulting in a high level of satisfaction of both patients and endoscopists[21].

Concerning endoscopic mucosal dissection (ESD) for early gastric cancer, a recent study in Japan revealed that ESD performed under sedation using continuous propofol infusion for early gastric cancer was as safe as ESD performed using intermittent midazolam injection. Moreover, patients treated with continuous propofol administration experienced a quicker recovery time than those treated with midazolam[90].

A more recent study, also from Japan, confirmed the results of the study by Kiriyama *et al*[90] and suggested that propofol is as safe and effective as midazolam during ESD. Despite these promising results, sedation guidelines for the use of propofol in early gastric cancer are needed[91].

Among the newer drugs used for sedation and analgesia during GI endoscopy, dexmedetomidine has been used in patients with early gastric cancer undergoing ESD. In a randomised study of 90 patients who underwent ESD treatment, sedation was achieved with either dexmedetomidine (3.0 μg/kg *i.v.* per hour over 5 min, followed by continuous infusion at 0.4 μg/kg per hour), propofol, or midazolam. It was shown that none of the dexmedetomidine-sedated patients developed a significant reduction in the oxygen saturation level. The rate of effective sedation was significantly higher in the dexmedetomidine group compared with the other two groups. It seems, therefore, that sedation with dexmedetomidine is safe and effective in patients with gastric tumours who are undergoing ESD[49].

ADVERSE EVENTS DURING SEDATION FOR ENDOSCOPY

Sedation is usually safe; however, complications may occur, although in various proportions depending on a

number of factors, including the type, dose and mode of administration of sedative drugs, as well as the patient's age and underlying chronic disorders. A large number of side effects, including hypotension, desaturation, bradycardia, hypertension, arrhythmia, aspiration, respiratory depression, vomiting, cardiac arrest, respiratory arrest, angina, hypoglycaemia, and/or allergic reaction, have been reported.

A study in patients submitted to colonoscopic examination showed that midazolam combined with propofol appeared to influence the pulse rate and blood pressure at a significantly higher rate than a combination with fentanyl or midazolam alone. The combination with fentanyl had a significantly lower effect on pulse rate and blood pressure[32].

Prolonged hypoxemia (oxygen saturation of < 90% for ⩾ 15 s) and apnoea (lack of respiratory activity for ⩾ 15 s) are not uncommon during moderate sedation for endoscopy. In a related study, it was noted that hypoxemia usually occurs within 5 min of medication administration or endoscope intubation and that only 1/3 of all apnoea/abnormal ventilation events lead to hypoxemia. Additionally, the total dose of meperidine/fentanyl and the total dose of midazolam are predictors of apnoea[92].

Dreaming is commonly reported after propofol-based sedation. In a relevant study, the per cent of patients reporting dreaming was 19%. It seems that this phenomenon appeared more frequently in patients who received high doses of propofol and in patients who had lower bispectral index values during sedation[93].

In a study of 17 999 endoscopic procedures performed over 8 years, the authors concluded that deep sedation during endoscopic procedures is safe[94]. They noted that adverse events occurred in a small proportion of patients (4.5%) and that six complications, i.e., hypotension, desaturation, bradycardia, hypertension, arrhythmia, and aspiration, occurred in more than 0.1% of patients.

Conigliaro *et al*[95] found a percentage of 0.47% of complications related to endoscopy in patients undergoing colonoscopy when sedation was used as recommended by the guidelines.

The administration of propofol as a sedative agent in GI endoscopy resulted in a significant reduction in mean arterial pressure compared with pre-intervention values, although severe hypotension (systolic blood pressure < 60 mmHg) was noted in 0.5% of patients. Oxygen saturation decreased from 96.5% to 94.4%, although a critical decrease in oxygen saturation (< 90%) was documented in only 2.4% of patients[28].

In cirrhotic outpatients undergoing upper GI endoscopy, sedation with a combination of propofol plus fentanyl or midazolam plus fentanyl revealed no significant differences in the rate of complications between the two groups (14% *vs* 7.3%). In this study, both sedation schemes appeared to be safe[69].

Another study revealed no significant differences in complication rates between propofol deep sedation and meperidine/midazolam administered for moderate sedation. In this study, the complication rate with propofol was 0.60%, compared with 1% in the historical case-control (meperidine/midazolam moderate sedation) group[96]. Among the 324 737 unique procedures performed in patients under conscious sedation, unplanned events were reported in 1.4% of the procedures, 0.9% of which were associated with unplanned cardiopulmonary events[97]. Ljubicić *et al*[98] observed a decrease in oxygen saturation to < 85% and a temporary decrease in heart rate to < 50 beats/min in 5.5 and 11.8% of patients receiving propofol for endoscopy, respectively. Finally, Amornyotin *et al*[87] noted significantly different overall complication rates of 18.2% and 42.9%, respectively, between patients receiving diluted or undiluted propofol for ERCP. Significant differences were also noted in the overall rate of cardiovascular events.

In conclusion, sedation in GI endoscopic procedures, even in time-consuming procedures, seems to be safe. The rate of complications, either cardiovascular or respiratory, could be characterised as "acceptable", provided that all prevention measures have been adopted and the endoscopy staff is suitably equipped, properly trained in the handling of possible complications, and ready to immediately apply rescue measures. Table 4 lists the main sedation-related adverse events occurring during endoscopy in clinical trials, while Table 5 shows the main adverse effects related to the administration of drugs used for sedation in GI endoscopy.

LEGAL ISSUES RELATED TO SEDATION IN GI ENDOSCOPY

Important medical and legal issues regarding sedation have been raised during recent years. Such issues include informed consent of the patient, difficulties in assessing withdrawal of consent in a sedated patient, and the need for sedation monitoring that meets accepted standard of care guidelines[99]. Other controversies possibly related to medico-legal aspects include both the use of propofol and the administration of sedation by anaesthesia personnel. The former controversy is extremely important from a legal point of view if the continuously increasing use of propofol in GI endoscopy by non-anaesth esiologists is taken into account. In a related article, Axon emphasises the possible clinical negligence that could be associated with sedation administration. Interestingly, while the law recognises the desirability of sedation in endoscopy procedures, the facts of a particular case will be scrutinised to determine possible responsibilities of the endoscopist if an adverse outcome occurs[100]. Some questions related to the administration of sedation during GI endoscopy are discussed below.

Should sedation be administered by an endoscopist gastroenterologist or an endoscopist nurse?

The optimal drug for sedation administered by non-anaesthesiologists should have certain properties, such as a

Table 4 Main adverse events related to sedation occurring during endoscopy in clinical trials

Ref.	Drug regimen	Percentage of side effects	Severe hypotension (< 60 mmHg)	Severe desaturation (< 90%)
Ljubicić *et al*[98]	Propofol	17.3% (including bradycardia: 11.8%)		5.5%
Conigliaro *et al*[95]	Midazolam	0.47%		
Gasparović *et al*[28]	Propofol	2.9%	0.5%	2.4%
Sharma *et al*[97]	Cardiopulmonary events	EGD: 0.6%; Colonoscopy: 1.1%; ERCP: 2.1%; EUS: 0.9%		
Nayar *et al*[96]	Propofol deep sedation *vs* moderate sedation	0.6% *vs* 1.0%	0.1%	0.1% (apnoea: 0.3%)
Correia *et al*[69]	Midazolam plus propofol *vs* midazolam plus fentanyl	14% *vs* 7.3%		
Amornyotin *et al*[87]	Diluted *vs* undiluted propofol for deep sedation	18.2% *vs* 42.9%	11.4% *vs* 31.0%	0 *vs* 2.4%
Wang *et al*[32]	Midazolam *vs* midazolam combined with either fentanyl or propofol		Midazolam combined with propofol resulted in hypotension and bradycardia more significantly than a combination with fentanyl or midazolam alone	

EUS: Endoscopic Ultrasound; EGD: Esophagogastroduodenoscopy; ERCP: Endoscopic retrograde cholangiopancreatography.

Table 5 Side effects related to the administration of drugs used for sedation in gastrointestinal endoscopy

Side effect	Midazolam	Propofol	Fentanyl
Hypotension	Yes	Yes	
Hypertension		Yes	
Heart rate alterations	Arrhythmia	Decrease	Arrhythmia
Respiratorydepression	Yes	Yes	Yes (particularly in the elderly)
Apnoea	Yes (in combination with fentanyl)	Yes	Yes (in combination with Midazolam)
Dystonia		Yes	Yes
Priapism		Yes	Yes (very rarely)
Pain on injection		Yes	
Lactic acidosis		Yes	
Intraocular pressure changes		Decrease	
Myoclonic movements		Yes	
Nervous system side effects	Yes (especially in the elderly)	Rare	Yes
Unusual dreams		Yes	
Hypersensitivity	Yes	Yes	Yes (rarely)
Liver damage		Yes	
Amnesia	Yes		
Impairment of cognitive functions - inability to drive safely	Yes		
Paradoxical behaviour	Yes	Yes	
Gastrointestinal effects (nausea, vomiting, hiccups, diarrhoea)	Yes	Yes	Yes
Sexual disinhibition	Yes		
Potential for abuse			Yes
Haemolysis			Yes (slow injection rates and/or mixture in isotonic fluid)

predictable pharmacokinetic profile, rapid onset of action, analgesic and anxiolytic effects, short recovery time, and minimal associated risks, thus making the presence of an anaesthesiologist unnecessary.

There is evidence suggesting that non-anaesthetists can administer sedative drugs, including propofol, safely and effectively in most cases[101].

In the last decade, a number of studies addressed the safety and efficacy of the administration of propofol during GI endoscopy by either physicians or trained nurses[102,103].

With regard to the occurrence of major side effects in these studies, there were no cases of death among patients submitted to endotracheal intubation. In a prospective trial involving 36 743 cases of nurse-administered propofol sedation, the authors concluded that adequately trained nurses and endoscopists can safely administer propofol[104].

Rex *et al*[105], in a safety review of 646 080 (223 656 published and 422 424 unpublished) endoscopist-directed propofol sedation cases, noted that endotracheal intubation and death occurred in 11 and 4 cases, respectively. They concluded that the endoscopist-directed administration of propofol appears to result in a lower mortality

rate than that of traditional sedation with benzodiazepines and opioids and a comparable rate to that of general anaesthesia administered by anaesthesiologists.

Nurse-administered propofol sedation for endoscopic procedures is safe when performed by personnel properly trained in airway handling and sedation with propofol and has considerable advantages compared with conventional sedation for endoscopy[106].

Finally, in a very recent study assessing the current use of propofol during colonoscopy screening in 29 countries, it was found that non-anaesthesiologist-administered propofol was used by 29.9% of respondents in 9 countries. Approximately 2/3 of the other endoscopists reported that they would consider implementing non-anaesthesiologist-administered propofol in low-risk patients. It was also found that propofol, benzodiazepine plus opioids and benzodiazepine alone were used in 45%, 31% and 14% of cases, respectively. Importantly, the main reasons for not considering non-anaesthesiologist-administered propofol implementation were medico-legal issues and cost[107]. We suppose that these issues have not been solved and will continue to be discussed in the future.

Should sedation be administered only by a specialist anaesthesiologist?

It is well established that most of the complications occurring during GI endoscopy, such as hypoxemia, hypoventilation, airway obstruction, apnoea, arrhythmias, hypotension and vasovagal episodes, are not related to the procedure itself, but rather to sedation. A recent trial compared endoscopist-administered propofol sedation for colonoscopy with anaesthetist-administered deep sedation. It was found that endoscopist-administered propofol sedation for colonoscopy yielded a better level of satisfaction and patient willingness to undergo further colonoscopies under the same conditions, as well as fewer side effects than anaesthetist-administered deep sedation[108].

Guidelines concerning the use of propofol have been delivered by most major endoscopic associations worldwide. The guidelines of the Endoscopic Section of the German Society for Digestive and Metabolic Diseases suggest that "for simple endoscopic examinations and in low-risk patients, sedation with propofol should be induced by a properly qualified physician and can then be monitored by an experienced person with appropriate training. The person must not have any other tasks while monitoring the sedation". Furthermore, they suggest that an anaesthesiologist should be required only in patients with a high-risk profile.

Four major United States GI Societies, the American Association for the Study of Liver Disease, American College of Gastroenterology, American Gastroenterological Association and American Society for Gastrointestinal Endoscopy, suggest that the administration of propofol is comparable to that of standard sedation with benzodiazepines by non-anaesthesiologists with respect to their safety and efficacy profile.

The guidelines of the European Society of Gastrointestinal Endoscopy, the European Society of Gastroenterology and Endoscopy Nurses and Associates, and the European Society of Anaesthesiology (produced by 32 individuals from 12 countries, published in 2010) can be summarised as follows:[109] "The consensus suggested that endoscopists and nurses with appropriate training can safely and effectively administer propofol to low-risk patients undergoing endoscopic procedures".

Therefore, the safety profile of non-anaesthesiologist-administered propofol sedation for GI endoscopy seems to be equivalent to that of standard sedation with respect to the risks of hypoxemia, hypotension and bradycardia in ERCP and EUS. Concerning upper and lower GI endoscopy, ERCP and EUS, the time for sedation induction and the recovery time using non-anaesthesiologist-administered propofol sedation are shorter compared with those associated with standard sedation, while non-anaesthesiologist-administered propofol sedation is most likely more cost-effective than standard sedation for ERCP and EUS.

However, the opinion of almost all anaesthesiology societies concerning the use of propofol by non-anaesthesiologists is definitely negative. They emphasise the fact that the manufacturers of propofol restrict its use solely to personnel trained in general anaesthesia and that the United States Food and Drug Administration denied a petition by gastroenterologists seeking the removal of this particular restriction. In a recent consensus statement, the European Society of Anaesthesiology, together with 20 European national anaesthesiology societies in Europe, published new guidelines, entitled "Non-anaesthesiologist Administration of Propofol for Gastrointestinal Endoscopy". They stated that due to its significant risks, propofol should be administered only by those trained in the administration of general anaesthesia[110]. Again, this is a topic of continued debate. International consensus by the major endoscopy societies of the world is urgently needed.

CONCLUSION

Currently, both diagnostic and therapeutic endoscopy is well tolerated and accepted by both patients and endoscopists due to the application of sedation by most centres in the world. During the last 15 years, dramatic changes have occurred in endoscopic procedures, mainly with regard to the sedation techniques and the sophisticated endoscopic instruments and equipment utilised. Today, a large number of drugs are available for achieving successful moderate and deep sedation, and other substances are in the clinical evaluation stage. Moderate sedation using midazolame and an opioid represents the standard method of sedation, although propofol is being increasingly used in many countries. We suggest that the use of this drug will be accepted by an increasing number of endoscopists and that it could become the

preferred sedation agent in the near future. Today, midazolam remains the benzodiazepine of choice, while the most popular opioids are pethidine and fentanyl. Safe sedation in special clinical circumstances, such as in obese, pregnant, and elderly individuals, as well as in patients with chronic lung, renal or liver disease, requires modification of the drug doses used for sedation. It is also crucial for endoscopists to be very familiar with the drug or combination of drugs that they are using in everyday clinical practice. However, the controversy regarding the administration of sedation by an endoscopist, anaesthesiologist or an experienced nurse continues. We emphasise that sedation under the supervision of a properly trained endoscopist could become the standard practice. Due to the legal issues related to the occurrence of unwanted effects of sedation, an updated international consensus regarding the use of sedative agents, especially propofol, is urgently needed.

REFERENCES

1 **Bell GD**. Preparation, premedication, and surveillance. *Endoscopy* 2004; **36**: 23-31 [PMID: 14722852]
2 **Cohen LB**. Sedation issues in quality colonoscopy. *Gastrointest Endosc Clin N Am* 2010; **20**: 615-627 [PMID: 20889067 DOI: 10.1016/j.giec.2010.07.003]
3 **Porostocky P**, Chiba N, Colacino P, Sadowski D, Singh H. A survey of sedation practices for colonoscopy in Canada. *Can J Gastroenterol* 2011; **25**: 255-260 [PMID: 21647459]
4 **Fanti L**, Agostoni M, Gemma M, Radaelli F, Conigliaro R, Beretta L, Rossi G, Guslandi M, Testoni PA. Sedation and monitoring for gastrointestinal endoscopy: A nationwide web survey in Italy. *Dig Liver Dis* 2011; **43**: 726-730 [PMID: 21640673 DOI: 10.1016/j.dld.2011.04.012]
5 **Paspatis GA**, Manolaraki MM, Tribonias G, Theodoropoulou A, Vardas E, Konstantinidis K, Chlouverakis G, Karamanolis DG. Endoscopic sedation in Greece: results from a nationwide survey for the Hellenic Foundation of gastroenterology and nutrition. *Dig Liver Dis* 2009; **41**: 807-811 [PMID: 19410522 DOI: 10.1016/j.dld.2009.03.003]
6 **Cohen LB**, Wecsler JS, Gaetano JN, Benson AA, Miller KM, Durkalski V, Aisenberg J. Endoscopic sedation in the United States: results from a nationwide survey. *Am J Gastroenterol* 2006; **101**: 967-974 [PMID: 16573781 DOI: 10.1111/j.1572-0241.2006.00500.x]
7 **Riphaus A**, Rabofski M, Wehrmann T. Endoscopic sedation and monitoring practice in Germany: results from the first nationwide survey. *Z Gastroenterol* 2010; **48**: 392-397 [PMID: 20140841 DOI: 10.1055/s-0028-1109765]
8 **Baudet JS**, Borque P, Borja E, Alarcón-Fernández O, Sánchez-del-Río A, Campo R, Avilés J. Use of sedation in gastrointestinal endoscopy: a nationwide survey in Spain. *Eur J Gastroenterol Hepatol* 2009; **21**: 882-888 [PMID: 19352194 DOI: 10.1097/MEG.0b013e328314b7ca]
9 **Heuss LT**, Froehlich F, Beglinger C. Changing patterns of sedation and monitoring practice during endoscopy: results of a nationwide survey in Switzerland. *Endoscopy* 2005; **37**: 161-166 [PMID: 15692932 DOI: 10.105s/s-2004-826143]
10 Safety and Sedation during Endoscopic Procedures. London: British Society of Gastroenterology, 2003
11 **Byrne MF**, Chiba N, Singh H, Sadowski DC. Propofol use for sedation during endoscopy in adults: a Canadian Association of Gastroenterology position statement. *Can J Gastroenterol* 2008; **22**: 457-459 [PMID: 18478130]
12 **Lichtenstein DR**, Jagannath S, Baron TH, Anderson MA, Banerjee S, Dominitz JA, Fanelli RD, Gan SI, Harrison ME, Ikenberry SO, Shen B, Stewart L, Khan K, Vargo JJ. Sedation and anesthesia in GI endoscopy. *Gastrointest Endosc* 2008; **68**: 815-826 [PMID: 18984096 DOI: 10.1016/j.gie.2008.09.029]
13 **Benson AA**, Cohen LB, Waye JD, Akhavan A, Aisenberg J. Endoscopic sedation in developing and developed countries. *Gut Liver* 2008; **2**: 105-112 [PMID: 20485619 DOI: 10.5009/gnl.2008.2.2.105]
14 **Manolaraki MM,** Stroumpos C, Paspatis GA. Sedation in Gastrointestinal Endoscopies [Part 1]. *Annals Gastroenterol* 2009; 22: 90-96
15 **Olkkola KT**, Ahonen J. Midazolam and other benzodiazepines. *Handb Exp Pharmacol* 2008; (182): 335-360 [PMID: 18175099 DOI: 10.1007/978-3-540-74806-9_16]
16 **Fakheri HT**, Kiasari AZ, Taghvaii T, Hosseini V, Mohammadpour RA, Nasrollah A, Kabirzadeh A, Shahmohammadi S. Assessment the effect of midazolam sedation on hypoxia during upper gastrointestinal endoscopy. *Pak J Biol Sci* 2010; **13**: 152-157 [PMID: 20437680 DOI: 10.3923/pjbs.2010.152.157]
17 **Ozel AM**, Oncü K, Yazgan Y, Gürbüz AK, Demirtürk L. Comparison of the effects of intravenous midazolam alone and in combination with meperidine on hemodynamic and respiratory responses and on patient compliance during upper gastrointestinal endoscopy: a randomized, double-blind trial. *Turk J Gastroenterol* 2008; **19**: 8-13 [PMID: 18386234]
18 **Cinar K**, Yakut M, Ozden A. Sedation with midazolam versus midazolam plus meperidine for routine colonoscopy: a prospective, randomized, controlled study. *Turk J Gastroenterol* 2009; **20**: 271-275 [PMID: 20084571]
19 **Hayee B**, Dunn J, Loganayagam A, Wong M, Saxena V, Rowbotham D, McNair A. Midazolam with meperidine or fentanyl for colonoscopy: results of a randomized trial. *Gastrointest Endosc* 2009; **69**: 681-687 [PMID: 19251010 DOI: 10.1016/j.gie.2008.09.033]
20 **Paspatis GA**, Manolaraki M, Xirouchakis G, Papanikolaou N, Chlouverakis G, Gritzali A. Synergistic sedation with midazolam and propofol versus midazolam and pethidine in colonoscopies: a prospective, randomized study. *Am J Gastroenterol* 2002; **97**: 1963-1967 [PMID: 12190161 DOI: 10.1111/j.1572-0241.2002.05908.x]
21 **Agostoni M**, Fanti L, Arcidiacono PG, Gemma M, Strini G, Torri G, Testoni PA. Midazolam and pethidine versus propofol and fentanyl patient controlled sedation/analgesia for upper gastrointestinal tract ultrasound endoscopy: a prospective randomized controlled trial. *Dig Liver Dis* 2007; **39**: 1024-1029 [PMID: 17913605 DOI: 10.1016/j.dld.2007.08.004]
22 **Barriga J**, Sachdev MS, Royall L, Brown G, Tombazzi CR. Sedation for upper endoscopy: comparison of midazolam versus fentanyl plus midazolam. *South Med J* 2008; **101**: 362-366 [PMID: 18360335 DOI: 10.1097/SMJ.0b013e318168521b]
23 **Mui LM**, Teoh AY, Ng EK, Lee YT, Au Yeung AC, Chan YL, Lau JY, Chung SC. Premedication with orally administered midazolam in adults undergoing diagnostic upper endoscopy: a double-blind placebo-controlled randomized trial. *Gastrointest Endosc* 2005; **61**: 195-200 [PMID: 15729225 DOI: 10.1016/S0016-5107(04)02590-8]
24 **Meining A**, Semmler V, Kassem AM, Sander R, Frankenberger U, Burzin M, Reichenberger J, Bajbouj M, Prinz C, Schmid RM. The effect of sedation on the quality of upper gastrointestinal endoscopy: an investigator-blinded, randomized study comparing propofol with midazolam. *Endoscopy* 2007; **39**: 345-349 [PMID: 17285514 DOI: 10.1055/s-2006-945195]
25 **Qadeer MA**, Vargo JJ, Khandwala F, Lopez R, Zuccaro G. Propofol versus traditional sedative agents for gastrointestinal endoscopy: a meta-analysis. *Clin Gastroenterol Hepatol* 2005; **3**: 1049-1056 [PMID: 16271333 DOI: 10.1016/S1542-356(05)00742-1]
26 **VanNatta ME**, Rex DK. Propofol alone titrated to deep sedation versus propofol in combination with opioids and/or

benzodiazepines and titrated to moderate sedation for colonoscopy. *Am J Gastroenterol* 2006; **101**: 2209-2217 [PMID: 17032185 DOI: 10.1111/j.1572-0241.2006.00760.x]
27 **Paspatis GA**, Manolaraki MM, Vardas E, Theodoropoulou A, Chlouverakis G. Deep sedation for endoscopic retrograde cholangiopancreatography: intravenous propofol alone versus intravenous propofol with oral midazolam premedication. *Endoscopy* 2008; **40**: 308-313 [PMID: 18058653 DOI: 10.1055/s-2007-995346]
28 **Gasparović S**, Rustemović N, Opacić M, Premuzić M, Korusić A, Bozikov J, Bates T. Clinical analysis of propofol deep sedation for 1,104 patients undergoing gastrointestinal endoscopic procedures: a three year prospective study. *World J Gastroenterol* 2006; **12**: 327-330 [PMID: 16482639]
29 **Horiuchi A**, Nakayama Y, Hidaka N, Ichise Y, Kajiyama M, Tanaka N. Low-dose propofol sedation for diagnostic esophagogastroduodenoscopy: results in 10,662 adults. *Am J Gastroenterol* 2009; **104**: 1650-1655 [PMID: 19513021 DOI: 10.1038/ajg.2009.250]
30 **Levitzky BE**, Lopez R, Dumot JA, Vargo JJ. Moderate sedation for elective upper endoscopy with balanced propofol versus fentanyl and midazolam alone: a randomized clinical trial. *Endoscopy* 2012; **44**: 13-20 [PMID: 22068700 DOI: 10.1055/s-0031-1291421]
31 **Hsieh YH**, Chou AL, Lai YY, Chen BS, Sia SL, Chen IC, Chang YL, Lin HJ. Propofol alone versus propofol in combination with meperidine for sedation during colonoscopy. *J Clin Gastroenterol* 2009; **43**: 753-757 [PMID: 19169146 DOI: 10.1097/MCG.0b013e3181862a8c]
32 **Wang F**, Shen SR, Xiao DH, Xu CX, Tang WL. Sedation, analgesia, and cardiorespiratory function in colonoscopy using midazolam combined with fentanyl or propofol. *Int J Colorectal Dis* 2011; **26**: 703-708 [PMID: 21409424 DOI: 10.1007/s00384-011-1162-3]
33 **Mandel JE**, Tanner JW, Lichtenstein GR, Metz DC, Katzka DA, Ginsberg GG, Kochman ML. A randomized, controlled, double-blind trial of patient-controlled sedation with propofol/remifentanil versus midazolam/fentanyl for colonoscopy. *Anesth Analg* 2008; **106**: 434-439 [PMID: 18227297 DOI: 10.1213/01.ane.0000297300.33441.32]
34 **Lee CK**, Lee SH, Chung IK, Lee TH, Park SH, Kim EO, Lee SH, Kim HS, Kim SJ. Balanced propofol sedation for therapeutic GI endoscopic procedures: a prospective, randomized study. *Gastrointest Endosc* 2011; **73**: 206-214 [PMID: 21168838 DOI: 10.1016/j.gie.2010.09.035]
35 **Singh H**, Poluha W, Cheung M, Choptain N, Baron KI, Taback SP. Propofol for sedation during colonoscopy. *Cochrane Database Syst Rev* 2008; (4): CD006268 [PMID: 18843709 DOI: 10.1002/14651858.CD006268]
36 **Robertson DJ**, Jacobs DP, Mackenzie TA, Oringer JA, Rothstein RI. Clinical trial: a randomized, study comparing meperidine (pethidine) and fentanyl in adult gastrointestinal endoscopy. *Aliment Pharmacol Ther* 2009; **29**: 817-823 [PMID: 19154568 DOI: 10.1111/j.1365-2036.2009.03943.x]
37 **McQuaid KR**, Laine L. A systematic review and meta-analysis of randomized, controlled trials of moderate sedation for routine endoscopic procedures. *Gastrointest Endosc* 2008; **67**: 910-923 [PMID: 18440381 DOI: 10.1016/l.gie.2007.12.046]
38 **Welchman S**, Cochrane S, Minto G, Lewis S. Systematic review: the use of nitrous oxide gas for lower gastrointestinal endoscopy. *Aliment Pharmacol Ther* 2010; **32**: 324-333 [PMID: 20491748 DOI: 10.1111/j.1365-2036.2010.04359.x]
39 **Aboumarzouk OM**, Agarwal T, Syed Nong Chek SA, Milewski PJ, Nelson RL. Nitrous oxide for colonoscopy. *Cochrane Database Syst Rev* 2011; (8): CD008506 [PMID: 21833967 DOI: 10.1002/14651858.CD008506]
40 **Maslekar S**, Gardiner A, Hughes M, Culbert B, Duthie GS. Randomized clinical trial of Entonox versus midazolam-fentanyl sedation for colonoscopy. *Br J Surg* 2009; **96**: 361-368 [PMID: 19283736 DOI: 10.1002/bjs.6467]
41 **Løberg M**, Furholm S, Hoff I, Aabakken L, Hoff G, Bretthauer M. Nitrous oxide for analgesia in colonoscopy without sedation. *Gastrointest Endosc* 2011; **74**: 1347-1353 [PMID: 22136779 DOI: 10.1016/j.gie.2011.07.071]
42 **Rogers WK**, McDowell TS. Remimazolam, a short-acting GABA(A) receptor agonist for intravenous sedation and/or anesthesia in day-case surgical and non-surgical procedures. *IDrugs* 2010; **13**: 929-937 [PMID: 21154153]
43 **Levitzky BE**, Vargo JJ. Fospropofol disodium injection for the sedation of patients undergoing colonoscopy. *Ther Clin Risk Manag* 2008; **4**: 733-738 [PMID: 19209255]
44 **Garnock-Jones KP**, Scott LJ. Fospropofol. *Drugs* 2010; **70**: 469-477 [PMID: 20205488 DOI: 10.2165/11204450-000000000-00000]
45 **Cohen LB**. Clinical trial: a dose-response study of fospropofol disodium for moderate sedation during colonoscopy. *Aliment Pharmacol Ther* 2008; **27**: 597-608 [PMID: 18194506 DOI: 10.1111/j.1365-2036.2008.03598.x]
46 **Cohen LB**, Cattau E, Goetsch A, Shah A, Weber JR, Rex DK, Kline JM. A randomized, double-blind, phase 3 study of fospropofol disodium for sedation during colonoscopy. *J Clin Gastroenterol* 2010; **44**: 345-353 [PMID: 19996984]
47 **Hoy SM**, Keating GM. Dexmedetomidine: a review of its use for sedation in mechanically ventilated patients in an intensive care setting and for procedural sedation. *Drugs* 2011; **71**: 1481-1501 [PMID: 21812509 DOI: 10.2165/11207190-000000000-00000]
48 **Dere K**, Sucullu I, Budak ET, Yeyen S, Filiz AI, Ozkan S, Dagli G. A comparison of dexmedetomidine versus midazolam for sedation, pain and hemodynamic control, during colonoscopy under conscious sedation. *Eur J Anaesthesiol* 2010; **27**: 648-652 [PMID: 20531094 DOI: 10.1097/EJA.0b013e3283347bfe]
49 **Takimoto K**, Ueda T, Shimamoto F, Kojima Y, Fujinaga Y, Kashiwa A, Yamauchi H, Matsuyama K, Toyonaga T, Yoshikawa T. Sedation with dexmedetomidine hydrochloride during endoscopic submucosal dissection of gastric cancer. *Dig Endosc* 2011; **23**: 176-181 [PMID: 21429025 DOI: 10.1111/j.1443-1661.2010.01080.x]
50 **Muller S**, Borowics SM, Fortis EA, Stefani LC, Soares G, Maguilnik I, Breyer HP, Hidalgo MP, Caumo W. Clinical efficacy of dexmedetomidine alone is less than propofol for conscious sedation during ERCP. *Gastrointest Endosc* 2008; **67**: 651-659 [PMID: 18291396 DOI: 10.1016/j.gie.2007.09.041]
51 **Usta B**, Türkay C, Muslu B, Gözdemir M, Kasapoglu B, Sert H, Demircioğlu RI, Karabayirli S. Patient-controlled analgesia and sedation with alfentanyl versus fentanyl for colonoscopy: a randomized double blind study. *J Clin Gastroenterol* 2011; **45**: e72-e75 [PMID: 21135703 DOI: 10.1097/MCG.0b013e318201fbce]
52 **Fanti L**, Agostoni M, Gemma M, Gambino G, Facciorusso A, Guslandi M, Torri G, Testoni PA. Remifentanil vs. meperidine for patient-controlled analgesia during colonoscopy: a randomized double-blind trial. *Am J Gastroenterol* 2009; **104**: 1119-1124 [PMID: 19337241 DOI: 10.1038/ajg.2009.53]
53 **Manolaraki MM**, Theodoropoulou A, Stroumpos C, Vardas E, Oustamanolakis P, Gritzali A, Chlouverakis G, Paspatis GA. Remifentanil compared with midazolam and pethidine sedation during colonoscopy: a prospective, randomized study. *Dig Dis Sci* 2008; **53**: 34-40 [PMID: 17476596 DOI: 10.1007/s10620-007-9818-0]
54 **Costa A**, Montalbano LM, Orlando A, Ingoglia C, Linea C, Giunta M, Mancuso A, Mocciaro F, Bellingardo R, Tinè F, D' Amico G. Music for colonoscopy: A single-blind randomized controlled trial. *Dig Liver Dis* 2010; **42**: 871-876 [PMID: 20452299 DOI: 10.1016/j.dld.2010.03.016]
55 **El-Hassan H**, McKeown K, Muller AF. Clinical trial: music reduces anxiety levels in patients attending for endoscopy. *Aliment Pharmacol Ther* 2009; **30**: 718-724 [PMID: 19604181 DOI: 10.1111/j.1365-2036.2009.0491.x]

56 **Meeuse JJ**, Koornstra JJ, Reyners AK. Listening to music does not reduce pain during sigmoidoscopy. *Eur J Gastroenterol Hepatol* 2010; **22**: 942-945 [PMID: 20110821 DOI: 10.1097/MEG.0b013e328336ec6e]

57 **Rudin D**, Kiss A, Wetz RV, Sottile VM. Music in the endoscopy suite: a meta-analysis of randomized controlled studies. *Endoscopy* 2007; **39**: 507-510 [PMID: 17554644 DOI: 10.1055/s-2007-966362]

58 **Tam WW**, Wong EL, Twinn SF. Effect of music on procedure time and sedation during colonoscopy: a meta-analysis. *World J Gastroenterol* 2008; **14**: 5336-5343 [PMID: 18785289 DOI: 10.3748/wjg.14.5336]

59 **Bechtold ML**, Puli SR, Othman MO, Bartalos CR, Marshall JB, Roy PK. Effect of music on patients undergoing colonoscopy: a meta-analysis of randomized controlled trials. *Dig Dis Sci* 2009; **54**: 19-24 [PMID: 18483858 DOI: 10.1007/s10620-008-0312-0]

60 **Smolen D**, Topp R, Singer L. The effect of self-selected music during colonoscopy on anxiety, heart rate, and blood pressure. *Appl Nurs Res* 2002; **15**: 126-136 [PMID: 12173164 DOI: 10.1053/apnr.2002.34140]

61 **Takahashi Y**, Tanaka H, Kinjo M, Sakumoto K. Sedation-free colonoscopy. *Dis Colon Rectum* 2005; **48**: 855-859 [PMID: 15768182 DOI: 10.1007/s10350-004-0860-0]

62 **Vargo JJ**. Sedation in the bariatric patient. *Gastrointest Endosc Clin N Am* 2011; **21**: 257-263 [PMID: 21569977 DOI: 10.1016/j.giec.2011.02.011]

63 **Schreiner MA**, Fennerty MB. Endoscopy in the obese patient. *Gastroenterol Clin North Am* 2010; **39**: 87-97 [PMID: 20202582 DOI: 10.1016/j.gtc.2009.12.009]

64 **Küper MA**, Kratt T, Kramer KM, Zdichavsky M, Schneider JH, Glatzle J, Stüker D, Königsrainer A, Brücher BL. Effort, safety, and findings of routine preoperative endoscopic evaluation of morbidly obese patients undergoing bariatric surgery. *Surg Endosc* 2010; **24**: 1996-2001 [PMID: 20135170 DOI: 10.1007/s00464-010-0893-5]

65 **Wani S**, Azar R, Hovis CE, Hovis RM, Cote GA, Hall M, Waldbaum L, Kushnir V, Early D, Mullady DK, Murad F, Edmundowicz SA, Jonnalagadda SS. Obesity as a risk factor for sedation-related complications during propofol-mediated sedation for advanced endoscopic procedures. *Gastrointest Endosc* 2011; **74**: 1238-1247 [PMID: 22136773 DOI: 10.1016/j.gie.2011.09.006]

66 **Madan AK**, Tichansky DS, Isom J, Minard G, Bee TK. Monitored anesthesia care with propofol versus surgeon-monitored sedation with benzodiazepines and narcotics for preoperative endoscopy in the morbidly obese. *Obes Surg* 2008; **18**: 545-548 [PMID: 18386111 DOI: 10.1007/s11695-007-9338-1]

67 **Bamji N**, Cohen LB. Endoscopic sedation of patients with chronic liver disease. *Clin Liver Dis* 2010; **14**: 185-194 [PMID: 20682228 DOI: 10.1016/j.cld.2010.03.003]

68 **Verbeeck RK**. Pharmacokinetics and dosage adjustment in patients with hepatic dysfunction. *Eur J Clin Pharmacol* 2008; **64**: 1147-1161 [PMID: 18762933 DOI: 10.1007/s00282-008-0553-z]

69 **Correia LM**, Bonilha DQ, Gomes GF, Brito JR, Nakao FS, Lenz L, Rohr MR, Ferrari AP, Libera ED. Sedation during upper GI endoscopy in cirrhotic outpatients: a randomized, controlled trial comparing propofol and fentanyl with midazolam and fentanyl. *Gastrointest Endosc* 2011; **73**: 45-51, 51.e1 [PMID: 21184869 DOI: 10.1016/j.gie.2010.09.025]

70 **Amorós A**, Aparicio JR, Garmendia M, Casellas JA, Martínez J, Jover R. Deep sedation with propofol does not precipitate hepatic encephalopathy during elective upper endoscopy. *Gastrointest Endosc* 2009; **70**: 262-268 [PMID: 19394004 DOI: 10.1016/j.gie.2008.10.038]

71 **Khamaysi I**, William N, Olga A, Alex I, Vladimir M, Kamal D, Nimer A. Sub-clinical hepatic encephalopathy in cirrhotic patients is not aggravated by sedation with propofol compared to midazolam: a randomized controlled study. *J Hepatol* 2011; **54**: 72-77 [PMID: 20934771 DOI: 10.1016/j.jhep.2010.06.023]

72 **Riphaus A**, Lechowicz I, Frenz MB, Wehrmann T. Propofol sedation for upper gastrointestinal endoscopy in patients with liver cirrhosis as an alternative to midazolam to avoid acute deterioration of minimal encephalopathy: a randomized, controlled study. *Scand J Gastroenterol* 2009; **44**: 1244-1251 [PMID: 19811337 DOI: 10.1080/00365520903194591]

73 **Sharma P**, Singh S, Sharma BC, Kumar M, Garg H, Kumar A, Sarin SK. Propofol sedation during endoscopy in patients with cirrhosis, and utility of psychometric tests and critical flicker frequency in assessment of recovery from sedation. *Endoscopy* 2011; **43**: 400-405 [PMID: 21547878 DOI: 10.1055/s-0030-1256182]

74 **Cappell MS**. Sedation and analgesia for gastrointestinal endoscopy during pregnancy. *Gastrointest Endosc Clin N Am* 2006; **16**: 1-31 [PMID: 16546020 DOI: 10.1016/j.giec.2006.01.007]

75 **O'mahony S**. Endoscopy in pregnancy. *Best Pract Res Clin Gastroenterol* 2007; **21**: 893-899 [PMID: 17889814 DOI: 10.1016/j.bpg.2007.05.007]

76 **Brothman AR**, Peehl DM, Patel AM, MacDonald GR, McNeal JE, Ladaga LE, Schellhammer PF. Cytogenetic evaluation of 20 cultured primary prostatic tumors. *Cancer Genet Cytogenet* 1991; **55**: 79-84 [PMID: 1913611 DOI: 10.1016/j.gie.2008.05.024]

77 **Chong VH**, Jalihal A. Endoscopic management of biliary disorders during pregnancy. *Hepatobiliary Pancreat Dis Int* 2010; **9**: 180-185 [PMID: 20382591]

78 **Cappell MS**. Risks versus benefits of gastrointestinal endoscopy during pregnancy. *Nat Rev Gastroenterol Hepatol* 2011; **8**: 610-634 [PMID: 21970872 DOI: 10.1038/nrgastro.2011.162]

79 **Lebwohl B**, Hassid B, Ludwin S, Lewis SK, Tennyson CA, Neugut AI, Green PH. Increased sedation requirements during endoscopy in patients with celiac disease. *Dig Dis Sci* 2012; **57**: 994-999 [PMID: 22052448 DOI: 10.1007/s10620-011-1959-5]

80 **Klotz U**. Pharmacokinetics and drug metabolism in the elderly. *Drug Metab Rev* 2009; **41**: 67-76 [PMID: 19514965 DOI: 10.1080/03602530902722679]

81 **Eisen GM,** Chutkan R, Goldstein JL, Petersen BT, Ryan ME, Sherman S, Vargo II J, Wright RA, Young HS, Catalano MF, Walter V. Guidelines for colorectal cancer screening and surveillance. *Gastrointestinal Endoscopy* 2000; **51:** 777-782

82 **Christe C**, Janssens JP, Armenian B, Herrmann F, Vogt N. Midazolam sedation for upper gastrointestinal endoscopy in older persons: a randomized, double-blind, placebo-controlled study. *J Am Geriatr Soc* 2000; **48**: 1398-1403 [PMID: 11083314]

83 **Kerker A**, Hardt C, Schlief HE, Dumoulin FL. Combined sedation with midazolam/propofol for gastrointestinal endoscopy in elderly patients. *BMC Gastroenterol* 2010; **10**: 11 [PMID: 20105314 DOI: 10.1186/1471-230x-10-11]

84 **Horiuchi A**, Nakayama Y, Tanaka N, Ichise Y, Katsuyama Y, Ohmori S. Propofol sedation for endoscopic procedures in patients 90 years of age and older. *Digestion* 2008; **78**: 20-23 [PMID: 18765935 DOI: 10.1159/000151765]

85 **Martínez JF,** Aparicio JR, Compañy L, Ruiz F, Gómez-Escolar L, Mozas I, Casellas JA. Safety of continuous propofol sedation for endoscopic procedures in elderly patients. *Rev Esp Enferm Dig* 2011; **103:** 76-82 [PMID: 21366368 DOI: 10.4321/S1130-01082011000200005]

86 **Kongkam P**, Rerknimitr R, Punyathavorn S, Sitthi-Amorn C, Ponauthai Y, Prempracha N, Kullavanijaya P. Propofol infusion versus intermittent meperidine and midazolam injection for conscious sedation in ERCP. *J Gastrointestin Liver Dis* 2008; **17**: 291-297 [PMID: 18836622]

87 **Amornyotin S**, Srikureja W, Chalayonnavin W, Kongphlay S. Dose requirement and complications of diluted and undi-

luted propofol for deep sedation in endoscopic retrograde cholangiopancreatography. *Hepatobiliary Pancreat Dis Int* 2011; **10**: 313-318 [PMID: 21669577 DOI: 10.1016/S1499-3872(11)60052-0]
88 **Mazanikov M**, Udd M, Kylänpää L, Lindström O, Aho P, Halttunen J, Färkkilä M, Pöyhiä R. Patient-controlled sedation with propofol and remifentanil for ERCP: a randomized, controlled study. *Gastrointest Endosc* 2011; **73**: 260-266 [PMID: 21295639 DOI: 10.1016/j.gie.2010.10.005]
89 **Che K**, Muckova N, Olafsson S, Srikureja W. Safety of same-day endoscopic ultrasound and endoscopic retrograde cholangiopancreatography under conscious sedation. *World J Gastroenterol* 2010; **16**: 3287-3291 [PMID: 20614484 DOI: 10.3748/wjg.v16.i26.3287]
90 **Kiriyama S**, Gotoda T, Sano H, Oda I, Nishimoto F, Hirashima T, Kusano C, Kuwano H. Safe and effective sedation in endoscopic submucosal dissection for early gastric cancer: a randomized comparison between propofol continuous infusion and intermittent midazolam injection. *J Gastroenterol* 2010; **45**: 831-837 [PMID: 20228999 DOI: 10.1007/s00535-010-0222-8]
91 **Yamagata T**, Hirasawa D, Fujita N, Suzuki T, Obana T, Sugawara T, Ohira T, Harada Y, Maeda Y, Koike Y, Suzuki K, Noda Y. Efficacy of propofol sedation for endoscopic submucosal dissection (ESD): assessment with prospective data collection. *Intern Med* 2011; **50**: 1455-1460 [PMID: 21757829 DOI: 10.2169/internalmedicine.50.4627]
92 **Qadeer MA**, Lopez AR, Dumot JA, Vargo JJ. Hypoxemia during moderate sedation for gastrointestinal endoscopy: causes and associations. *Digestion* 2011; **84**: 37-45 [PMID: 21304242 DOI: 10.1159/000321621]
93 **Eer AS**, Padmanabhan U, Leslie K. Propofol dose and incidence of dreaming during sedation. *Eur J Anaesthesiol* 2009; **26**: 833-836 [PMID: 19528807 DOI: 10.1097/EJA.0b013e32832c500c]
94 **Agostoni M**, Fanti L, Gemma M, Pasculli N, Beretta L, Testoni PA. Adverse events during monitored anesthesia care for GI endoscopy: an 8-year experience. *Gastrointest Endosc* 2011; **74**: 266-275 [PMID: 21704990 DOI: 10.1016/j.gie.2011.04.028]
95 **Conigliaro R**, Rossi A. Implementation of sedation guidelines in clinical practice in Italy: results of a prospective longitudinal multicenter study. *Endoscopy* 2006; **38**: 1137-1143 [PMID: 17111337 DOI: 10.1055/s-2006-944842]
96 **Nayar DS**, Guthrie WG, Goodman A, Lee Y, Feuerman M, Scheinberg L, Gress FG. Comparison of propofol deep sedation versus moderate sedation during endosonography. *Dig Dis Sci* 2010; **55**: 2537-2544 [PMID: 20635148 DOI: 10.1007/s10620-010-1308-0]
97 **Sharma VK**, Nguyen CC, Crowell MD, Lieberman DA, de Garmo P, Fleischer DE. A national study of cardiopulmonary unplanned events after GI endoscopy. *Gastrointest Endosc* 2007; **66**: 27-34 [PMID: 17591470 DOI: 10.1016/j.gie.2006.12.040]
98 **Ljubicić N**, Supanc V, Roić G, Sharma M. Efficacy and safety of propofol sedation during urgent upper gastrointestinal endoscopy--a prospective study. *Coll Antropol* 2003; **27**: 189-195 [PMID: 12974146]
99 **Feld AD**. Endoscopic sedation: medicolegal considerations. *Gastrointest Endosc Clin N Am* 2008; **18**: 783-788 [PMID: 18922415 DOI: 10.1016/j.giec.2008.06.009]
100 **Axon AE**. The use of propofol by gastroenterologists: medico-legal issues. *Digestion* 2010; **82**: 110-112 [PMID: 20407258 DOI: 10.1159/000285570]
101 **Thomson A**, Andrew G, Jones DB. Optimal sedation for gastrointestinal endoscopy: review and recommendations. *J Gastroenterol Hepatol* 2010; **25**: 469-478 [PMID: 20370725 DOI: 10.1111/j.1440-2009.06174.x]
102 **Vargo JJ**, Zuccaro G, Dumot JA, Shermock KM, Morrow JB, Conwell DL, Trolli PA, Maurer WG. Gastroenterologist-administered propofol versus meperidine and midazolam for advanced upper endoscopy: a prospective, randomized trial. *Gastroenterology* 2002; **123**: 8-16 [PMID: 12105827 DOI: 10.1053/gast.2002.34232]
103 **Walker JA**, McIntyre RD, Schleinitz PF, Jacobson KN, Haulk AA, Adesman P, Tolleson S, Parent R, Donnelly R, Rex DK. Nurse-administered propofol sedation without anesthesia specialists in 9152 endoscopic cases in an ambulatory surgery center. *Am J Gastroenterol* 2003; **98**: 1744-1750 [PMID: 12907328 DOI: 10.1016/S0002-9270(03)00496-9]
104 **Rex DK**, Heuss LT, Walker JA, Qi R. Trained registered nurses/endoscopy teams can administer propofol safely for endoscopy. *Gastroenterology* 2005; **129**: 1384-1391 [PMID: 16285939 DOI: 10.1053/j.gastro.2005.08.014]
105 **Rex DK**, Deenadayalu VP, Eid E, Imperiale TF, Walker JA, Sandhu K, Clarke AC, Hillman LC, Horiuchi A, Cohen LB, Heuss LT, Peter S, Beglinger C, Sinnott JA, Welton T, Rofail M, Subei I, Sleven R, Jordan P, Goff J, Gerstenberger PD, Munnings H, Tagle M, Sipe BW, Wehrmann T, Di Palma JA, Occhipinti KE, Barbi E, Riphaus A, Amann ST, Tohda G, McClellan T, Thueson C, Morse J, Meah N. Endoscopist-directed administration of propofol: a worldwide safety experience. *Gastroenterology* 2009; **137**: 1229-1237; quiz 1518-1519 [PMID: 19549528 DOI: 10.1053/j.gastro.2009.06.042]
106 **Jensen JT**, Vilmann P, Horsted T, Hornslet P, Bodtger U, Banning A, Hammering A. Nurse-administered propofol sedation for endoscopy: a risk analysis during an implementation phase. *Endoscopy* 2011; **43**: 716-722 [PMID: 21811940 DOI: 10.1055/s-0030-1256515]
107 **Riphaus A**, Macias-Gomez C, Devière J, Dumonceau JM. Propofol, the preferred sedation for screening colonoscopy, is underused. Results of an international survey. *Dig Liver Dis* 2012; **44**: 389-392 [PMID: 22119619 DOI: 10.1016/j.dld.2011.10.019]
108 **Poincloux L**, Laquière A, Bazin JE, Monzy F, Artigues F, Bonny C, Abergel A, Dapoigny M, Bommelaer G. A randomized controlled trial of endoscopist vs. anaesthetist-administered sedation for colonoscopy. *Dig Liver Dis* 2011; **43**: 553-558 [PMID: 21450542 DOI: 10.1016/j.dld.2011.02.007]
109 **Dumonceau JM**, Riphaus A, Aparicio JR, Beilenhoff U, Knape JT, Ortmann M, Paspatis G, Ponsioen CY, Racz I, Schreiber F, Vilmann P, Wehrmann T, Wientjes C, Walder B. European Society of Gastrointestinal Endoscopy, European Society of Gastroenterology and Endoscopy Nurses and Associates, and the European Society of Anaesthesiology Guideline: Non-anesthesiologist administration of propofol for GI endoscopy. *Endoscopy* 2010; **42**: 960-974 [PMID: 21072716 DOI: 10.1055/s-0030-1255728]
110 **Cohen LB**, Ladas SD, Vargo JJ, Paspatis GA, Bjorkman DJ, Van der Linden P, Axon AT, Axon AE, Bamias G, Despott E, Dinis-Ribeiro M, Fassoulaki A, Hofmann N, Karagiannis JA, Karamanolis D, Maurer W, O'Connor A, Paraskeva K, Schreiber F, Triantafyllou K, Viazis N, Vlachogiannakos J. Sedation in digestive endoscopy: the Athens international position statements. *Aliment Pharmacol Ther* 2010; **32**: 425-442 [PMID: 20456310 DOI: 10.1111/j.1365-2036.2010.04352.x]

P- Reviewers Paspatis GA, De Palma GD, Lakatos PL, Nakajima A, Takuya W **S- Editor** Jiang L **L- Editor** A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i4.482

World J Gastroenterol 2013 January 28; 19(4): 482-491
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

ORIGINAL ARTICLE

Age-dependent slowing of enteric axonal transport in insulin-resistant mice

Kathy J LePard, Joseph Cellini

Kathy J LePard, Joseph Cellini, Department of Physiology, Chicago College of Osteopathic Medicine, Midwestern University, Downers Grove, IL 60515, United States
Author contributions: Cellini J performed the experiments and assisted in editing the manuscript; LePard KJ and Cellini J analyzed the data; LePard KJ designed the research study and wrote the manuscript.
Supported by Office of Research and Sponsored Programs, Midwestern University, Downers Grove, IL, United States
Correspondence to: Kathy J LePard, PhD, Associate Professor of Physiology, Department of Physiology, Chicago College of Osteopathic Medicine, Midwestern University, Downers Grove, IL 60515, United States. klepar@midwestern.edu
Telephone: +1-630-5156391 Fax: +1-630-5156375
Received: June 18, 2012 Revised: November 20, 2012
Accepted: December 15, 2012
Published online: January 28, 2013

Abstract

AIM: To investigate retrograde tracer transport by gastric enteric neurons in insulin resistant mice with low or high glycosylated hemoglobin (Hb).

METHODS: Under anesthesia, the retrograde tracer fluorogold was superficially injected into the fundus or antrum using a microsyringe in KK Cg-Ay/J mice prior to onset of type 2 diabetes mellitus (T2DM; 4 wk of age), at onset of T2DM (8 wk of age), and after 8, 16, or 24 wk of untreated T2DM and in age-matched KK/HIJ mice. Six days later, mice were sacrificed by CO_2 narcosis followed by pneumothorax. Stomachs were removed and fixed. Sections from fundus, corpus and antrum were excised and mounted on a glass slide. Tracer-labeled neurons were viewed using a microscope and manually counted. Data were expressed as the number of neurons in short and long descending and ascending pathways and in local fundus and antrum pathways, and the number of neurons in all regions labeled after injection of tracer into either the fundus or the antrum.

RESULTS: By 8 wk of age, body weights of KKAy mice (n = 12, 34 ± 1 g) were heavier than KK mice (n = 17, 29 ± 1 g; F (4, 120) = 4.414, P = 0.002] and glycosylated Hb was higher [KK: (n = 7), 4.97% ± 0.04%; KKAy: (n = 6), 6.57% ± 0.47%; F (1, 26) = 24.748, P < 0.001]. The number of tracer labeled enteric neurons was similar in KK and KKAy mice of all ages in the short descending pathway [F (1, 57) = 2.374, P = 0.129], long descending pathway [F (1, 57) = 0.922, P = 0.341], local fundus pathway [F (1, 53) = 2.464, P = 0.122], local antrum pathway [F (1, 57) = 0.728, P = 0.397], and short ascending pathway [F (1, 53) = 2.940, P = 0.092]. In the long ascending pathway, fewer tracer-labeled neurons were present in KKAy as compared to KK mice [KK: (n = 34), 302 ± 17; KKAy: (n = 29), 230 ± 15; F (1, 53) = 8.136, P = 0.006]. The number of tracer-labeled neurons was decreased in all mice by 16 wk as compared to 8 wk of age in the short descending pathway [8 wk: (n = 15), 305 ± 26; 16 wk: (n = 13), 210 ± 30; F (4, 57) = 9.336, P < 0.001], local antrum pathway [8 wk: (n = 15), 349 ± 20; 16 wk: (n = 13), 220 ± 33; F (4, 57) = 8.920, P < 0.001], short ascending pathway [8 wk: (n = 14), 392 ± 15; 16 wk: (n = 14), 257 ± 33; F (4, 53) = 17.188, P < 0.001], and long ascending pathway [8 wk: (n = 14), 379 ± 39; 16 wk: (n = 14), 235 ± 26; F (4, 53) = 24.936, P < 0.001]. The number of tracer-labeled neurons decreased at 24 wk of age in the local fundus pathway [8 wk: (n = 14), 33 ± 11; 24 wk: (n = 12), 3 ± 2; F (4, 53) = 5.195, P = 0.001] and 32 wk of age in the long descending pathway [8 wk: (n = 15), 16 ± 3; 32 wk: (n = 12), 3 ± 2; F (4, 57) = 2.944, P = 0.028]. The number of tracer-labeled enteric neurons was correlated to final body weight for local fundus and ascending pathways [KK: (n = 34), r = -0.746, P < 0.001; KKAy: (n = 29), r = -0.842, P < 0.001] as well as local antrum and descending pathways [KK (n = 36), r = -0.660, P < 0.001; KKAy (n = 31), r = -0.622, P < 0.001]. In contrast, glycosylated Hb was not significantly correlated to number of tracer-labeled neurons [KK (n = 17), r = -0.164, P = 0.528; KKAy (n

= 16), r = -0.078, P = 0.774].

CONCLUSION: Since uncontrolled T2DM did not uniformly impair tracer transport in gastric neurons, long ascending neurons may be more susceptible to persistent hyperglycemia and low effective insulin.

© 2013 Baishideng. All rights reserved.

Key words: Autonomic neuropathy; Retrograde transport; KKAy mice; Fluorogold; Type 2 diabetes mellitus

LePard KJ, Cellini J. Age-dependent slowing of enteric axonal transport in insulin-resistant mice. *World J Gastroenterol* 2013; 19(4): 482-491 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i4/482.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i4.482

INTRODUCTION

Diabetes mellitus (DM), characterized by persistent hyperglycemia and a deficiency of effective plasma insulin, can result in neuropathy of autonomic neurons. In rodent models of type 1 DM (T1DM) and type 2 DM (T2DM), neuropathies of the parasympathetic vagus nerve[1-3] and enteric neurons located within the wall of the gut[4,5] have been described. Many factors likely contribute to the development of autonomic neuropathy[6,7] including accumulation of sorbitol in nerves[8,9], oxidative stress[10,11], and somal deficiency of neurotrophins[7,12-14].

Neurotrophins, proteins that support the proper functioning of neurons, are retrogradely transported from the innervated organ to the soma by an axonal active transport mechanism. Active axonal transport of proteins in the vagus nerve was impaired in animal models of DM. Early studies using nerve ligation demonstrated reduced axonal transport of opiate receptors[15] and choline acetyltransferase[8] in the vagus nerve of streptozotocin (STZ) rats with partial reversal by immediate treatment with insulin[15] or an aldose reductase inhibitor[8]. Retrograde transport by the vagus nerve of endogenous neurotrophins[8] or experimental neuronal tracers injected into the stomach[16,17] was impaired after 16 or 24 wk of uncontrolled hypoinsulinemia and hyperglycemia in STZ rats. In a rodent model of T2DM, the db/db mouse, axonal transport of acetylcholinesterase[18,19] and choline acetyltransferase[20] in peripheral nerves was impaired, but only after at least 25 wk[18,19], approximately double the time observed in STZ models of T1DM[16,17,21]. To date, active axonal transport of proteins by enteric neurons located within the wall of the gut has not been evaluated in rodent models of DM. We hypothesized that retrograde transport of the neural tracer fluorogold (FG) would be impaired in enteric neurons, but only after prolonged insulin resistance and hyperglycemia.

Coordinated contraction and relaxation of fundus, corpus and antrum is required for normal storage and emptying of gastric contents[22,23]. Enteric neural control of contraction and relaxation of gastric smooth muscle varied by region in rodent models of DM[24,25]. Therefore, tracer transport in ascending and descending enteric neurons innervating the fundus and antrum, respectively, were evaluated.

These studies used an obese mouse model of T2DM, the KKAy mouse. As the result of an agouti mutation on a diabetes susceptible background (KK strain), the KKAy mouse overeats normal chow, accumulates adipose tissue, and develops overt hyperglycemia. Since both mouse groups are insulin resistant[26-28], but only the KKAy mouse group develops persistent hyperglycemia[26], the contribution of overt hyperglycemia to enteric retrograde axonal transport could be determined. We hypothesized that the combination of insulin resistance and overt hyperglycemia in KKAy mice would accelerate the onset of impaired FG transport by enteric neurons as compared to KK mice.

MATERIALS AND METHODS

Animals

Female KK Cg-Ay/J mice (KKAy, strain 2468, n = 60), an obese model of T2DM, and age-matched female KK/HIJ mice (KK, stock number 2106, n = 70), were obtained at 3-6 wk of age (Jackson Laboratories, Bar Harbor, ME, United States). Initially, KKAy mice were screened for onset of T2DM by urine glucose. Since most KKAy mice became diabetic at 8 wk of age, onset of T2DM was defined as 8 wk of age and T2DM was untreated for 8, 16, or 24 wk. Body weight was recorded weekly. At sacrifice, drops of whole blood were used to determine glycosylated hemoglobin (Hb) (A1C Now+® Multi Test A1C System; Bayer, Tarrytown, NY, United States) and stress-induced glucose (BD Logic blood glucose monitor; Becton, Dickinson, and Co., Franklin Lakes, NJ, United States). Urine was drained from the bladder using a syringe and tested for glucose using uristix (Bayer). Urine was considered positive for glucose if glucose was $\geqslant$ 500 mg/dL.

Injection of FG into stomach

Surgeries were performed prior to onset of T2DM (4 wk of age), at onset of T2DM (8 wk of age), and after 8, 16, or 24 wk of untreated T2DM (i.e., 16, 24, and 32 wk of age, respectively). The number of mice at each age is shown in Table 1. Both KKAy and age-matched KK mice surgeries were performed on the same day using the same tracer solution. Surgeries were performed under ketamine (100-150 mg/kg, *ip*; Fort Dodge Animal Health, Fort Dodge, IA, United States) and xylaxine (8 mg/kg, *ip*; Vedco, St. Joseph, MO, United States) anesthesia. The neural tracer FG was used to assess active axonal retrograde transport in enteric neural pathways.

The tracer solution containing 4% FG (hydroxystilbamidine methanesulfonate; Invitrogen Molecular Probes) in dimethyl sulfoxide was superficially injected into the

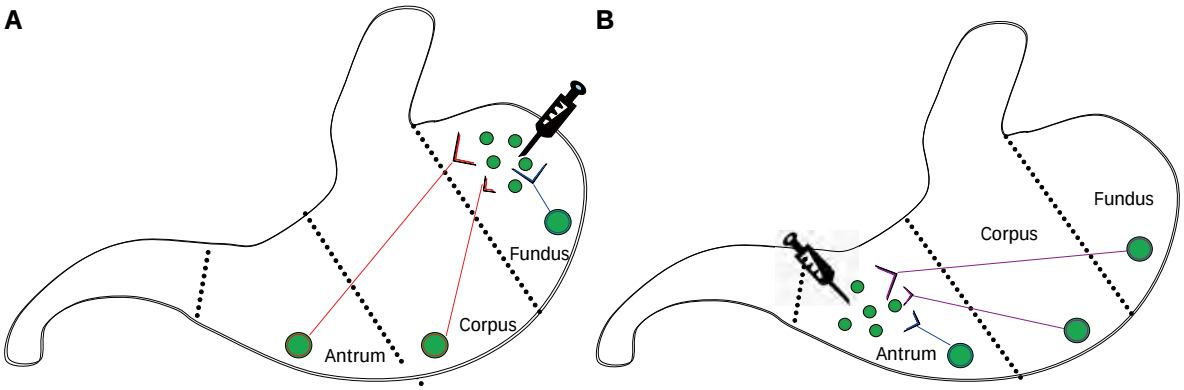


Figure 1 Cartoon of ascending, descending and local enteric pathways in the stomach. Enteric nerve terminals endocytosed the tracer fluorogold (small green circles) and it was transported back to cell bodies (large green circles) located in the fundus, corpus, or antrum. A: The retrograde tracer fluorogold was injected into the fundus. Red neurons were in the ascending pathway while blue neurons were in the local fundic pathway. The approximate distances from fundus injection sites were 0.6, 2.2, and 3.3 cm for fundus, corpus, and antrum sections, respectively; B: The retrograde tracer fluorogold was injected into the antrum. Purple neurons were in the descending pathway while blue neurons were in the local antral pathway. The approximate distances from antrum injection sites were 3.2, 1.1, and 0.4 cm for fundus, corpus, and antrum sections, respectively.

ventral fundus or ventral antrum at 5 sites, 2 μL each, using a microsyringe (702RN, 25 μL; Hamilton, Reno, NV, United States) with replaceable needle (3/8, point style 2, 33 G, Hamilton). An analgesic (buprenorphine, 0.05 mg/kg, *sc*; Hospira, Inc., Lake Forest, IL, United States) was administered immediately after surgery. Ketoprofen (5 mg/kg, *ip*; Fort Dodge Animal Health) was administered 6 h after surgery and the following day.

To promote selective labeling, care was taken to inject the tracer into the gastric wall and avoid leakage of the tracer into the abdominal cavity. Other experiments performed in the lab using the same injection technique have shown that ventral injection of tracer into the stomach wall resulted in unilateral FG labeling of efferent vagal neurons in the brainstem dorsal motor nucleus[29]. If tracer were not confined to the stomach wall, brainstem labeling would be bilateral.

Stomach removal

Six days after tracer injection, mice were sacrificed by CO_2 narcosis followed by pneumothorax. Stomachs were removed, placed in oxygenated Krebs solution, trimmed, opened along the lesser curvature, and emptied of contents. The fundus was cut away from the corpus/antrum. Tissues were stretched and pinned, mucosa side up, in a sylgard-lined Petri dish then fixed overnight at 37 ℃ in 4% paraformaldehyde in 0.1 mol/L PBS. The mucosa was removed. Using a razor blade, the center of each fundus, corpus, and antrum region along the greater curvature was cut out and mounted on a glass slide then coverslipped. The approximate distances of the excised sections from fundus injection sites were 0.6, 2.2, and 3.3 cm for fundus, corpus, and antrum sections, respectively. The approximate distances from antrum injection sites were 3.2, 1.1, and 0.4 cm for fundus, corpus sections, and antrum sections, respectively.

Counting FG-labeled neurons

Tracer-labeled neurons were viewed at 20× using a microscope (Leica DMIL with UV light source; Leica Microsystems Inc., Buffalo Grove, IL, United States) with attached digital camera (MagnaFire; Optronics, Goleta, CA, United States) using the Chroma filter for FG (11006V3 Gold: Ex. 300-390, Em. ≥ 515). Digital images (1280 by 1024 pixels) were 0.327 μm/pixel. Digital images were in 32 bit RGB color at an image resolution of 100 dpi and a computer screen resolution of 92 dpi. Labeled neurons were manually counted with the assistance of ImagePro Plus (version 4.5.0.29, Media Cybernetics, Silver Spring, MD, United States). The numbers of labeled neurons in 20 adjacent images (418.6 μm by 334.8 μm) of the fundus and in 12 adjacent images of the corpus and antrum (total area: 2.8, 1.7, and 1.7 mm^2, respectively) were counted and data were expressed as (1) total number of tracer-labeled neurons in that pathway; and (2) total number of neurons in all three regions labeled after tracer injection into the fundus or antrum.

Description of ascending, descending, and local enteric neural pathways

Six pathways in the stomach were assessed in this study (Figure 1). Enteric neurons in ascending pathways had cell bodies in the corpus (short ascending pathways) or antrum (long ascending pathways) and nerve terminals in the ventral fundus, while those in descending pathways had cell bodies in the fundus (long descending pathways) or corpus (short descending pathways) and nerve terminals in the ventral antrum. Enteric neurons in fundic local pathways had both cell bodies and nerve terminals in the ventral fundus, while those in antral local pathways had both cell bodies and nerve terminals in the ventral antrum.

Statistical analysis

All data were expressed as mean ± SE. Data were statistically analyzed using SPSS (version 18.0, Chicago, IL). Independent factors for analysis of variance included KK or KKAy, age (4, 8, 16, 24, or 32 wk) or pathway (ascending or descending). Both main effects of one

Table 1 Number of mice at each age with tracer injection into the fundus or antrum

Age (wk)	Fundus	Antrum
KK mice		
4	6	6
8	8	9
16	8	7
24	6	8
32	6	6
KKAy mice		
4	6	6
8	6	6
16	6	6
24	6	7
32	5	6

independent factor and interactions of two independent factors were evaluated and Sidak correction was used for post-hoc analysis. The ratios of KK and KKAy mice with positive urine glucose were compared using χ^2. Correlations were performed using Pearson correlation (r), linear regression (r^2) was performed using SigmaPlot (version 12.0, Systat Software, Inc., San Jose, CA). The number inside the bar in the graphs indicates the number of animals in the group. A P value < 0.05 was considered statistically significant with $^aP < 0.05$, $^bP < 0.01$.

RESULTS

Animal characteristics

Body weights of KK and KKAy mice were similar at 4 wk of age (Table 2), then KKAy mice became heavier [interaction: KK/KKAy and age F (4, 120) = 4.414, P = 0.002]. Body weight plateaued in KK and KKAy mice at 24 and 16 wk of age, respectively.

Glucose control was impaired in KKAy mice (Table 2). Glycosylated Hb, an index of average plasma glucose over the previous 40 d for a mouse[30], was elevated in KKAy as compared to KK mice [main effect F (1, 26) = 24.748, P < 0.001] but did not change with age [main effect: F (3, 26) = 1.916, P = 0.152]. For all mice 8 wk of age and older, glycosylated Hb was higher in KKAy mice (6.53 ± 0.31, n = 17) than in KK mice (5.02 ± 0.04, n = 17). The ability to raise blood glucose in response to stress was greater in KKAy as compared to KK mice. Stress-induced hyperglycemia was highest at 16 wk in KKAy mice (535 ± 47, n = 7), but remained steady in KK mice (297 ± 20, n = 9) [interaction: KK/KKAy and age F (4, 67) = 2.609, P = 0.043]. Glucose was detected in the urine of some KKAy mice prior to onset of T2DM, but was not detected in any KK mice.

Tracer-labeled neurons in enteric pathways

Six days after injection of tracer into the fundus or antrum, FG accumulated in the cell bodies of enteric neurons of the fundus, corpus, and antrum. Tracer-labeled enteric neurons of the corpus are shown in Figure 2.

In the long ascending pathway (Figure 3A), significantly fewer FG-labeled enteric neurons were detected in KKAy as compared to KK mice. For both KK and KKAy mice, the number of FG-labeled neurons was reduced by 16 wk of age and reached its lowest value at 24 wk of age.

In the short ascending pathway (Figure 3B), there was a trend for fewer FG-labeled enteric neurons in KKAy as compared to KK mice. By 16 wk of age, the numbers of FG-labeled neurons were reduced as compared to 4 and 8 wk of age. The numbers of FG-labeled neurons remained low at 24 and 32 wk of age.

In the long (Figure 3D) and short (Figure 3E) descending pathways, similar numbers of FG-labeled enteric neurons were detected in KK as compared to KKA^y mice. The total number of FG-labeled enteric neurons reached its lowest value at 24 wk of age for the short descending pathway and 32 wk of age for the long descending pathway.

A similar dominance of ascending projections as compared to descending projections reported in guinea pig stomach[31,32] was observed in this mouse study. At 4 wk of age, more FG-labeled neurons were in the ascending pathways as compared to the descending pathways for KK and KKAy mice [main effect: F (1, 20) = 98.981, P < 0.001; KK: ascending (n = 6), 858 ± 45; descending (n = 6), 397 ± 55; KKAy: ascending (n = 6), 800 ± 37; descending (n = 6), 384 ± 37].

Similar numbers of FG-labeled fundic (Figure 3C) and antral (Figure 3F) enteric neurons were detected in KK as compared to KKAy mice. In the local fundic pathway, the

Table 2 Animal characteristics

	Body weight (g)	HbA1c (%)	Urine glucose[1]
4 wk of age			
KK	19.2 ± 0.4 (n = 12)	< 4.0 (n = 3)	0/6
KKAy	19.9 ± 0.5 (n = 12)	< 4.0 (n = 3)	2/6
8 wk of age			
KK	29 ± 1[d] (n = 17)	5.0 ± 0.0 (n = 7)	0/6
KKAy	34 ± 1[b,d] (n = 12)	6.6 ± 0.5[b] (n = 6)	1/3[1]
16 wk of age			
KK	35 ± 1[d,f] (n = 15)	5.1 ± 0.1 (n = 3)	0/13
KKAy	43 ± 1[b,d,f] (n = 12)	7.5 ± 0.7[b] (n = 3)	8/11[1]
24 wk of age			
KK	40 ± 1[d,f,h] (n = 14)	5.3 (n = 2)	0/12
KKAy	45 ± 1[b,d,f] (n = 13)	7.0 ± 0.9[b] (n = 3)	7/11[1]
32 wk of age			
KK	39 ± 2[d,f] (n = 12)	5.0 ± 0.1 (n = 5)	0/9
KKAy	47 ± 1[b,d,f] (n = 11)	5.6 ± 0.5[b] (n = 5)	3/11

[1]Number of mice with urine glucose ⩾ 500 mg/dL out of the total number of mice tested for urine glucose. [b]P < 0.01 *vs* KK; [d]P < 0.01 *vs* 4 wk; [f]P < 0.01 *vs* 8 wk; [h]P < 0.01 *vs* 16 wk. HbA1c: Haemoglobin A1c.

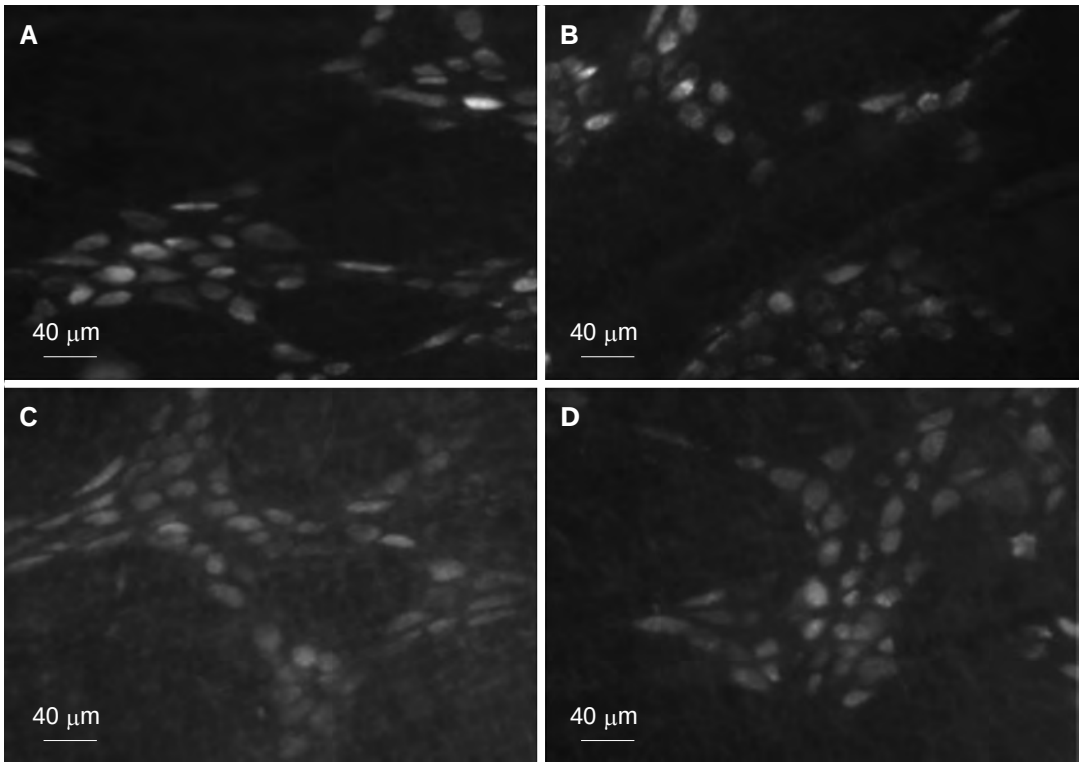


Figure 2 Fluorogold-labeled enteric neurons in gastric corpus. A: Fluorogold-labeled ascending enteric neurons in KK mice; B: Fluorogold-labeled ascending enteric neurons in KKAy mice; C: Fluorogold-labeled descending enteric neurons in KK mice; D: Fluorogold-labeled descending enteric neurons in KKAy mice. The brightness of neurons varied depending on the amount of fluorogold transported to the cell body, but most neurons in the ganglia were labeled with fluorogold.

total number of FG-labeled enteric neurons was variable, reaching its highest value at 8 wk and its lowest value at 24 wk of age. In contrast, in the antral pathway, the total number of FG-labeled enteric neurons was decreased by 16 wk in KK and KKA^{y} mice.

Correlation of FG-labeled neurons with body weight or blood glucose

The total number of FG-labeled neurons in all sections after injection into either the fundus or antrum was significantly correlated to mouse final body weight, but not stress-elevated blood glucose (Figure 4). As KK mice aged and body weight increased, the total number of enteric neurons labeled by fundus injection (Figure 4A) decreased (n = 34, r = -0.746, P < 0.001). In the heavier KKAy mice, the correlation was stronger (n = 29, r = -0.842, P < 0.001). As a result of injection into the antrum (Figure 4B), the total number of FG-labeled neurons similarly decreased with body weight in both groups of mice [KK (n = 36), r = -0.660, P < 0.001; KKAy (n = 31), r = -0.622, P < 0.001]. In contrast, stress-elevated blood glucose was not correlated with the number of FG-labeled neurons [fundus injection (Figure 4C): KK (n = 17), r = -0.097, P = 0.712; KKAy (n = 15), r = 0.328, P = 0.232; antrum injection (Figure 4D): KK (n = 25), r = 0.174, P = 0.407; KKAy (n = 20), r = 0.308, P = 0.187]. In addition, HbA1c was not correlated to number of FG-labeled neurons for antrum injection [KK (n = 17), r = -0.164, P = 0.528; KKAy (n = 16), r = -0.078, P = 0.774]. Data was not available for fundus injection.

DISCUSSION

The principal findings of these studies were that (1) insulin resistance, in the absence of persistent hyperglycemia, slowed retrograde FG transport in KK mice at 16 wk of age, the approximate age of onset of insulin resistance[26-28]; and (2) persistent hyperglycemia, as indicated by elevated HbA1c, significantly slowed tracer transport in long ascending pathways in KKAy as compared to KK mice, but the age of onset was unchanged. Persistent hyperglycemia did not significantly accelerate the age of onset of neuropathy in KKAy as compared to KK mice.

Reduced numbers of FG-labeled neurons may be due to many factors related to enteric neuropathy. Enteric neurons undergoing neuropathy may take up less tracer because of limited endocytosis[33]. Retrograde transport of tracer may be impaired due to glycosylation of axonal cytoskeletal proteins[34-36]. The density of nerve terminals in the tracer injection field may be reduced due to degeneration of nerve terminal processes[3,37,38] or to low rates of neuronal apoptosis[1,39] as a consequence of metabolic disturbances[40]. In addition, impaired active axonal transport of growth factors to the neuronal soma, including insulin and neurotrophins[11,14,41-44], may both promote and exacerbate neuropathy[6,7].

Specific mechanism(s) in distinct populations of neurons may contribute more significantly to neuropathy. Many factors play a role in the development of diabetic neuropathy[6,7,45], but treatments targeting a single mechanism do not prevent neuropathy of all autonomic neu-

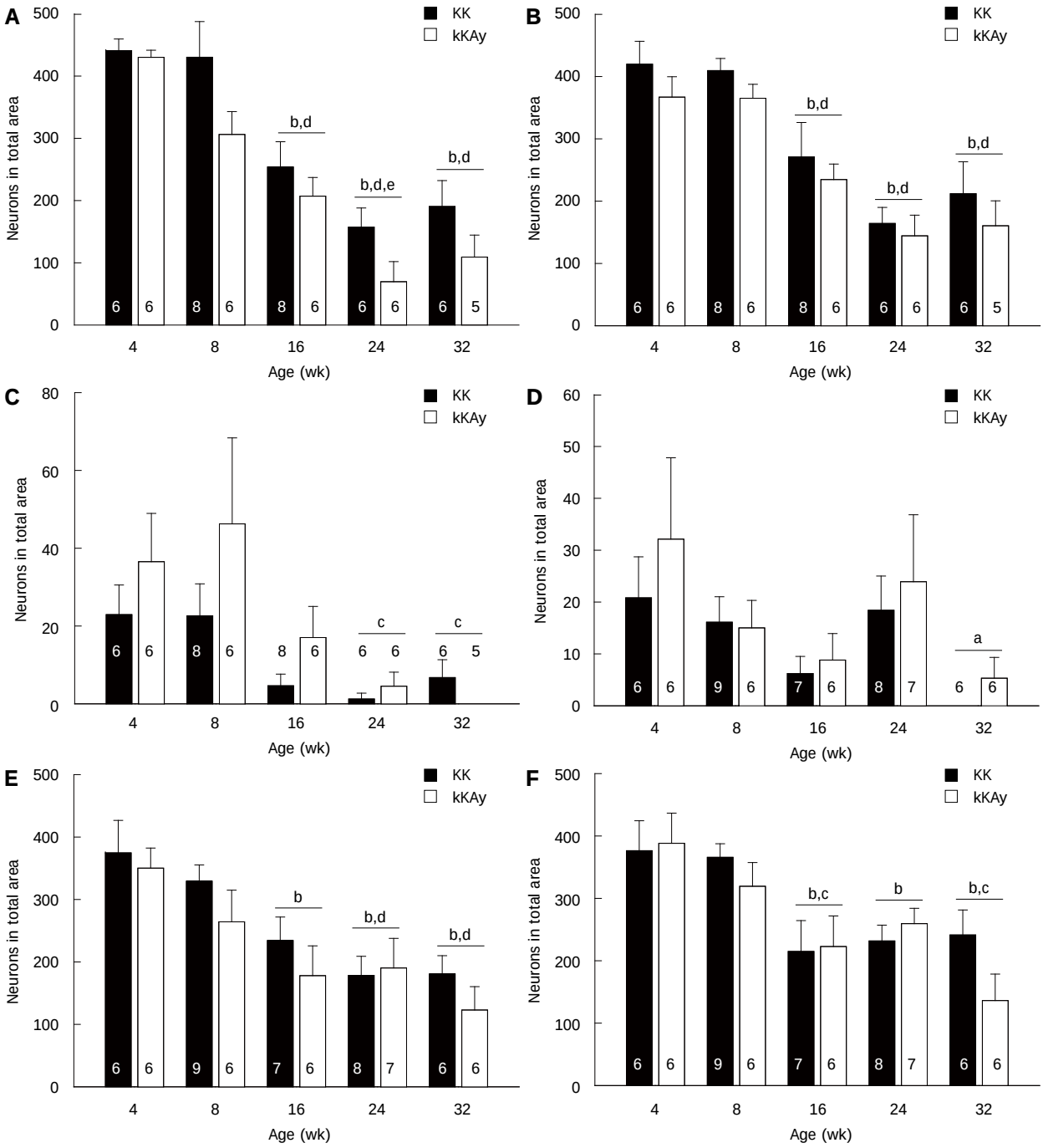


Figure 3 Number of fluorogold-labeled neurons in enteric neural pathways in 4-32 wk old KK and KKAy mice. A: Long ascending pathway: The number of fluorogold (FG)-labeled neurons was significantly decreased in KKAy as compared to KK mice [main effect: F (1, 53) = 8.136, P = 0.006]. The numbers of FG-labeled neurons began to decrease with age in KK and KKAy mice at 16 wk of age with the fewest neurons detected at 24 wk of age [main effect: F (4, 53) = 24.936, P < 0.001]; B: Short ascending pathway: There was a trend for the numbers of FG-labeled neurons to be reduced in KKAy as compared to KK mice [main effect: F (1, 53) = 2.940, P = 0.092]. The number of FG-labeled neurons decreased with age in KK and KKAy mice [main effect: F (4, 53) = 17.188, P < 0.001]. By 16 wk of age, the numbers of FG-labeled neurons were reduced and remained low; C: Fundus: Similar numbers of FG-labeled neurons were observed in KK and KKAy mice [main effect: F (1, 53) = 2.464, P = 0.122]. In KK and KKAy mice, the numbers of labeled FG neurons were highest at 8 wk and lowest at 24 and 32 wk [main effect: F (4, 53) = 5.195, P = 0.001]; D: Long descending pathway: Similar numbers of FG-labeled neurons were observed in KK and KKAy mice [main effect: F (1, 57) = 0.922, P = 0.341]. By 32 wk of age, the numbers of FG-labeled neurons were reduced as compared to younger ages [main effect: F (4, 57) = 2.944, P = 0.028]; E: Short descending pathway: Similar numbers of FG-labeled neurons were observed in KK and KKAy mice [main effect: F (1, 57) = 2.374, P = 0.129]. The numbers of FG-labeled neurons began to decrease in KK and KKAy mice at 16 wk of age with the fewest neurons detected at 24 wk of age [main effect: F (4, 57) = 9.336, P < 0.001]; F: Antrum: Similar numbers of FG-labeled neurons were observed in KK and KKAy mice [main effect: F (1, 57) = 0.728, P = 0.397]. By 16 wk of age, the numbers of FG-labeled neurons were reduced as compared to 4 and 8 wk of age [main effect: F (4, 57) = 8.920, P < 0.001]. ^{a}P < 0.05, ^{b}P < 0.01 *vs* 4 wk; ^{c}P < 0.05, ^{d}P < 0.01 *vs* 8 wk; ^{e}P < 0.05 *vs* 16 wk.

rons[46-48]. In this study, long ascending enteric neurons, but not long descending enteric neurons, displayed significantly slower tracer transport in KKAy as compared to KK mice despite similar axonal lengths. Ascending enteric neurons are predominantly cholinergic[31,49-51]. Impaired axonal transport has been reported for acetylcho-

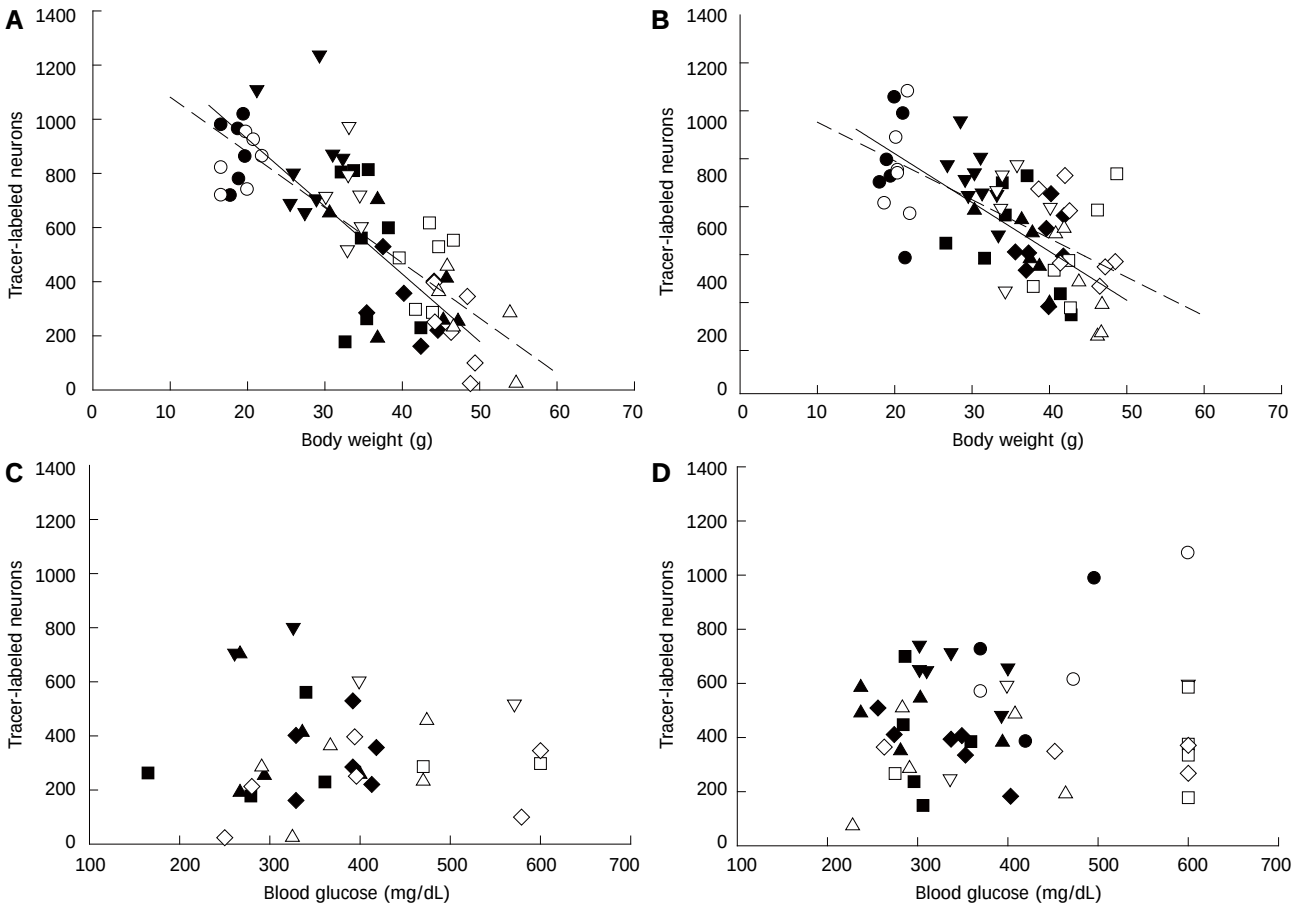


Figure 4 Correlation of body weight or stress-elevated blood glucose with number of tracer-labeled enteric neurons. A: Body weight was correlated with number of neurons labeled by fundus injection (KK mice, r^2 = 0.4362; KKAy mice, r^2 = 0.3868); B: Body weight was correlated with number of neurons labeled by antrum injection (KK mice, r^2 = 0.5565; KKAy mice, r^2 = 0.7087); C: Stress-elevated blood glucose was not correlated with number of neurons labeled by fundus injection (KK mice, r^2 = 0.0093; KKAy mice, r^2 = 0.108); D: Stress-elevated blood glucose was not correlated with number of neurons labeled by antrum injection (KK mice, r^2 = 0.0301; KKAy mice, r^2 = 0.0947). Legend: Black symbols and solid line, KK mice; white symbols and dashed line, KKAy mice; circle, 4 wk; inverted triangle, 8 wk; square, 16 wk; diamond, 24 wk; upright triangle, 32 wk.

linesterase[18,19] and muscarinic receptors[15] and debated for choline acetyltransferase[8,20,43,52] in rodent models of DM. Hence, active tracer transport in this subpopulation of autonomic neurons with a cholinergic phenotype may be more susceptible to combined persistent hyperglycemia and low effective insulin.

Age-related degeneration of axonal branches[37,40] may contribute to the reduction in the number of FG-labeled neurons observed in older as compared to younger mice. In young NMRI mice, the number of myenteric neurons per ganglion section did not change between 1 and 3 mo of age[53]. Similarly, in this study, neuron density was unchanged from 4 to 8 wk of age. In rats, gastric myenteric neurons have been reported to be less susceptible to age-related degeneration than intestinal myenteric neurons[54,55]. The density of gastric myenteric neurons was unchanged until 27 mo of age, when density was reduced in the forestomach, but not corpus or antrum[55]. In this study, the initial reduction in FG labeled neurons was observed at 16 wk of age in KK and KKA^y mice. Hence, in mice with insulin resistance, the reduced delivery of target organ trophic factors back to the cell soma may be associated with increased susceptibility of enteric neurons, especially those with cholinergic phenotype[54], to age-related degeneration[37,56].

A heavier body weight, reflecting a higher degree of adiposity and therefore insulin resistance, contributed more strongly to enteric neuropathy than severity of hyperglycemia. The correlation of total FG-labeled neurons with body weight was stronger for heavier KKAy as compared to lighter KK mice. In contrast, the severity of hyperglycemia was not correlated to total FG-labeled neurons. Other studies have also reported that insulin deficiency, but not hyperglycemia, was associated with severity of peripheral neuropathy[14,38,57-59].

An increase in stomach size may result in a lower density of nerve terminals in the injection field for FG endocytosis and subsequent reduction in FG labeling. The weights of stomachs from 16 wk old KK and KKAy mice were similar [personal observation, KK (n = 4), 277 ± 40 mg; KKAy (n = 4), 263 ± 33 mg, P = 0.80] suggesting that stomachs from KKAy mice were not hypertrophied.

Insulin resistance itself in the absence of persistent hyperglycemia slowed tracer transport in 16-wk-old KK mice. The addition of persistent hyperglycemia in KKAy mice further slowed tracer transport in only long ascend-

ing enteric neurons without accelerating the age of onset. Slowed active tracer transport in enteric neurons implies that retrograde transport of endogenous neurotrophins required for maintenance of normal nerve function may also be impaired[6,21,33,60]. Given the codependence of neurons and the target tissue on neurotrophins to preserve organ function[6], neuropathy resulting from impaired retrograde transport of neurotrophins may result in imprecise coordination of gastric regions by ascending and descending inhibitory and excitatory enteric reflexes[61,62], thereby contributing to asynchronous gastric motor function[63,64].

COMMENTS

Background

Diabetes mellitus (DM), characterized by persistent hyperglycemia and a deficiency of effective plasma insulin, can result in neuropathy of autonomic neurons. In rodent models of type 1 DM (T1DM) and type 2 DM (T2DM), neuropathies of the parasympathetic vagus nerve and enteric neurons located within the wall of the gut have been described. Many factors likely contribute to the development of autonomic neuropathy including accumulation of sorbitol in nerves, oxidative stress, and somal deficiency of neurotrophins.

Research frontiers

Under anesthesia, the retrograde tracer fluorogold was superficially injected into the fundus or antrum using a microsyringe in KK Cg-Ay/J mice prior to onset of T2DM (4 wk of age), at onset of T2DM (8 wk of age), and after 8, 16, or 24 wk of untreated T2DM and in age-matched KK/HlJ mice. Six days later, mice were sacrificed by CO_2 narcosis followed by pneumothorax. Data were expressed as the number of neurons in short and long descending and ascending pathways and in local fundus and antrum pathways, and the number of neurons in all regions labeled after injection of tracer into either the fundus or the antrum.

Innovations and breakthroughs

The principal findings of these studies were that (1) insulin resistance, in the absence of persistent hyperglycemia, slowed retrograde fluorogold transport in KK mice at 16 wk of age, the approximate age of onset of insulin resistance; and (2) persistent hyperglycemia, as indicated by elevated HbA1c, significantly slowed tracer transport in long ascending pathways in KKAy as compared to KK mice, but the age of onset was unchanged. Persistent hyperglycemia did not significantly accelerate the age of onset of neuropathy in KKAy as compared to KK mice.

Applications

Insulin resistance itself in the absence of persistent hyperglycemia slowed tracer transport in 16-wk-old KK mice. The addition of persistent hyperglycemia in KKAy mice further slowed tracer transport in only long ascending enteric neurons without accelerating the age of onset. Slowed active tracer transport in enteric neurons implies that retrograde transport of endogenous neurotrophins required for maintenance of normal nerve function may also be impaired.

Peer review

The manuscript is formally well written and figures are clear and understandable. Experimental design and methodology are suitable for publication.

REFERENCES

1 **Tay SS**, Wong WC. Short- and long-term effects of streptozotocin-induced diabetes on the dorsal motor nucleus of the vagus nerve in the rat. *Acta Anat* (Basel) 1994; **150**: 274-281 [PMID: 7839795 DOI: 10.1159/000147630]

2 **Kniel PC**, Junker U, Perrin IV, Bestetti GE, Rossi GL. Varied effects of experimental diabetes on the autonomic nervous system of the rat. *Lab Invest* 1986; **54**: 523-530 [PMID: 3702343]

3 **Robertson DM**, Sima AA. Diabetic neuropathy in the mutant mouse [C57BL/ks(db/db)]: a morphometric study. *Diabetes* 1980; **29**: 60-67 [PMID: 6991317 DOI: 10.2337/diabetes.29.1.60]

4 **Chandrasekharan B**, Srinivasan S. Diabetes and the enteric nervous system. *Neurogastroenterol Motil* 2007; **19**: 951-960 [PMID: 17971027 DOI: 10.1111/j.1365-2982.2007.01023.x]

5 **Bagyánszki M**, Bódi N. Diabetes-related alterations in the enteric nervous system and its microenvironment. *World J Diabetes* 2012; **3**: 80-93 [PMID: 22645637 DOI: 10.4239/wjd.v3.i5.80]

6 **Pittenger G**, Vinik A. Nerve growth factor and diabetic neuropathy. *Exp Diabesity Res* 2003; **4**: 271-285 [PMID: 14668049 DOI: 10.1155/EDR.2003.271]

7 **Sima AA**. New insights into the metabolic and molecular basis for diabetic neuropathy. *Cell Mol Life Sci* 2003; **60**: 2445-2464 [PMID: 14625688 DOI: 10.1007/s00018-003-3084-x]

8 **Tomlinson DR**, Townsend J, Fretten P. Prevention of defective axonal transport in streptozocin-diabetic rats by treatment with "Statil" (ICI 128436), an aldose reductase inhibitor. *Diabetes* 1985; **34**: 970-972 [PMID: 2412918 DOI: 10.2337/diabetes.34.10.970]

9 **Belai A**, Calcutt NA, Carrington AL, Diemel LT, Tomlinson DR, Burnstock G. Enteric neuropeptides in streptozotocin-diabetic rats; effects of insulin and aldose reductase inhibition. *J Auton Nerv Syst* 1996; **58**: 163-169 [PMID: 8738309 DOI: 10.1016/0165-1838(95)00129-8]

10 **Hounsom L**, Corder R, Patel J, Tomlinson DR. Oxidative stress participates in the breakdown of neuronal phenotype in experimental diabetic neuropathy. *Diabetologia* 2001; **44**: 424-428 [PMID: 11357471 DOI: 10.1007/s001250051638]

11 **Vincent AM**, Brownlee M, Russell JW. Oxidative stress and programmed cell death in diabetic neuropathy. *Ann NY Acad Sci* 2002; **959**: 368-383 [PMID: 11976211 DOI: 10.1111/j.1749-6632.2002.tb02108.x]

12 **Tomlinson DR**, Mayer JH. Defects of axonal transport in diabetes mellitus--a possible contribution to the aetiology of diabetic neuropathy. *J Auton Pharmacol* 1984; **4**: 59-72 [PMID: 6201487 DOI: 10.1111/j.1474-8673.1984.tb00434.x]

13 **Liu W**, Yue W, Wu R. Effects of diabetes on expression of glial fibrillary acidic protein and neurotrophins in rat colon. *Auton Neurosci* 2010; **154**: 79-83 [PMID: 20042376 DOI: 10.1016/j.autneu.2009.12.003]

14 **Pierson CR**, Zhang W, Murakawa Y, Sima AA. Insulin deficiency rather than hyperglycemia accounts for impaired neurotrophic responses and nerve fiber regeneration in type 1 diabetic neuropathy. *J Neuropathol Exp Neurol* 2003; **62**: 260-271 [PMID: 12638730]

15 **Laduron PM**, Janssen PF. Impaired axonal transport of opiate and muscarinic receptors in streptozocin-diabetic rats. *Brain Res* 1986; **380**: 359-362 [PMID: 2428426 DOI: 10.1016/0006-8993(86)90235-0]

16 **Lee PG**, Cai F, Helke CJ. Streptozotocin-induced diabetes reduces retrograde axonal transport in the afferent and efferent vagus nerve. *Brain Res* 2002; **941**: 127-136 [PMID: 12031555 DOI: 10.1016/S0006-8993(02)02645-8]

17 **Odekunle A**. Impairment of transneuronal traffic in Streptozotocin-induced diabetes, a WGA-HRP neurohistochemical study in the rat. *J Biomed Sci* 2006; **13**: 675-680 [PMID: 16850180 DOI: 10.1007/s11373-006-9097-3]

18 **Vitadello M**, Couraud JY, Hässig R, Gorio A, Di Giamberardino L. Axonal transport of acetylcholinesterase in the diabetic mutant mouse. *Exp Neurol* 1983; **82**: 143-147 [PMID: 6195008 DOI: 10.1016/0014-4886(83)90249-2]

19 **Norido F**, Canella R, Zanoni R, Gorio A. Development of diabetic neuropathy in the C57BL/Ks (db/db) mouse and its treatment with gangliosides. *Exp Neurol* 1984; **83**: 221-232 [PMID: 6692864 DOI: 10.1016/0014-4886(84)90094-3]

20 **Calcutt NA**, Willars GB, Tomlinson DR. Axonal transport of choline acetyltransferase and 6-phosphofructokinase activities in genetically diabetic mice. *Muscle Nerve* 1988; **11**: 1206-1210 [PMID: 2467203 DOI: 10.1002/mus.880111204]

21 **Lee PG**, Hohman TC, Cai F, Regalia J, Helke CJ. Streptozotocin-induced diabetes causes metabolic changes and alterations in neurotrophin content and retrograde transport in the cervical vagus nerve. *Exp Neurol* 2001; **170**: 149-161 [PMID: 11421592 DOI: 10.1006/exnr.2001.7673]

22 **Cannon WB,** Lieb CW. The receptive relaxation of the stomach. *Am J Physiol* 1911; **29:** 267-273

23 **Cannon WB.** The nature of gastric peristalsis. *Am J Physiol* 1911; **29:** 250-266

24 **Cellini J**, DiNovo K, Harlow J, LePard KJ. Regional differences in neostigmine-induced contraction and relaxation of stomach from diabetic guinea pig. *Auton Neurosci* 2011; **160**: 69-81 [PMID: 21075692 DOI: 10.1016/j.autneu.2010.10.006]

25 **James AN**, Ryan JP, Crowell MD, Parkman HP. Regional gastric contractility alterations in a diabetic gastroparesis mouse model: effects of cholinergic and serotoninergic stimulation. *Am J Physiol Gastrointest Liver Physiol* 2004; **287**: G612-G619 [PMID: 15107299 DOI: 10.1152/ajpgi.00431.2003]

26 **Iwatsuka H**, Shino A, Suzuoki Z. General survey of diabetic features of yellow KK mice. *Endocrinol Jpn* 1970; **17**: 23-35 [PMID: 5468422 DOI: 10.1507/endocrj1954.17.23]

27 **Nakamura M**, Yamada K. Studies on a diabetic (KK) strain of the mouse. *Diabetologia* 1967; **3**: 212-221 [PMID: 4907141 DOI: 10.1007/BF01222198]

28 **Reddi AS**, Camerini-Davalos RA. Hereditary diabetes in the KK mouse: an overview. *Adv Exp Med Biol* 1988; **246**: 7-15 [PMID: 3074669 DOI: 10.1007/978-1-4684-5616-5_2]

29 **Fullmer R,** LePard KJ. Gastric vagal fluorogold labeled neurons are reduced in obese diabetic agouti mice. In: Masters Thesis of Biomedical Sciences Program in the College of Health Sciences at Midwestern University. Downers Grove, IL: Midwestern University, 2009

30 **Van Putten LM**. The life span of red cells in the rat and the mouse as determined by labeling with DFP32 in vivo. *Blood* 1958; **13**: 789-794 [PMID: 13560578]

31 **Schemann M**, Reiche D, Michel K. Enteric pathways in the stomach. *Anat Rec* 2001; **262**: 47-57 [PMID: 11146428]

32 **Michel K**, Reiche D, Schemann M. Projections and neurochemical coding of motor neurones to the circular and longitudinal muscle of the guinea pig gastric corpus. *Pflugers Arch* 2000; **440**: 393-408 [PMID: 10954325 DOI: 10.1007/s004240000299]

33 **Tomlinson DR**, Fernyhough P, Diemel LT, Maeda K. Deficient neurotrophic support in the aetiology of diabetic neuropathy. *Diabet Med* 1996; **13**: 679-681 [PMID: 8840106 DOI: 10.1002/(SICI)1096-9136(199607)13]

34 **Cullum NA**, Mahon J, Stringer K, McLean WG. Glycation of rat sciatic nerve tubulin in experimental diabetes mellitus. *Diabetologia* 1991; **34**: 387-389 [PMID: 1715829 DOI: 10.1007/BF00403175]

35 **Jeyabal PV**, Kumar R, Gangula PR, Micci MA, Pasricha PJ. Inhibitors of advanced glycation end-products prevent loss of enteric neuronal nitric oxide synthase in diabetic rats. *Neurogastroenterol Motil* 2008; **20**: 253-261 [PMID: 17971026 DOI: 10.1111/j.1365-2982.2007.01018.x]

36 **McLean WG**. The role of axonal cytoskeleton in diabetic neuropathy. *Neurochem Res* 1997; **22**: 951-956 [PMID: 9239750]

37 **Raff MC**, Whitmore AV, Finn JT. Axonal self-destruction and neurodegeneration. *Science* 2002; **296**: 868-871 [PMID: 11988563 DOI: 10.1126/science.1068613]

38 **Sima AA**, Zhang W, Xu G, Sugimoto K, Guberski D, Yorek MA. A comparison of diabetic polyneuropathy in type II diabetic BBZDR/Wor rats and in type I diabetic BB/Wor rats. *Diabetologia* 2000; **43**: 786-793 [PMID: 10907124 DOI: 10.1007/s001250051376]

39 **Guo C**, Quobatari A, Shangguan Y, Hong S, Wiley JW. Diabetic autonomic neuropathy: evidence for apoptosis in situ in the rat. *Neurogastroenterol Motil* 2004; **16**: 335-345 [PMID: 15198656 DOI: 10.111/j.1365-2982.2004.00524.x]

40 **Wiley JW**. Aging and neural control of the GI tract: III. Senescent enteric nervous system: lessons from extraintestinal sites and nonmammalian species. *Am J Physiol Gastrointest Liver Physiol* 2002; **283**: G1020-G1026 [PMID: 12381514 DOI: 10.1152/ajpgi.00224.2002]

41 **Burnstock G**, Mirsky R, Belai A. Reversal of nerve damage in streptozotocin-diabetic rats by acute application of insulin in vitro. *Clin Sci* (Lond) 1988; **75**: 629-635 [PMID: 3061714]

42 **Brussee V**, Cunningham FA, Zochodne DW. Direct insulin signaling of neurons reverses diabetic neuropathy. *Diabetes* 2004; **53**: 1824-1830 [PMID: 15220207 DOI: 10.2337/diabetes.53.7.1824]

43 **Jakobsen J**, Brimijoin S, Skau K, Sidenius P, Wells D. Retrograde axonal transport of transmitter enzymes, fucose-labeled protein, and nerve growth factor in streptozotocin-diabetic rats. *Diabetes* 1981; **30**: 797-803 [PMID: 7274587]

44 **Schmidt RE**, Modert CW, Yip HK, Johnson EM. Retrograde axonal transport of intravenously administered 125I-nerve growth factor in rats with streptozotocin-induced diabetes. *Diabetes* 1983; **32**: 654-663 [PMID: 6345245 DOI: 10.2337/diabetes.32.7.654]

45 **Sima AA**. Pathological mechanisms involved in diabetic neuropathy: can we slow the process? *Curr Opin Investig Drugs* 2006; **7**: 324-337 [PMID: 16625819]

46 **Shotton HR**, Clarke S, Lincoln J. The effectiveness of treatments of diabetic autonomic neuropathy is not the same in autonomic nerves supplying different organs. *Diabetes* 2003; **52**: 157-164 [PMID: 12502507 DOI: 10.2337/diabetes.52.1.157]

47 **Shotton HR**, Adams A, Lincoln J. Effect of aminoguanidine treatment on diabetes-induced changes in the myenteric plexus of rat ileum. *Auton Neurosci* 2007; **132**: 16-26 [PMID: 16987713 DOI: 10.1016/j.autneu.2006.08.007]

48 **Shotton HR**, Broadbent S, Lincoln J. Prevention and partial reversal of diabetes-induced changes in enteric nerves of the rat ileum by combined treatment with alpha-lipoic acid and evening primrose oil. *Auton Neurosci* 2004; **111**: 57-65 [PMID: 15109939 DOI: 10.1016/j.autneu.2004.02.004]

49 **Pfannkuche H**, Reiche D, Firzlaff U, Sann H, Schemann M. Enkephalin-immunoreactive subpopulations in the myenteric plexus of the guinea-pig fundus project primarily to the muscle and not to the mucosa. *Cell Tissue Res* 1998; **294**: 45-55 [PMID: 9724455 DOI: 10.1007/s004410051155]

50 **Reiche D**, Schemann M. Mucosa of the guinea pig gastric corpus is innervated by myenteric neurones with specific neurochemical coding and projection preferences. *J Comp Neurol* 1999; **410**: 489-502 [PMID: 10404414 DOI: 10.1002/(SICI)1096-9861(19990802)410]

51 **Schemann M**, Schaaf C. Differential projection of cholinergic and nitroxidergic neurons in the myenteric plexus of guinea pig stomach. *Am J Physiol* 1995; **269**: G186-G195 [PMID: 7544534]

52 **Mayer JH**, Herberg L, Tomlinson DR. Axonal transport and nerve conduction and their relation to nerve polyol and myo-inositol levels in spontaneously diabetic BB/D rats. *Neurochem Pathol* 1984-1985; **2**: 285-293 [PMID: 6085698]

53 **El-Salhy M**, Sandström O, Holmlund F. Age-induced changes in the enteric nervous system in the mouse. *Mech Ageing Dev* 1999; **107**: 93-103 [PMID: 10197791 DOI: 10.1016/S0047-6374(98)00142-0]

54 **Phillips RJ**, Kieffer EJ, Powley TL. Aging of the myenteric plexus: neuronal loss is specific to cholinergic neurons. *Auton Neurosci* 2003; **106**: 69-83 [PMID: 12878075 DOI: 10.1016/S1566-0702(03)00072-9]

55 **Phillips RJ**, Powley TL. As the gut ages: timetables for aging of innervation vary by organ in the Fischer 344 rat. *J Comp Neurol* 2001; **434**: 358-377 [PMID: 11331534 DOI: 10.1002/cne.1182]

56 **Cowen T**. Selective vulnerability in adult and ageing mammalian neurons. *Auton Neurosci* 2002; **96**: 20-24 [PMID: 11911498 DOI: 10.1016/S1566-0702(01)00376-9]

57 **Romanovsky D**, Cruz NF, Dienel GA, Dobretsov M. Mechanical hyperalgesia correlates with insulin deficiency in normoglycemic streptozotocin-treated rats. *Neurobiol Dis* 2006; **24**: 384-394 [PMID: 16935517 DOI: 10.1016/j.nbd.2006.07.009]

58 **Hoybergs YM**, Meert TF. The effect of low-dose insulin on mechanical sensitivity and allodynia in type I diabetes neuropathy. *Neurosci Lett* 2007; **417**: 149-154 [PMID: 17412508 DOI: 10.1016/j.neulet.2007.02.087]

59 **Schmidt RE**, Dorsey DA, Beaudet LN, Parvin CA, Zhang W, Sima AA. Experimental rat models of types 1 and 2 diabetes differ in sympathetic neuroaxonal dystrophy. *J Neuropathol Exp Neurol* 2004; **63**: 450-460 [PMID: 15198124]

60 **Li Y**, Owyang C. Musings on the wanderer: what's new in our understanding of vago-vagal reflexes? V. Remodeling of vagus and enteric neural circuitry after vagal injury. *Am J Physiol Gastrointest Liver Physiol* 2003; **285**: G461-G469 [PMID: 12909562 DOI: 10.1152/ajpgi.00119.2003]

61 **Yuan SY**, Brookes SJ, Costa M. Distension-evoked ascending and descending reflexes in the isolated guinea-pig stomach. *J Auton Nerv Syst* 1997; **62**: 94-102 [PMID: 9021655 DOI: 10.1016/S0165-1838(96)00115-4]

62 **Hennig GW**, Brookes SJ, Costa M. Excitatory and inhibitory motor reflexes in the isolated guinea-pig stomach. *J Physiol* 1997; **501** (Pt 1): 197-212 [PMID: 9175003 DOI: 10.1111/j.1469-7793.1997.197bo.x]

63 **Yamamoto T**, Watabe K, Nakahara M, Ogiyama H, Kiyohara T, Tsutsui S, Tamura S, Shinomura Y, Hayashi N. Disturbed gastrointestinal motility and decreased interstitial cells of Cajal in diabetic db/db mice. *J Gastroenterol Hepatol* 2008; **23**: 660-667 [PMID: 18341539 DOI: 10.1111/j.1440-1746.2008.05326.x]

64 **Song GQ**, Chen JD. Synchronized gastric electrical stimulation improves delayed gastric emptying in nonobese mice with diabetic gastroparesis. *J Appl Physiol* 2007; **103**: 1560-1564 [PMID: 17717123 DOI: 10.1152/japplphysiol.00319.2007]

P- Reviewers Binder HJ, Wex T
S- Editor Gou SX **L- Editor** A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i4.492

World J Gastroenterol 2013 January 28; 19(4): 492-502
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

ORIGINAL ARTICLE

Combined early fluid resuscitation and hydrogen inhalation attenuates lung and intestine injury

Wei Liu, Li-Ping Shan, Xue-Song Dong, Xiao-Wei Liu, Tao Ma, Zhi Liu

Wei Liu, Xue-Song Dong, Xiao-Wei Liu, Tao Ma, Zhi Liu, Department of Emergency Medicine, the First Affiliated Hospital of China Medical University, Shenyang 110001, Liaoning Province, China
Li-Ping Shan, Department of Urologic Surgery, Shengjing Hospital, China Medical University, Shenyang 110004, Liaoning Province, China
Author contributions: Liu W designed and did the experiment assisted by Shan LP, Dong XS, Liu XW and Ma T; Liu W drafted the manuscript; Liu Z supervised and revised the manuscript; all authors have read and approved the final version.
Supported by National Natural Science Foundation of China, No. 81101415
Correspondence to: Dr. Zhi Liu, Professor, Department of Emergency Medicine, the First Affiliated Hospital of China Medical University, No. 155, Nanjing Beijie, Shenyang 110001, Liaoning Province, China. zhiliu2012@gmail.com
Telephone: +86-24-83283055 Fax: +86-24-83282011
Received: July 5, 2012 Revised: November 20, 2012
Accepted: November 24, 2012
Published online: January 28, 2013

Abstract

AIM: To study the effects of combined early fluid resuscitation and hydrogen inhalation on septic shock-induced lung and intestine injuries.

METHODS: Wistar male rats were randomly divided into four groups: control group (Group A, *n* = 15); septic shock group (Group B, *n* = 15); early fluid resuscitation-treated septic shock group (Group C, *n* = 15); and early fluid resuscitation and inhalation of 2% hydrogen-treated septic shock group (Group D, *n* = 15). The activity of hydroxyl radicals, myeloperoxidase (MPO), superoxide dismutase (SOD), diamine oxidase (DAO), and the concentration of malonaldehyde (MDA) in the lung and intestinal tissue were assessed according to the corresponding kits. Hematoxylin and eosin staining was carried out to detect the pathology of the lung and intestine. The expression levels of interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF)-α in lung and intestine tissue were detected by enzyme-linked immunosorbent assay method. The expression levels of Fas and Bcl2 in lung tissues were determined by immunohistochemistry and Western blotting.

RESULTS: Septic shock elicited a significant increase in the levels of MDA (10.17 ± 1.12 nmol/mg protein *vs* 2.98 ± 0.64 nmol/mg protein) and MPO (6.79 ± 1.02 U/g wet tissue *vs* 1.69 ± 0.14 U/g wet tissue) in lung tissues. These effects were not significantly decreased by Group C pretreatment, but were significantly reduced by Group D pretreatment (MDA: 4.45 ± 1.13 nmol/mg protein *vs* 9.56 ± 1.37 nmol/mg protein; MPO: 2.58 ± 0.21 U/g wet tissue *vs* 6.02 ± 1.16 U/g wet tissue). The activity of SOD (250.32 ± 8.56 U/mg protein *vs* 365.78 ± 10.26 U/mg protein) in lung tissues was decreased after septic shock, and was not significantly increased by Group C pretreatment, but was significantly enhanced by Group D pretreatment (331.15 ± 9.64 U/mg protein *vs* 262.98 ± 5.47 U/mg protein). Histological evidence of lung hemorrhage, neutrophil infiltration and overexpression of IL-6, IL-8, and TNF-α was observed in lung tissues, all of which were attenuated by Group C and further alleviated by Group D pretreatment. Septic shock also elicited a significant increase in the levels of MDA, MPO and DAO (6.54 ± 0.68 kU/L *vs* 4.32 ± 0.33 kU/L) in intestinal tissues, all of which were further increased by Group C, but significantly reduced by Group D pretreatment. Increased Chiu scoring and overexpression of IL-6, IL-8 and TNF-α were observed in intestinal tissues, all of which were attenuated by Group C and further attenuated by Group D pretreatment.

CONCLUSION: Combined early fluid resuscitation and hydrogen inhalation may protect the lung and intestine of the septic shock rats from the damage induced by oxidative stress and the inflammatory reaction.

© 2013 Baishideng. All rights reserved.

Key words: Early fluid resuscitation; Inhalation of hydrogen gas; Septic shock; Lung; Intestine; Oxidative damage

Liu W, Shan LP, Dong XS, Liu XW, Ma T, Liu Z. Combined early fluid resuscitation and hydrogen inhalation attenuates lung and intestine injury. *World J Gastroenterol* 2013; 19(4): 492-502 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i4/492.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i4.492

INTRODUCTION

Septic shock is a common complication of infection, severe trauma, and major operation in patients. Septic shock is an autoimmune injury of the body, and the pathogenesis is very complicated. The immunocyte is activated in patients with septic shock, and the respiratory burst creates massive amounts of reactive oxygen species (ROS) which cannot be cleaned by the antioxidant defense systems[1,2]. Oxidative stress induced by ROS can increase lipid peroxidation, and can also increase the permeability of the alveolar epithelial cells by destroying the cell membrane. Simultaneously, the aggregation of neutrophilic leukocytes in the lung further induces the respiratory burst. The imbalance of antioxidant defense systems against oxidative stress then further damages the alveolar epithelial cells.

In order to ensure the blood supply of major organs, including the heart and brain, in patients with septic shock, blood flow is redistributed. There is a significant decrease in blood flow of the gastrointestinal tract which induces severe ischemia, hypoxia, and reperfusion injury. Inflammatory factors are activated which induce further injury in the gastrointestinal tract, and may consequently produce multiple organ dysfunction syndrome (MODS)[3]. Despite recent advances in antibiotic therapy and intensive care, sepsis is still considered to be one of the most common causes of death in intensive care units. The main therapy for septic shock is hemostasis and fluid resuscitation which can prevent MODS by improving hemodynamics[4]. However, fluid resuscitation is not effective in microcirculation disturbance and hypoxia. So, new and effective therapies for septic shock should be developed to improve the patient outcomes.

Recently, it has been suggested that molecular hydrogen exerts a therapeutic antioxidant activity by selectively reducing hydroxyl radicals (•OH, the most cytotoxic ROS) and effectively protects against organ damage[5-7]. Xie *et al*[1,8,9] also report that hydrogen inhalation can significantly decrease levels of oxidative product, increase activities of antioxidant enzymes, and reduce levels of high-mobility group box 1 in serum and lung tissue, thus improving the survival rate of mice with sepsis. However, there are no reports combining fluid resuscitation and hydrogen inhalation as treatment for septic shock. Therefore, the present study was designed to investigate the protective effects of combined early fluid resuscitation and 2% hydrogen on the lung and intestine in a lipopolysaccharide (LPS)-induced septic shock rat model.

MATERIALS AND METHODS

Animals and chemicals

Male Wistar rats weighing 180-200 g were used for the study. They were obtained from the Laboratory Animal Center (China Medical University, Shenyang, China). LPS (L-2880 from *Escherichia coli* serotype 055:B5) was obtained from Sigma Chemical Co. (St. Louis, MO, United States). The •OH, myeloperoxidase (MPO), superoxidase dismutase (SOD), and malonaldehyde (MDA) assay kits were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The interleukin (IL)-6, IL-8, and tumor necrosis factor (TNF)-α enzyme-linked immunosorbent assay (ELISA) kits, rabbit polyclonal anti-Bcl-2 and rabbit polyclonal anti-Fas were purchased from Wuhan Boster Bio-engineering Limited Company (Wuhan, China).

Experimental design

Sixty male Wistar rats were divided into four groups randomly, 15 rats in each: control group (Group A); septic shock group (Group B); early fluid resuscitation + septic shock group (Group C); and early fluid resuscitation + inhalation of 2% hydrogen + septic shock group (Group D). Rats were anesthetized by intraperitoneal injection of 10% chloral hydrate (300 mg/kg) and tracheally ventilated using a Colombus ventilator (HX-300, TaiMeng Technologies, China), with a respiratory frequency of 100 breaths/min and a tidal volume of 10 mL/kg. The inhaled gas used for Group D was a hydrogen-air mixture (2% hydrogen in air), and the inhaled gas for other groups was room air. Left common carotid artery was cannulated to monitor the heart rate and mean arterial pressure (MAP). The femoral vein was cannulated for drug administration and fluid resuscitation. An electric heater was used to maintain body temperature. Half an hour after the rats reached a stable status, except for the rats in Group A, LPS was administered at a dose of 15 mg/kg by slow intravenous injection (not less than 2 min) to establish the septic shock rat model. Then, 0.2 mL saline was used to ensure that the LPS remaining in the syringe was completely injected[10]. Group A rats received intravenous injections of the same volume of saline. Fluid challenge (10 mL/kg per 15 min) was performed when MAP or arterial blood flow decreased to 80% of baseline values. If the percent change in pulse pressure > 13% with a persistent decrease in MAP or arterial blood flow, the fluid challenge was repeated. Norepinephrine was introduced when MAP remained low despite a normal percent change in pulse pressure (< 13%). Norepinephrine concentration was either progressively increased or decreased between 0.5 and 6 μg/kg.min, as needed, to maintain the MAP at ± 10% of baseline value[10]. Vital signs, fluid volume and the amount of norepinephrine were also recorded.

Preparation of lung tissues

The rats were sacrificed 2 h after the septic shock rat model was established. Arterial blood (1 mL) was collected for assessing the arterial blood gases. The heart and lung were removed through a thoracotomy and the dorsal lobe of the right lung was cut into three blocks (0.1 cm × 0.1 cm × 0.1 cm). The excess liquid of the first block was drained by the filter paper, and wet weight (W) was recorded. Then the same block was dried in a drying oven at a temperature of 70 ℃ for 24 h to measure dry weight (D). The second block was fixed with 4% paraformaldehyde at a temperature of 4 ℃, and then, the block was embedded in paraffin and sliced for hematoxylin and eosin (HE) staining and immunohistochemical study. The other lung tissue block was frozen at -70 ℃ for Western blotting analysis. The right hilum was ligated, and 2 mL of saline solution was used for the left lung lavage, and about 3.0-3.6 mL of bronchial alveolar lavage fluid (BALF) was collected. The BALF was centrifuged at 1500 × *g* for 4 min, and the supernatant was collected and stored at -20 ℃ for inflammatory mediator analysis.

Preparation of small intestine tissues

The rats were sacrificed 2 h after the septic shock rat model was established. Arterial blood (1 mL) was collected for assessing the arterial blood gases. Another 4 mL arterial blood was centrifuged at 2000 rpm for 10 min, and the supernatant serum was collected and stored at -20 ℃. A laparotomy was performed, two small intestinal segments were resected from 10 cm distal to the ligament of Treitz, and the intestinal segments were gently washed using saline rinse cooled to 4 ℃. These two small intestine segments (cut to 1 cm × 1 cm) were fixed with 4% paraformaldehyde and 2.5% glutaraldehyde, respectively. The other small intestine segments used for assessing the oxidative stress were frozen at -70 ℃.

Determination of oxidative damage

Small intestinal tissues and lung tissues were homogenated, centrifuged at 12 000 × *g* for 20 min, and the supernatant serum was collected. The OH, MDA, SOD, and MPO activities were measured according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

Measurement of serum diamine oxidase activity

The frozen serum was thawed on ice, and centrifuged at 1000 × *g* for 15 min at 4 ℃. The serum diamine oxidase (DAO) activity was detected with dianisidine developer, and the serum DAO activity assay was performed according to the manufacturer's instructions (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

HE staining of tissues

Both the small intestinal tissue and lung tissues were fixed with 4% paraformaldehyde for more than 24 h. After dehydrating and embedding, the tissues were cut into 5 μm slices. The slices were stained with HE. The pathogenesis of lung injury and small intestinal mucosal injury was observed under microscope. The intestinal mucosal injury was evaluated by the Chiu scoring system. The Chiu's score was graded as: Grade 0: normal mucosa; Grade 1: formation of subepithelial detachments at the tip of the villi with capillary congestion; Grade 2: subepithelial detachments exert a moderate amount of upward push on the mucosal epithelium; Grade 3: large subepithelial detachments exert a massive amount of upward push on the mucosal epithelium along the villi and a few denuded villus tips are observed; Grade 4: the villi are denuded to the level of lamina propria and dilated capillaries; Grade 5: presence of ulceration, disintegration of lamina propria, and hemorrhage[11]. Ten fields for each sample were observed, and the average score was recorded as the pathology score of the small intestinal tissue.

Changes in the organelles of small intestine

The small intestine segments were fixed with 2.5% glutaraldehyde. After dehydration, the samples were embedded in epoxy resins, and then were cut into ultrathin sections. The changes in the organelles were observed under transmission electron microscope.

Measurement of serum inflammatory mediators

The frozen serum was thawed on ice, and centrifuged at 1000 × *g* for 15 min at 4 ℃. Levels of IL-6, IL-8 and TNF-α were detected by ELISA according to the manufacturer's instructions (Wuhan Boster Bio-engineering Limited Company, Wuhan, China).

Immunohistochemical study

Tissue sections (slice thickness 3 μm) were dried for 4 h at 60 ℃-65 ℃, and then were deparaffinized and rehydrated. Sections were immersed in phosphate buffered saline (PBS) three times (5 min/time). Then antigen retrieval was performed followed by cooling at room temperature. The slides were immersed in PBS three times (5 min/time). After that, the sections were incubated with methanol containing 3% hydrogen peroxide for 20 min, and then were left at room temperature for 10 min followed by washing with PBS three times (5 min/time). The slides were blocked with normal goat serum solution at room temperature for 10 min, and the superfluous serum surrounding slides was wiped off. After that, the slides were incubated with primary antibody (50 μL) at 4 ℃ overnight. After washing with PBS three times (5 min/time), sections were incubated in horseradish peroxidase-polymer goat anti-Ms/Rb immunoglobulin G at room temperature for 20 min followed by rinsing in PBS three times (5 min/time). The slides were developed with 3,3'-diaminobenzidine solution for about 3-5 min, rinsed with tap water, and then counterstained with hematoxylin. Acid alcohol solution (1%) was used for differentiation for 1 s, followed by rinsing with tap water for 10-15 min. Finally, specimens were dehydrated and mounted, and examined with a microscope equipped with an image analysis system. Yellow or brownish-yellow staining represents positive staining.

Table 1 Comparison of blood gas and fluid volume among four groups (mean ± SD)

Group	pH value	MAP (mmHg)	Heart rate	PaO_2 (mmHg)	$PaCO_2$ (mmHg)	Lactate (mmol/L)	Fluid volumes (mL)	Norepinephrine (μg/kg per minute)
A	7.38 ± 0.12	101 ± 8	240 ± 13	93.9 ± 2.23	38.65 ± 1.78	1.50 ± 0.34	0	0
B	6.89 ± 0.13[a]	37 ± 11[a]	65 ± 20[a]	50.19 ± 3.78[a]	34.54 ± 1.89	6.98 ± 2.45[a]	0	0
C	7.21 ± 0.15[c]	95 ± 10[c]	260 ± 15[c]	62.34 ± 2.46[c]	35.09 ± 2.01	4.37 ± 1.36[c]	12 ± 3[c]	3.5 ± 1.8[c]
D	7.32 ± 0.17[e]	97 ± 11	255 ± 12	88.98 ± 3.17[e]	36.49 ± 1.84	3.59 ± 1.53[c]	5 ± 2[e]	0.8 ± 0.3[e]

[a]$P < 0.05$ *vs* Group A; [c]$P < 0.05$ *vs* Group B; [e]$P < 0.05$ *vs* Group C. MAP: Mean arterial pressure.

Table 2 Comparison of wet/dry wight and redox indicator among four groups (mean ± SD)

Group	Wet/dry wight	Inhibition of •OH (U/mg protein)	MDA (nmol/mg protein)	MPO (U/g wet tissue)	SOD (U/mg protein)
A	3.85 ± 0.34	87.52 ± 3.45	2.98 ± 0.64	1.69 ± 0.14	365.78 ± 10.26
B	6.89 ± 0.23[a]	12.89 ± 1.52[a]	10.17 ± 1.12[a]	6.79 ± 1.02[a]	250.32 ± 8.56[a]
C	6.96 ± 0.25	13.14 ± 2.24	9.56 ± 1.37	6.02 ± 1.16	262.98 ± 5.47
D	4.62 ± 0.27[c]	66.31 ± 2.98[c]	4.45 ± 1.13[c]	2.58 ± 0.21[c]	331.15 ± 9.64[c]

[a]$P < 0.05$ *vs* Group A; [c]$P < 0.05$ *vs* Group C. •OH: Hydroxyl radical; MDA: Malonaldehyde; MPO: Mmyeloperoxidase; SOD: Superoxide dismutase.

Western blotting analysis

Levels of Fas and Bcl2 proteins in lung tissue were quantified by Western blotting as described previously with some minor modifications[12]. Briefly, about 10 mg frozen lung tissue were immersed in the tissue lysates at a ratio of 1:10 w/v, then submitted to ultrasound homogenation for 1 min followed by centrifugation at 13 000 rpm for 5 min at 4 ℃. The supernatant was collected, and total protein concentration was measured by the Bradford method. About 15 μg total proteins was loaded in each well and separated on 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis for 1 h. After running, the proteins were transferred onto the polyvinylidene difluoride membranes (Millipore, United States). The membranes were blocked with 5% fat-free milk, and then were incubated with Tris-buffered-daline Tween20-containing the primary antibodies (1:500 dilution) overnight at 4 ℃. After extensive washing, the second antibody was added, and the immunocomplexes were then detected using an enhanced chemiluminescence Western blotting detection kit (GE, Healthcare). The relative densities of the protein bands were analyzed using the Quantity One software from Bio-Rad. Data were represented in relative arbitrary units.

Statistical analysis

All the data analyses were carried out using the statistical analysis software SPSS 13.0 for Windows (SPSS Inc., Chicago, IL, United States). Data were presented as the mean ± SD. Significant differences between the groups were analyzed by analysis of variance, and differences were considered significant at $P < 0.05$.

RESULTS

Fluid volume and blood gas parameters

Compared with Group A, the pH, MAP, heart rate and oxygen partial pressure of Group B were significantly decreased; however, a significant increase in lactate value was observed ($P < 0.05$). The pH, MAP, heart rate and oxygen partial pressure were increased obviously, and the lactate value was clearly decreased in Group C as compared with Group B. Furthermore, a significant increase in pH value and oxygen partial pressure and decrease in lactate value were also observed in Group D compared with Group C ($P < 0.05$). There was no significant difference in MAP and heart rate between Group D and Group C. Total fluid volume and the amount of norepinephrine used in Group D were significantly less than in Group C ($P < 0.05$). There was no significant difference in partial pressure of carbon dioxide among the four groups ($P > 0.05$; Table 1).

OH, MDA, MPO and SOD levels and lung W/D values

In Group B, the levels of OH, MDA, and MPO were significantly increased, and the level of SOD was significantly decreased compared with Group A ($P < 0.05$). Compared with Group B, lower levels of OH, MDA and MPO, and a higher level of SOD in Group C were also observed, but there was no significant difference between the two groups ($P > 0.05$). However, compared with Group C, there were also significant decreases in the levels of OH, MDA and MPO, and a significant increase in the SOD level in Group D ($P < 0.05$). The lung W/D value in Group B was significantly increased compared with group A ($P > 0.05$), and a higher lung W/D value was observed in Group C compared with Group B, but there was no significant difference between the two groups ($P > 0.05$). However, there was a significant decrease in the lung W/D value in Group D compared with Group C ($P < 0.05$; Table 2).

HE staining of lung tissues

HE-stained lung tissue sections were observed under

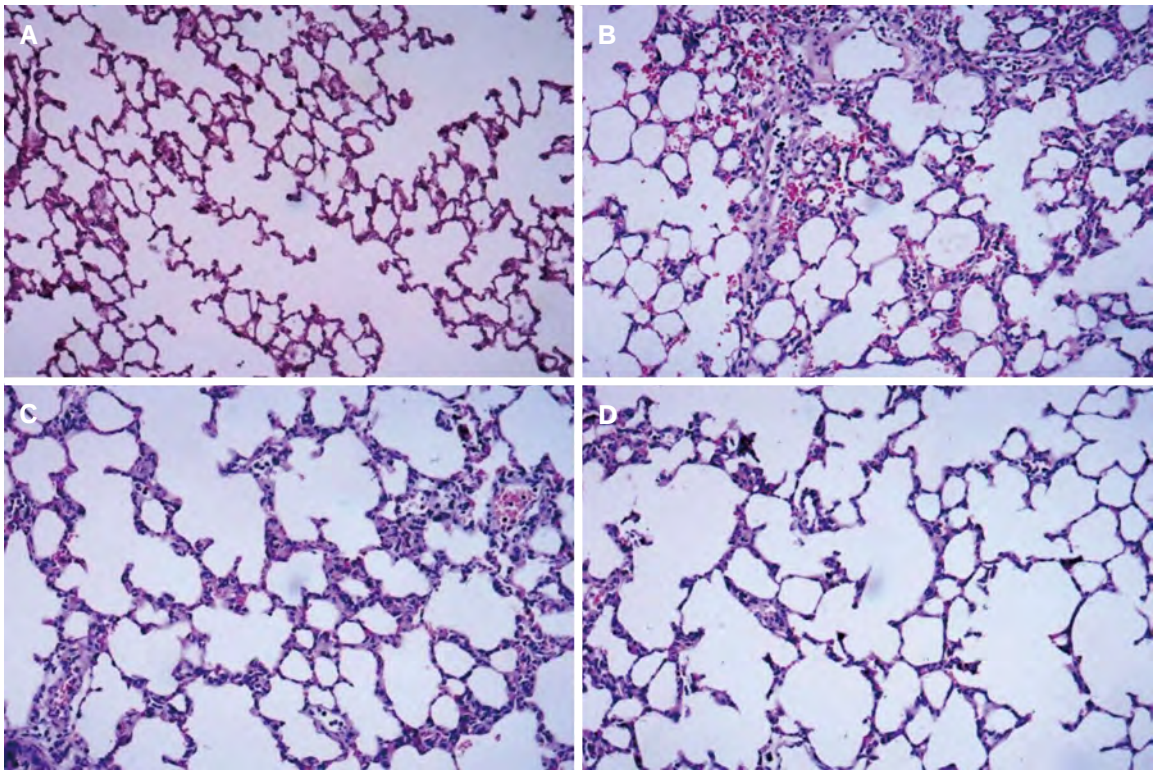


Figure 1 Hematoxylin and eosin staining of dorsal lobe of right lung tissue. A: Control group; B: Septic shock group; C: The early fluid resuscitation-treated group; D: Combined early fluid resuscitation and 2% hydrogen inhalation-treated group. Lung tissues were fixed with 4% paraformaldehyde for more than 24 h. After dehydrating and embedding, they were cut into 5 μm slices, and were stained with hematoxylin and eosin. The pathogenesis of lung injury was observed under a microscope (original magnification × 200). Normal alveolar structure was found in Group A. In Group B, disorders of the alveolar structures, severe neutrophil infiltration in the alveoli and alveolar capillary congestion were observed. In Group C, the extent of neutrophil accumulation and the alveolar-capillary exudate were reduced compared with Group B. However, significant decreases in alveolar damage were found in Group D, and there was significant improvement in alveolar edema compared with Group C.

Table 3 Level of inflammatory mediators in the bronchial alveolar lavage fluid of four groups (mean ± SD)

Group	IL-6 (pg/mL)	IL-8 (pg/mL)	TNF-α (pg/mL)
A	35.88 ± 3.12	24.29 ± 4.23	19.65 ± 1.78
B	446.89 ± 37.24[a]	411.19 ± 32.78[a]	158.54 ± 16.89[a]
C	387.79 ± 31.58[c]	347.34 ± 29.37[c]	104.09 ± 9.18[c]
D	118.32 ± 11.38[e]	96.45 ± 10.25[e]	47.58 ± 3.65[e]

[a]$P < 0.05$ *vs* Group A; [c]$P < 0.05$ *vs* Group B; [e]$P < 0.05$ *vs* Group C. TNF-α: Tumor necrosis factor-α; IL: Interleukin.

a light microscope. The normal alveolar structure was found in Group A, and no hyperemia, neutrophil infiltration, and interstitial edema in the interstitium were observed (Figure 1A). In Group B, disorders of the alveolar structures, the collapse of alveoli, loss of integrity of the alveolar wall, severe neutrophil infiltration in the alveoli, and alveolar capillary congestion were observed, and the alveolar walls were thickened by edema (Figure 1B). In Group C, the extent of neutrophil accumulation and the alveolar-capillary exudate were reduced compared with Group B, but there was no significant improvement in alveolar edema (Figure 1C). Compared with Group B, significant decreases in alveolar damage were found in Group D (Figure 1D), and there was significant improvement in alveolar edema compared with Group C.

Cytokine profiles in lung tissues

Compared with Group A, significant increases in levels of IL-6, IL-8 and TNF-α were found in Group B ($P < 0.05$). The expression of IL-6, IL-8 and TNF-α was significantly decreased in Group C as compared with Group B. In addition, the expression of IL-6, IL-8 and TNF-α was even lower in Group D than in Group C, and the difference was significant (Table 3).

Expression of Fas and Bcl2-immunoreactivity in lung tissues

The positive expression of Fas and Bcl2 protein in lung tissue was demonstrated by the presence of brown-yellow granules, mostly located in the nucleolus cytoplasm (Figure 2). Most bronchial epithelial cells, alveolar epithelial cells and inflammatory cells were Fas-positive and Bcl-positive cells, and Fas and Bcl2 were expressed at low levels in normal lung tissue (Figure 2A and E). The expression of Fas proteins was up-regulated, while the expression of Bcl2 proteins was down-regulated in Group B (Figure 2B and F) compared with Group A. In Group C, however, the expression of Fas proteins was down-regulated, and the expression of Bcl2 proteins was up-regulated (Figure 2C and G) compared with Group B ($P < 0.05$). In addition, the lower expression of Fas proteins and the higher expression of Bcl2 proteins were

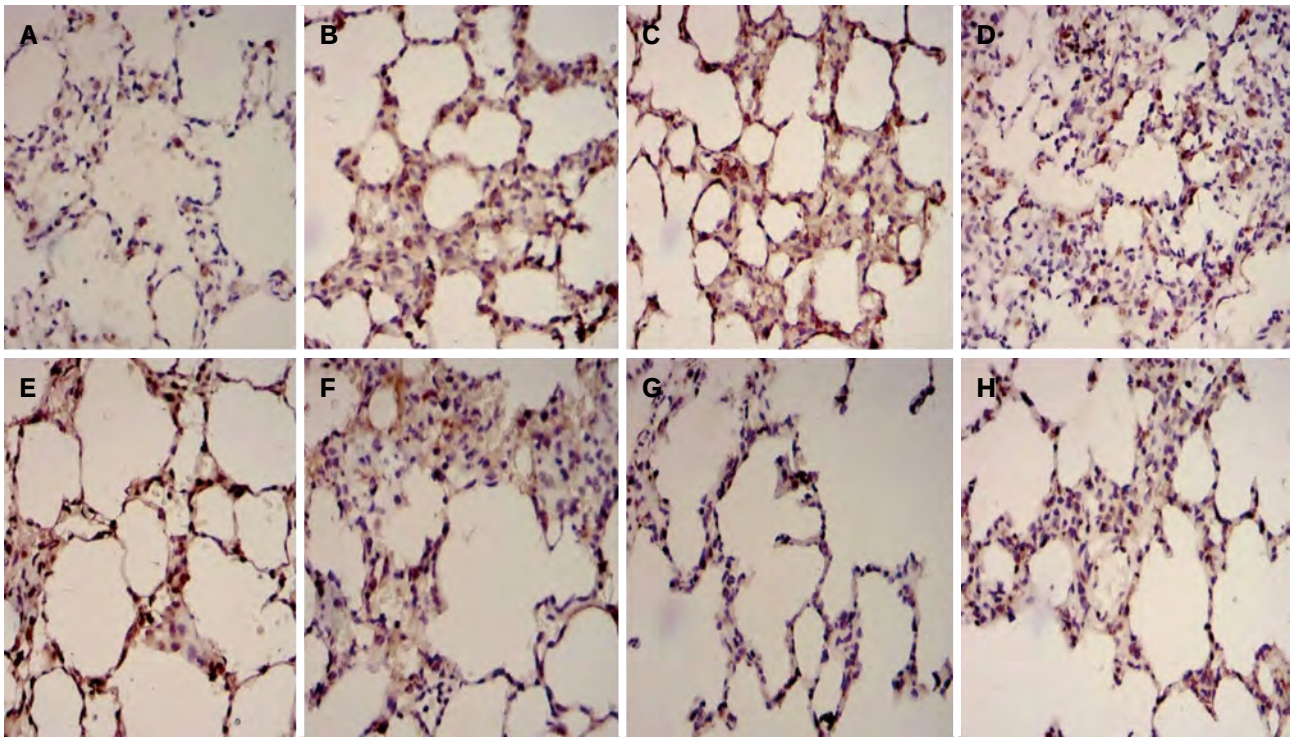


Figure 2 Immunohistochemical examples for Fas and Bcl2 in dorsal lobe of right lung tissue. A: Fas in control group; B: Fas in septic shock group; C: Fas in early fluid resuscitation-treated group; D: Fas in early fluid resuscitation + 2% hydrogen inhalation-treated group; E: Bcl2 in control group; F: Bcl2 in septic shock group; G: Bcl2 in early fluid resuscitation-treated group; H: Bcl2 in early fluid resuscitation + 2% hydrogen inhalation-treated group. Examples were examined under a light microscope equipped with an image analysis system. Yellow or brownish-yellow staining represents positive staining. The expression of Fas proteins was up-regulated, while the expression of Bcl2 proteins was down-regulated in Group B compared with Group A. In Group C, however, the expression of Fas proteins was down-regulated, and the expression of Bcl2 proteins was up-regulated compared with Group B. In addition, the lower expression of Fas proteins and the higher expression of Bcl2 proteins were observed in Group D compared with Group C.

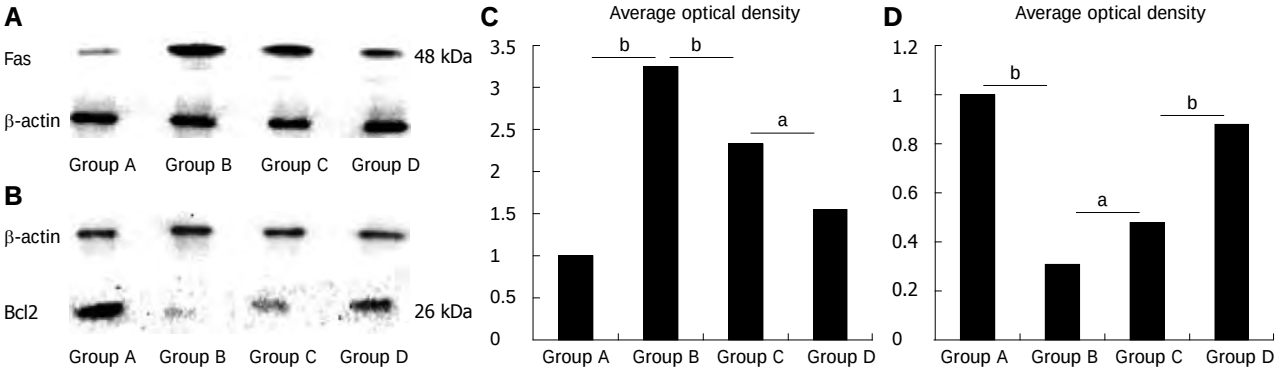


Figure 3 Levels of Fas and Bcl2 proteins in dorsal lobe of right lung tissues quantified by Western blotting. β-actin was used as a loading control. The relative Fas (A) and Bcl2 (B) protein levels of lung tissues from the four groups were expressed as arbitrary units after normalization with β-actin (C: Fas; D: Bcl2). Data were considered significant at $^{a}P < 0.05$ and highly significant at $^{b}P < 0.01$ *vs* the control group. The level of Fas protein was up-regulated, while the level of Bcl2 protein was down-regulated in Group B *vs* Group A. However, in Group C the level of Fas proteins was down-regulated, and the level of Bcl2 proteins was up-regulated *vs* Group B ($P < 0.01$). In addition, the lower level of Fas protein and the higher level of Bcl2 protein was found in Group D compared with Group C ($P < 0.05$).

observed in Group D (Figure 2D and H) compared with Group C ($P < 0.05$; Table 4).

Levels of Fas and Bcl2 protein in lung tissues

The level of Fas protein was up-regulated, while the level of Bcl2 protein was down-regulated in Group B compared with Group A ($P < 0.01$). However, in Group C, level of Fas protein was down-regulated, and the level of Bcl2 protein was up-regulated compared with Group B ($P < 0.01$; Figure 3). In addition, a lower level of Fas protein and a higher level of Bcl2 protein were found in Group D compared with Group C ($P < 0.05$; Figure 3).

Levels of DAO in intestinal tissues

The level of DAO in Group B was significantly increased compared with Group A ($P < 0.05$), and the level of DAO was even higher in Group C ($P < 0.05$). But the level of DAO in Group D was significantly decreased

Table 4 Comparison of average optical density and the positive area ratio of Fas and Bcl2 among the four groups (n = 15, mean ± SD)

Group	Average optical density (10^{-3})		Positive area ratio (%)	
	Fas	Bcl2	Fas	Bcl2
A	0.802 ± 0.221	1.260 ± 0.128	2.737 ± 0.881	11.012 ± 0.792
B	1.549 ± 0.343[a]	0.638 ± 0.072[a]	6.441 ± 0.331[a]	4.121 ± 0.313[a]
C	1.329 ± 0.311[c]	1.103 ± 0.099[c]	4.876 ± 0.887[c]	4.376 ± 0.250[c]
D	1.255 ± 0.400[e]	1.141 ± 0.043[e]	3.247 ± 0.621[e]	10.432 ± 0.791[e]

[a]P < 0.05 *vs* Group A; [c]P < 0.05 *vs* Group B; [e]P < 0.05 *vs* Group C.

Table 5 Levels of serum diamine oxidase and inflammatory mediators in the bronchial alveolar lavage fluid of four groups (mean ± SD)

Group	DAO (kU/L)	IL-6 (pg/mL)	IL-8 (pg/mL)	TNF-α (pg/mL)
A	4.32 ± 0.33	50.38 ± 3.24	35.93 ± 4.37	25.32 ± 2.18
B	6.54 ± 0.68[a]	478.33 ± 30.78[a]	458.19 ± 31.32[a]	167.41 ± 14.47[a]
C	6.89 ± 0.77[c]	394.21 ± 28.15[c]	395.34 ± 28.46[c]	121.05 ± 8.01[c]
D	5.14 ± 0.44[c,e]	145.26 ± 12.33[e]	123.12 ± 10.23[e]	36.27 ± 3.54[e]

[a]P < 0.05 *vs* Group A; [c]P < 0.05 *vs* Group B; [e]P < 0.05 *vs* Group C. TNF-α: Tumor necrosis factor-α; IL: Interleukin; DAO: Diamine oxidase.

Table 6 Chiu's score and levels of malonaldehyd and myeloperoxidase of small intestine (mean ± SD)

Group	Chiu's score	MDA (nmol/mg protein)	MPO (U/g wet tissue)
A	0.37 ± 0.33	2.09 ± 0.33	1.55 ± 0.12
B	3.30 ± 0.89[a]	6.19 ± 1.38[a]	6.54 ± 0.99[a]
C	3.79 ± 1.12[c]	6.89 ± 1.26[c]	7.05 ± 1.01[c]
D	2.12 ± 0.67[c,e]	2.98 ± 0.45[c,e]	2.32 ± 0.34[c,e]

[a]P < 0.05 *vs* Group A; [c]P < 0.05 *vs* Group B; [e]P < 0.05 *vs* Group C. MDA: Malonaldehyd; MPO: Myeloperoxidase.

compared with both Group B and Group C (P < 0.05; Table 5).

HE staining of small intestinal tissues

In Group A, the structure of the small intestinal mucosa was intact, and normal intestinal mucosa was observed (Figure 4A). In Group B, glands of the small intestine were significantly damaged, and severe edema of mucosal villi, neutrophil infiltration, epithelial cell sloughing off, and structural changes in the epithelium of the small intestine and even small bowel ulceration were observed (Figure 4B). The damage mentioned above was far more severe in Group C (Figure 4C). But in Group D, the damage was significantly reduced (Figure 4D). The Chiu's score of Group B was significantly higher than Group A (P < 0.05), and the Chiu's score of Group C was even higher compared with Group B. But the Chiu's score of Group D was significantly decreased compared with Group B and Group C (Table 6).

Transmission electron microscopic analysis of small intestinal samples

In Group A, normal microvilli on the surface of epithelial cells of the intestine were observed. The mitochondria, lysosome and rough endoplasmic reticulum maintained normal morphology (Figure 5A). In Group B, disarrangement of the epithelial surface and intestinal microvillus reduction were observed. Mitochondria present in the cytoplasm also showed vacuolization, and the cristae of mitochondria were reduced. Heterochromatin nuclei showed margination phenomena, membrane rupture, and widened nuclear gap (Figure 5B). The microvilli on the surface of epithelial cells of the intestine were sparse in Group C; there was obvious reduction of the cristae of mitochondria, severe vacuolization of mitochondria and an abundance of marginated heterochromatin nuclei. The amount of rough endoplasmic reticulum was reduced, and more severe rough endoplasmic reticulum swelling and expansion were observed (Figure 5C). In Group D, the microvilli were missing to a small extent, and the heterochromatin nuclei showed only mild margination (Figure 5D).

Levels of MDA and SOD in small intestinal tissues

The levels of MDA and SOD in Group B were significantly increased compared with Group A (P < 0.05). Levels of MDA and SOD were higher in Group C than in Group B. In Group D, MDA and SOD were significantly decreased compared with both Group B and Group C (Table 6).

Cytokine profiles in small intestinal tissues

Levels of IL-6, IL-8 and TNF-α were significantly increased in Group B compared with Group A (P < 0.05). The expression of IL-6, IL-8 and TNF-α was significantly lower in Group C than in group B. In addition, the expression of IL-6, IL-8 and TNF-α in Group D was even lower compared with Group C, and the difference was significant (P < 0.05; Table 5).

DISCUSSION

In the present study, we investigated the effects of combined early fluid resuscitation and 2% hydrogen on the lung and small intestine in a LPS-induced septic shock rat model. Our study showed that early fluid resuscitation and inhalation of 2% hydrogen could decrease the oxidative damage and inhibit the over-expression of inflammatory factors (IL-6, IL-8 and TNF-α) in the lung tissue of the septic shock rats. This treatment could also down-regulate the expression of proapoptotic protein Fas, and up-regulate the expression of anti-apoptotic protein Bcl2 in the lung tissue. These data suggest that the application of combined early fluid resuscitation and inhalation of 2% hydrogen may protect the lung from septic shock damage by inhibiting the inflammatory reaction and apoptosis in comparison with traditional fluid resuscitation alone. Early fluid resuscitation and inhalation of 2% hydrogen not only decreased the oxidative damage and

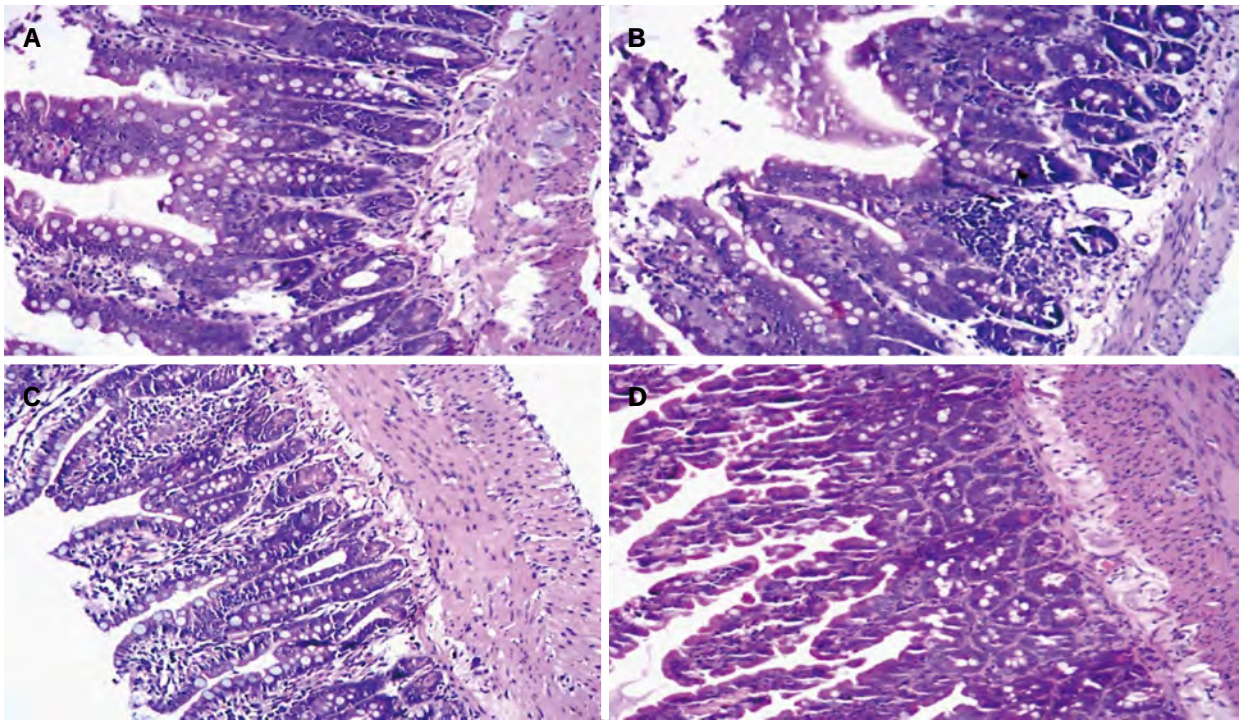


Figure 4 Hematoxylin and eosin staining of small intestine tissue. A: Control group; B: Septic shock group; C: Early fluid resuscitation-treated group; D: Early fluid resuscitation + 2% hydrogen inhalation-treated group. Small intestinal tissues were fixed with 4% paraformaldehyde for more than 24 h. After dehydrating and embedding, they were cut into 5 μm slices, and were stained with hematoxylin and eosin. The pathogenesis of small intestine injury was observed under a light microscope (original magnification × 200). In Group A, normal intestinal mucosa was observed. In Group B, glands of small intestine were significantly damaged, and severe edema of mucosal villi, neutrophil infiltration, epithelial cell sloughing off, and even small bowel ulceration were observed. The damage mentioned above was far more severe in Group C. But in Group D, the damage was significantly reduced.

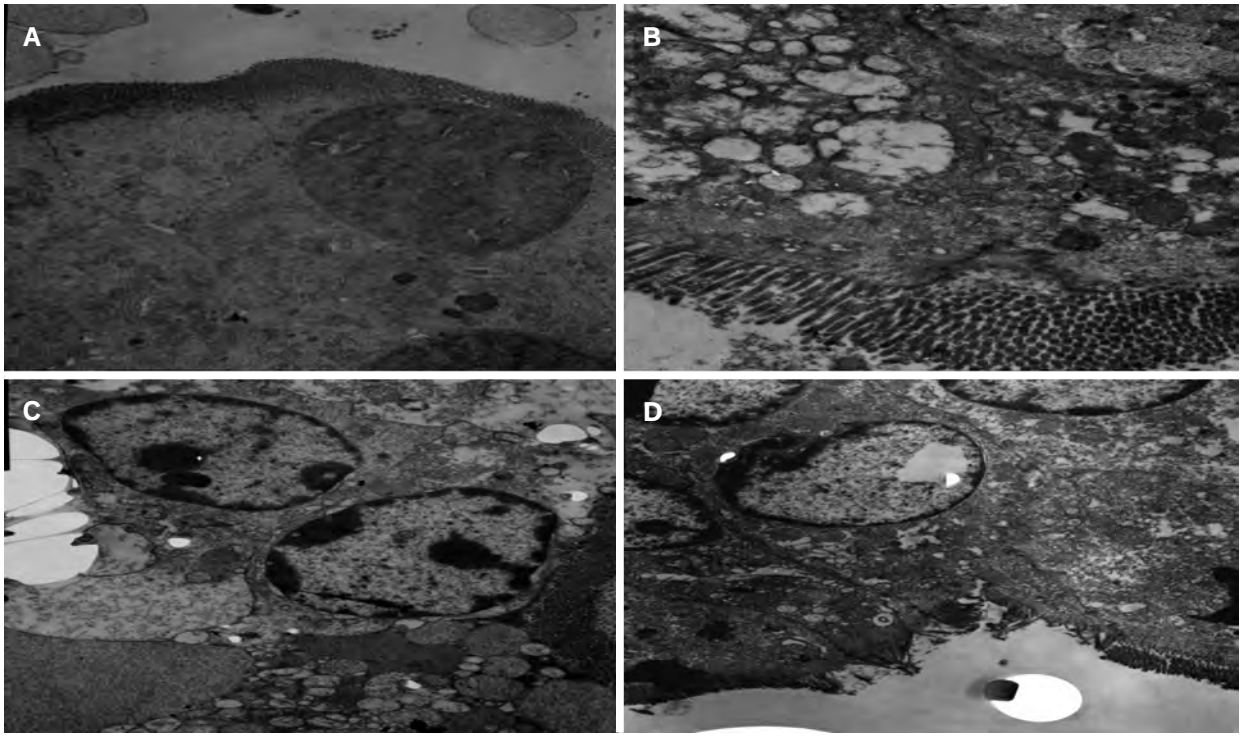


Figure 5 Changes in the organelles of small intestinal tissues under transmission electron microscope. A: Control group; B: Septic shock group; C: Early fluid resuscitation-treated group; D: Early fluid resuscitation + 2% hydrogen inhalation-treated group. The changes in the organelles were observed under transmission electron microscope. In Group A, normal microvilli on the surface of epithelial cells of the intestine were observed. In Group B, intestinal microvillus reduction was observed. Mitochondria showed vacuolization and heterochromatin nuclei showed margination phenomena. The microvilli on the surface of epithelial cells of the intestine were sparse in Group C, and reduction of the cristae of mitochondria, an abundance of marginated heterochromatin nuclei, and severe rough endoplasmic reticulum swelling and expansion were observed. In Group D, the microvilli were missing to a small extent, and the heterochromatin nuclei showed only mild margination.

the levels of IL-6, IL-8 and TNF-α in plasma, but also protected the intestinal mucosa from mechanical injury.

Effects of combined early fluid resuscitation and hydrogen inhalation on lung injury in a LPS-induced septic shock rat model

Previous studies have indicated that MPO is a peroxidase enzyme released by activated polymorphonuclear neutrophils, which can be used as an indicator of the level of tissue neutrophils. MDA is one of the toxic metabolites of ROS induced lipid peroxidation, and SOD is one of the most important free radical scavenging enzymes. Levels of MDA and SOD can reflect the extent of oxidative stress. In the present study, the levels of MDA, SOD and MPO were used as an indicator for oxidative stress[13-15]. The results showed that the levels of MDA and MPO were significantly increased, however, the level of SOD was significantly decreased in the septic shock rats, and severe neutrophil infiltrates were observed in the alveoli. These data suggest that changes in the lung oxidant-antioxidant status may be associated with lung injury in septic shock rats. There was no obvious improvement in oxidative stress in the traditional fluid resuscitation-treated group compared with the septic shock group, but there was significant improvement in the indicators above in the early fluid resuscitation and inhalation of 2% hydrogen treatment group. Early fluid resuscitation and inhalation of 2% hydrogen attenuated septic shock-induced organ injury, and decreased neutrophil infiltrate in the alveoli. Significant improvements in alveolar edema and reduced alveolar damage were also observed. Previous studies have indicated that hydrogen, as a selective antioxidant with a therapeutic antioxidant activity, can easily pass through the cell membrane and reach the organelles[16,17]. Hydrogen can effectively protect against organ damage induced by selectively clearing •OH. These findings indicate that 2% hydrogen may selectively reduce •OH and provide a beneficial effect on septic shock.

Amplification of inflammatory responses and cell apoptosis also play an important role in the pathogenesis of sepsis-induced acute lung injury (ALI)[18,19]. In the present study, the levels of IL-6, IL-8 and TNF-α in BALF were significantly increased in the LPS-induced ALI rats compared with the control group. Our data showed that the early fluid resuscitation and inhalation of 2% hydrogen treatment reduced the levels of IL-6, IL-8 and TNF-α in BALF in comparison with the traditional fluid resuscitation-treated group, and these results are consistent with the previous study[1]. In the process of sepsis-induced ALI, the expression of Fas protein in the lung epithelial cell was up-regulated, and blocking FAS-mediated apoptosis could reduce the LSP-induced lung injury[20]. As one of the most important anti-apoptotic factors, Bcl-2 plays an important role in regulating the progress of apoptosis, and it is considered as a cytoprotective factor[21]. In the present study, decreases in lung cell apoptosis were observed in the early fluid resuscitation-treated group, and further decreases in lung cell apoptosis were observed in the early fluid resuscitation and inhalation of 2% hydrogen treated group. These results demonstrated that co-treatment could down-regulate the levels of Fas protein and up-regulate the levels of Bcl2 protein, which may inhibit ALI by inducing apoptosis, and may protect lung function.

Effects of combined early fluid resuscitation and inhalation of 2% hydrogen on small intestine in a LPS-induced septic shock rat model

Clinical observations and animal experiments have shown that the gastrointestinal tract is one of the most important target organs during septic shock. In early shock, blood flow in the intestinal wall is significantly decreased because of the redistribution of blood, and intestinal mucosal barrier dysfunction is induced by hypoxia and ischemia. Previous studies show that intestinal mucosal edema and bacterial translocation are closely related to the occurrence of MODS[22]. In the present study, bowel mucosal structural changes were observed in the septic shock rats, including significant damage of the small intestine, severe edema of mucosal villi, sloughing off of epithelial cells, structural changes in the epithelium of the small intestine and even small bowel ulceration. Serum DAO activity, which was used as a marker of intestinal integrity, was significantly increased[23]. All these indicators suggest that the intestinal mucosal mechanical barrier is severely damaged during septic shock. Early fluid resuscitation treatment alone was not effective in reducing pathological changes of the small intestine; on the contrary, pathological changes were more severe, including more severe bowel wall edema, higher Chiu's score, and higher level of serum DAO activity in the early fluid resuscitation-treated group compared with the septic shock group. But 2% hydrogen gas treatment significantly reduced the pathological changes of the small intestine, indicating that early fluid resuscitation and inhalation of 2% hydrogen could protect the intestinal mucosal mechanical barrier, inhibit bacterial translocation, and then effectively prevent enterogenous sepsis, and protect the function of other organs in the body[24,25].

Increased endothelial cell ischemia and hypoxia were observed during septic shock. The endothelial cells could release large amounts of cytokine and chemokine, and induce large amounts of neutrophil aggregation. During the removal of harmful pathogens, large amounts of oxygen free radical, which increase the intestinal damage, were released[26]. After fluid resuscitation treatment, large amounts of oxygen free radical were generated in the course of ischemia and reperfusion injury. Thus, oxygen free radicals were considered as one of the major causes of intestinal damage during septic shock. Our data indicate that oxygen free radicals were involved in the severe intestinal mucosal damage, and pathological changes were more severe after fluid resuscitation treatment. Early fluid resuscitation and inhalation of 2% hydrogen could significantly decrease the level of MDA and MPO,

and mitigate the oxidative damage, indicating that 2% hydrogen may selectively reduce •OH, reducing ischemia/reperfusion-induced organ damage.

Intestinal mucosal injury can lead to the release of inflammatory mediators which play an important role in the process of shock-induced intestinal tissue injury[27]. The inflammatory mediators were one of the initiation factors of MODS, and could worsen organ dysfunction[28]. During the shock period, the intestinal tract is the main organ to produce TNF-α[29]. Release of TNF-α increases chemotaxis, aggregation and adhesion to endothelial cells of neutrophils. Then more inflammatory mediators such as IL-6 and IL-8 are also released, leading to aggravated intestinal mucosal injury[30]. In the present study, the levels of TNF-α, IL-6 and IL-8 in serum were significantly increased in septic shock rats, but the levels of TNF-α, IL-6 and IL-8 in serum were decreased after early fluid resuscitation treatment, while early fluid resuscitation and inhalation of 2% hydrogen gas further decreased the levels of TNF-α, IL-6 and IL-8 in serum compared with early fluid resuscitation only. These data demonstrated that the association of early fluid resuscitation and inhalation of 2% hydrogen gas may protect small intestine function by reducing inflammatory mediators.

In summary, combined early fluid resuscitation and inhalation of 2% hydrogen is beneficial for septic shock and septic shock-associated lung or small intestine injury, which is associated with a decrease of oxidative stress and inhibition of the inflammatory response. We conclude that combined early fluid resuscitation and inhalation of 2% hydrogen may be an effective therapeutic strategy for ALI and MODS in patients with septic shock.

COMMENTS

Background

Sepsis shock is a common complication of infection, severe trauma, and major operation in patients. However, the effective therapy method needs to be developed.

Research frontiers

Early fluid resuscitation has been demonstrated to have some protective effects on the sepsis shock -induced acute lung injury, but the effective is not significant. Recent studies indicate that hydrogen inhalation can significantly decrease the oxidative stress in septic animal model. The objective of this study is to explore the effects of combined early fluid resuscitation and hydrogen inhalation on septic shock induced lung and intestine injuries.

Innovations and breakthroughs

This is the first study to report that combined early fluid resuscitation and hydrogen inhalation exerts a potency to effectively attenuate lung and intestine injuries.

Applications

The paper provides the experiment basis for the clinical application of early fluid resuscitation and hydrogen inhalation to prevent sepsis induced lung and intestine injury.

Terminology

Sepsis shock is a common complication of infection, severe trauma, and major operation in patients. Sepsis shock leads to severe, hypoxia, and reperfusion injury of lung and intestine ischemia. The inflammatory factors in these tissues will be activated and may consequently develop the multiple organ dysfunction syndrome, which is associated with high morbidity and mortality rates. Early fluid resuscitation and hydrogen have the potential to clear these inflammatory factors.

Peer review

This study shows that combined early fluid resuscitation and hydrogen inhalation can significantly decrease septic shock induced lung and intestine injuries compared with early fluid resuscitation alone. The study is innovative and with potential therapeutic interest.

REFERENCES

1 **Xie K**, Yu Y, Pei Y, Hou L, Chen S, Xiong L, Wang G. Protective effects of hydrogen gas on murine polymicrobial sepsis via reducing oxidative stress and HMGB1 release. *Shock* 2010; **34**: 90-97 [PMID: 19997046 DOI: 10.1097/SHK.0b013e3181cdc4ae]

2 **Biswal S**, Remick DG. Sepsis: redox mechanisms and therapeutic opportunities. *Antioxid Redox Signal* 2007; **9**: 1959-1961 [PMID: 17711390 DOI: 10.1089/ars.2007.1808]

3 **Velasco N**. [Gut barrier in the critically ill patient: facts and trends]. *Rev Med Chil* 2006; **134**: 1033-1039 [PMID: 17130993 DOI: 10.4067/S0034-98872006000800014]

4 **Brandt S**, Regueira T, Bracht H, Porta F, Djafarzadeh S, Takala J, Gorrasi J, Borotto E, Krejci V, Hiltebrand LB, Bruegger LE, Beldi G, Wilkens L, Lepper PM, Kessler U, Jakob SM. Effect of fluid resuscitation on mortality and organ function in experimental sepsis models. *Crit Care* 2009; **13**: R186 [PMID: 19930656 DOI: 10.1186/cc8179]

5 **Ohsawa I**, Ishikawa M, Takahashi K, Watanabe M, Nishimaki K, Yamagata K, Katsura K, Katayama Y, Asoh S, Ohta S. Hydrogen acts as a therapeutic antioxidant by selectively reducing cytotoxic oxygen radicals. *Nat Med* 2007; **13**: 688-694 [PMID: 17486089 DOI: 10.1038/nm1577]

6 **Li J**, Wang C, Zhang JH, Cai JM, Cao YP, Sun XJ. Hydrogen-rich saline improves memory function in a rat model of amyloid-beta-induced Alzheimer's disease by reduction of oxidative stress. *Brain Res* 2010; **1328**: 152-161 [PMID: 20171955 DOI: 10.1016/j.brainres.2010.02.046]

7 **Ohta S**. Molecular hydrogen is a novel antioxidant to efficiently reduce oxidative stress with potential for the improvement of mitochondrial diseases. *Biochim Biophys Acta* 2012; **1820**: 586-594 [PMID: 21621588 DOI: 10.1016/j.bbagen.2011.05.0066]

8 **Xie K**, Yu Y, Zhang Z, Liu W, Pei Y, Xiong L, Hou L, Wang G. Hydrogen gas improves survival rate and organ damage in zymosan-induced generalized inflammation model. *Shock* 2010; **34**: 495-501 [PMID: 20351628]

9 **Xie K**, Yu Y, Huang Y, Zheng L, Li J, Chen H, Han H, Hou L, Gong G, Wang G. Molecular hydrogen ameliorates lipopolysaccharide-induced acute lung injury in mice through reducing inflammation and apoptosis. *Shock* 2012; **37**: 548-555 [PMID: 22508291 DOI: 10.1097/SHK.0b013e31824ddc81]

10 **Sennoun N**, Montemont C, Gibot S, Lacolley P, Levy B. Comparative effects of early versus delayed use of norepinephrine in resuscitated endotoxic shock. *Crit Care Med* 2007; **35**: 1736-1740 [PMID: 17522580 DOI: 10.1097/01.CCM.0000269028.28521.08]

11 **Chiu CJ**, McArdle AH, Brown R, Scott HJ, Gurd FN. Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic, and metabolic reappraisal. *Arch Surg* 1970; **101**: 478-483 [PMID: 5457245 DOI: 10.1001/archsurg.1970.01340280030009]

12 **Towbin H**, Staehelin T, Gordon J. Electrophoretic transfer of proteins from polyacrylamide gels to nitrocellulose sheets: procedure and some applications. *Proc Natl Acad Sci USA* 1979; **76**: 4350-4354 [PMID: 388439]

13 **An KW**, Shin HS, Choi CY. Physiological responses and expression of metallothionein (MT) and superoxide dismutase (SOD) mRNAs in olive flounder, Paralichthys olivaceus exposed to benzo[a]pyrene. *Comp Biochem Physiol B Biochem Mol Biol* 2008; **149**: 534-539 [PMID: 18203642 DOI: 10.1016/j.cbpb.2007.12.004]

14 **McFarland VA**, Inouye LS, Lutz CH, Jarvis AS, Clarke JU, McCant DD. Biomarkers of oxidative stress and genotoxicity in livers of field-collected brown bullhead, Ameiurus nebulosus. *Arch Environ Contam Toxicol* 1999; **37**: 236-241 [PMID: 10398774 DOI: 10.1007/s002449900510]

15 **Oldham KM**, Bowen PE. Oxidative stress in critical care: is antioxidant supplementation beneficial? *J Am Diet Assoc* 1998; **98**: 1001-1008 [PMID: 9739800 DOI: 10.1016/S0002-8223(98)00230-2]

16 **Fukuda K**, Asoh S, Ishikawa M, Yamamoto Y, Ohsawa I, Ohta S. Inhalation of hydrogen gas suppresses hepatic injury caused by ischemia/reperfusion through reducing oxidative stress. *Biochem Biophys Res Commun* 2007; **361**: 670-674 [PMID: 17673169 DOI: 10.1016/j.bbrc.2007.07.088]

17 **Nakao A**, Toyoda Y, Sharma P, Evans M, Guthrie N. Effectiveness of hydrogen rich water on antioxidant status of subjects with potential metabolic syndrome-an open label pilot study. *J Clin Biochem Nutr* 2010; **46**: 140-149 [PMID: 20216947 DOI: 10.3164/jcbn.09-100]

18 **Chen H**, Zhang L, Jin Z, Jin E, Fujiwara M, Ghazizadeh M, Asoh S, Ohta S, Kawanami O. Anti-apoptotic PTD-FNK protein suppresses lipopolysaccharide-induced acute lung injury in rats. *Exp Mol Pathol* 2007; **83**: 377-384 [PMID: 17959170 DOI: 10.1016/j.yexmp.2007.08.016]

19 **Hotchkiss RS**, Karl IE. The pathophysiology and treatment of sepsis. *N Engl J Med* 2003; **348**: 138-150 [PMID: 12519925 DOI: 10.1056/NEJMra021333]

20 **Matute-Bello G**, Lee JS, Liles WC, Frevert CW, Mongovin S, Wong V, Ballman K, Sutlief S, Martin TR. Fas-mediated acute lung injury requires Fas expression on nonmyeloid cells of the lung. *J Immunol* 2005; **175**: 4069-4075 [PMID: 16148156]

21 **O'Reilly MA**, Staversky RJ, Huyck HL, Watkins RH, LoMonaco MB, D'Angio CT, Baggs RB, Maniscalco WM, Pryhuber GS. Bcl-2 family gene expression during severe hyperoxia induced lung injury. *Lab Invest* 2000; **80**: 1845-1854 [PMID: 11140697 DOI: 10.1038/labinvest.3780195]

22 **Grocott MP**, Mythen MG, Gan TJ. Perioperative fluid management and clinical outcomes in adults. *Anesth Analg* 2005; **100**: 1093-1106 [PMID: 15781528 DOI: 10.1213/01.ANE.0000148691.33690.AC]

23 **Luk GD**, Bayless TM, Baylin SB. Plasma postheparin diamine oxidase. Sensitive provocative test for quantitating length of acute intestinal mucosal injury in the rat. *J Clin Invest* 1983; **71**: 1308-1315 [PMID: 6406546 DOI: 10.1172/JCI110881]

24 **Abu-Rustum RS**, Abu-Rustum SE, Abdo BK, Jamal MH. Inner myometrial laceration causing a massive postpartum hemorrhage: a case report. *J Reprod Med* 2006; **51**: 135-137 [PMID: 16572915]

25 **Magann EF**, Evans S, Chauhan SP, Lanneau G, Fisk AD, Morrison JC. The length of the third stage of labor and the risk of postpartum hemorrhage. *Obstet Gynecol* 2005; **105**: 290-293 [PMID: 15684154 DOI: 10.1097/01.AOG.0000151993.83276.70]

26 **Simpson R**, Alon R, Kobzik L, Valeri CR, Shepro D, Hechtman HB. Neutrophil and nonneutrophil-mediated injury in intestinal ischemia-reperfusion. *Ann Surg* 1993; **218**: 444-453; discussion 453-454 [PMID: 8215636 DOI: 10.1097/00000658-199310000-00005]

27 **Tamion F**, Richard V, Lacoume Y, Thuillez C. Intestinal preconditioning prevents systemic inflammatory response in hemorrhagic shock. Role of HO-1. *Am J Physiol Gastrointest Liver Physiol* 2002; **283**: G408-G414 [PMID: 12121889 DOI: 10.1152/ajpgi.00348.2001]

28 **Sobhian B**, Jafarmadar M, Redl H, Bahrami S. Hemorrhage- and resuscitation-related alterations in gastrointestinal circulation: effect of a low dose of L-NMMA. *Shock* 2005; **23**: 243-247 [PMID: 15718922 DOI: 10.1097/01.shk.0000150775.93741.6f]

29 **Tani T**, Fujino M, Hanasawa K, Shimizu T, Endo Y, Kodama M. Bacterial translocation and tumor necrosis factor-alpha gene expression in experimental hemorrhagic shock. *Crit Care Med* 2000; **28**: 3705-3709 [PMID: 11098977]

30 **Weinbroum AA**, Hochhauser E, Rudick V, Kluger Y, Karchevsky E, Graf E, Vidne BA. Multiple organ dysfunction after remote circulatory arrest: common pathway of radical oxygen species? *J Trauma* 1999; **47**: 691-698 [PMID: 10528603 DOI: 10.1097/00005373-199910000-00]

P- Reviewer Schmidt R **S- Editor** Gou SX
L- Editor A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i4.503

World J Gastroenterol 2013 January 28; 19(4): 503-510
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Development and validation of a registry-based definition of eosinophilic esophagitis in Denmark

Evan S Dellon, Rune Erichsen, Lars Pedersen, Nicholas J Shaheen, John A Baron, Henrik T Sørensen, Mogens Vyberg

Evan S Dellon, Nicholas J Shaheen, Center for Esophageal Diseases and Swallowing, and Center for Gastrointestinal Biology and Disease, Division of Gastroenterology and Hepatology, Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, NC 27599-7080, United States
Evan S Dellon, Nicholas J Shaheen, John A Baron, Center for Gastrointestinal Biology and Disease, Division of Gastroenterology and Hepatology, Department of Medicine, University of North Carolina School of Medicine, Chapel Hill, NC 27599-7080, United States
Rune Erichsen, Lars Pedersen, Henrik T Sørensen, Department of Clinical Epidemiology, Aarhus University Hospital, 8000 Aarhus, Danmark
Mogens Vyberg, Institute of Pathology, Aalborg Hospital, Aarhus University Hospital, 8000 Aarhus, Danmark
Author contributions: All the authors designed the study, interpreted the data, and approved the final manuscript draft; Vyberg M performed histologic analysis; Dellon ES, Erichsen R and Pedersen L performed statistical analysis; Dellon ES drafted the paper with critical review from Erichsen R, Pedersen L, Shaheen NJ, Baron JA, Sørensen HT and Vyberg M.
Supported by Pilot/feasibility Grant from the UNC Center for Gastrointestinal Biology and Disease, NIH P30 DK34987; and NIH award K23DK090073 (in part)
Correspondence to: Evan S Dellon, MD, MPH, Center for Esophageal Diseases and Swallowing, Division of Gastroenterology and Hepatology, Department of Medicine, University of North Carolina School of Medicine, CB#7080, Bioinformatics Building, 130 Mason Farm Road, UNC-CH, Chapel Hill, NC 27599-7080, United States. edellon@med.unc.edu
Telephone: +1-919-9662513 Fax: +1-919-8432508
Received: September 12, 2012 Revised: November 15, 2012
Accepted: November 24, 2012
Published online: January 28, 2013

Abstract

AIM: To develop and validate a case definition of eosinophilic esophagitis (EoE) in the linked Danish health registries.

METHODS: For case definition development, we queried the Danish medical registries from 2006-2007 to identify candidate cases of EoE in Northern Denmark. All International Classification of Diseases-10 (ICD-10) and prescription codes were obtained, and archived pathology slides were obtained and re-reviewed to determine case status. We used an iterative process to select inclusion/exclusion codes, refine the case definition, and optimize sensitivity and specificity. We then re-queried the registries from 2008-2009 to yield a validation set. The case definition algorithm was applied, and sensitivity and specificity were calculated.

RESULTS: Of the 51 and 49 candidate cases identified in both the development and validation sets, 21 and 24 had EoE, respectively. Characteristics of EoE cases in the development set [mean age 35 years; 76% male; 86% dysphagia; 103 eosinophils per high-power field (eos/hpf)] were similar to those in the validation set (mean age 42 years; 83% male; 67% dysphagia; 77 eos/hpf). Re-review of archived slides confirmed that the pathology coding for esophageal eosinophilia was correct in greater than 90% of cases. Two registry-based case algorithms based on pathology, ICD-10, and pharmacy codes were successfully generated in the development set, one that was sensitive (90%) and one that was specific (97%). When these algorithms were applied to the validation set, they remained sensitive (88%) and specific (96%).

CONCLUSION: Two registry-based definitions, one highly sensitive and one highly specific, were developed and validated for the linked Danish national health databases, making future population-based studies feasible.

© 2013 Baishideng. All rights reserved.

Key words: Eosinophilic esophagitis; Denmark; Epidemiology; Case definition; Sensitivity; Specificity

Dellon ES, Erichsen R, Pedersen L, Shaheen NJ, Baron JA, Sørensen HT, Vyberg M. Development and validation of a registry-based definition of eosinophilic esophagitis in Denmark. *World J Gastroenterol* 2013; 19(4): 503-510 Available from:

URL: http://www.wjgnet.com/1007-9327/full/v19/i4/503.htm
DOI: http://dx.doi.org/10.3748/wjg.v19.i4.503

INTRODUCTION

Eosinophilic esophagitis (EoE) is a chronic immune-mediated disorder characterized by symptoms of esophageal dysfunction and infiltration of the esophageal mucosa by eosinophils[1]. While the first case was described in 1978[2], a set of case series in the early 1990s highlighted what are now viewed as the classic clinicopathologic features of EoE[3-5]. Since that time, there has been a remarkable increase in the incidence and prevalence of EoE[6-14], EoE has been detected in up to 16% of patients undergoing upper endoscopy for dysphagia[15-17], and EoE is now the leading cause of food impaction in patients presenting to emergency departments[18-20]. Most data regarding the evolving epidemiology of EoE originate from tertiary centers[7-13], and there have been few population-based studies of EoE across centers or at a national level.

The national population-based medical registries of Denmark, which link medical records, diagnostic codes, pathology findings, and prescription medications *via* an individual identifier assigned to every person in the country[21-24], offer a unique opportunity to systematically study the epidemiology of EoE. However, no validated registry-based case definitions of EoE exist, and this limitation hampers any attempt to accurately study EoE in Denmark and other countries where national health databases are maintained.

The aims of this study were to characterize candidate and definite EoE cases in Northern Denmark detected through a search of the health registries, and to develop and validate a registry-based case definition of EoE in the linked Danish clinical, pathology, and pharmacy databases that could be used for future epidemiologic study.

MATERIALS AND METHODS

Denmark health registries

This retrospective study of the Danish medical registries was approved by both the University of North Carolina IRB and the Danish Data Protection Agency (record number 2010-41-4986). Denmark, with its stable population of approximately 5.5 million people, is well-suited for epidemiological studies[21-24]. The establishment of the Civil Registration System in 1968 allows information about the same person to be linked across independent registries by using the Civilian Registration Number, a unique identifier assigned to every person in the country[23].

Three of the comprehensive national medical registries in Denmark were utilized to generate the necessary data for this study. These included: the Danish National Registry of Patients, which houses International Classification of Diseases-10 (ICD-10) codes dating from 1994, hospital admission and discharge codes, surgical procedure codes, and outpatient visit codes[25]; the National Pathology Registry, which contains Systematized Nomenclature of Medicine (SNOMED) codes for pathologic specimens dating from 1997[24]; and the Aarhus University Prescription Database, which has outpatient prescription data for Northern Denmark using Anatomical Therapeutic Chemical (ATC) classification system codes[26].

For the purposes of this study we limited our analysis to Northern Denmark. This geographic area contains 1.8 million inhabitants, approximately 1/3 of the Danish population, and allowed ready access to the required pathology specimens for case verification, as described below.

Case definition development

This study utilized two independent sets of patients, one set in which to develop the case definition and one set in which to validate it.

For case definition development, we queried the National Pathology Registry from 2006-2007 in Northern Denmark to identify candidate cases of EoE. These were patients with esophageal eosinophilia as defined by the combination of SNOMED codes for esophageal biopsies (T62xxx) and tissue eosinophilia (M47150). These codes were assigned by the clinical pathologist at the time of specimen examination and interpretation. Next, all ICD-10 diagnostic and ATC prescription codes for these patients were obtained from the National Registry of Patients and the Aarhus University Prescription Database, respectively. Finally, the original archived pathology slides were obtained and, using a previously validated protocol[27], were re-reviewed by the study pathologist (Vyberg M) to determine the maximum eosinophil count [eosinophils per high-power field (eos/hpf); hpf = 0.24 mm^2].

From this pool of subjects with esophageal eosinophilia, we identified the subset of patients with a confirmed diagnosis of EoE as per consensus diagnostic guidelines[1,28]. Specifically, these patients had symptoms of esophageal dysfunction, a maximum eosinophil count $\geqslant$ 15 eos/hpf on pathology re-review, and no other competing causes of esophageal eosinophilia identified. While the current consensus guidelines allow reflux and EoE to overlap[1], we attempted to minimize this overlap by ensuring that there was not a mixed inflammatory infiltrate on the re-reviewed esophageal biopsies and by assessing use of anti-acid mediations in the months preceding the diagnostic endoscopy (see below). These subjects with confirmed EoE comprised the reference standard for the case definition development set.

We used an iterative process to develop a case definition of EoE based on previously described methodology for gastrointestinal disorders used in the Danish and other administrative databases[29-31]. First, we empirically selected a combination of SNOMED, ICD-10 and ATC codes that were potentially pertinent to the diagnosis of EoE and could exclude disorders on the differential diagnosis for esophageal eosinophilia as well as patients with non-EoE conditions with similar symptom profiles. Next, the National Pathology Registry, National Registry of Pa-

Table 1 Characteristics of the case definition development set (2006-2007) and validation set (2008-2009) *n* (%)

	Development set (2006-2007)				Validation set (2007-2008)			
	Overall (*n* = 51)	EoE cases (*n* = 21)	Sensitive definition (*n* = 31)	Specific definition (*n* = 9)	Overall (*n* = 49)	EoE cases (*n* = 24)	Sensitive definition (*n* = 26)	Specific definition (*n* = 6)
Age, yr (mean ± SD)	42 ± 19	35 ± 16	40 ± 18	38 ± 19	48 ± 21	42 ± 15	49 ± 19	49 ± 19
Male	38 (75)	16 (76)	22 (71)	7 (78)	36 (73)	20 (83)	21 (81)	4 (67)
Dysphagia	24 (47)	18 (86)	28 (90)	8 (89)	19 (39)	16 (67)	18 (69)	3 (50)
Esophageal foreign body	15 (29)	11 (52)	14 (45)	3 (33)	8 (16)	2 (8)	4 (15)	1 (17)
Rhinitis/sinusitis	5 (10)	2 (10)	2 (6)	1 (11)	0 (0)	0 (0)	0 (0)	0 (0)
Asthma	3 (6)	1 (5)	1 (3)	0 (0)	2 (4)	2 (8)	2 (8)	1 (17)
PPI use	38 (75)	17 (81)	21 (68)	9 (100)	33 (67)	12 (50)	21 (81)	6 (100)
Maximum eosinophil count, eos/hpf (mean ± SD)	71 ± 53	103 ± 54	84 ± 58	93 ± 83	68 ± 71	77 ± 62	60 ± 35	43 ± 31

EoE: Eosinophilic esophagitis; PPI: Proton-pump inhibitor; eos/hpf: Eosinophils per high-power field.

tients, and Aarhus University Prescription Database were queried using this combination of codes to provide a set of possible EoE patients. Then, using the confirmed EoE cases previously identified as the reference standard, the sensitivity and specificity of this initial search strategy were determined within the population of patients with esophageal eosinophilia. Sensitivity was calculated by dividing the number of true positive EoE cases identified using the case definition by the total number of reference standard EoE cases. Specificity was calculated by dividing the number of true negative subjects identified using the case definition by the total number of subjects without EoE. By examining the coding and patient characteristics of those classified as false positive and false negative, the administration case definition was further refined, the databases were re-queried with the updated definition, and new operating characteristics were calculated. This process continued until sensitivity and specificity were optimized.

Case definition validation

Using the same methodology as described for case definition development, the case definition was validated in an independent population in Northern Denmark from 2008-2009. In brief, candidate cases of EoE were identified based on SNOMED codes, and a reference standard set of patients with confirmed EoE in the new time frame was created after re-review of original pathology slides and all coding data. The administrative case definition algorithm was then applied to the National Pathology Registry, National Registry of Patients, and Aarhus University Prescription Database, the set of possible EoE cases was generated, and sensitivity and specificity were calculated using the confirmed EoE cases as the reference standard.

RESULTS

Characteristics of the development set

There were 51 patients with esophageal eosinophilia identified in Northern Denmark from 2006-2007, and 21 (41%) had a confirmed diagnosis of EoE (Table 1). This group comprised the reference standard for the development set. Patients with esophageal eosinophilia had a mean age of 42 years, 75% were male, and almost half had dysphagia. While the EoE patients had a similar proportion of males (76%), they were somewhat younger (35 years), and 86% had dysphagia. The maximum eosinophil count was 71 eos/hpf in all patients with esophageal eosinophilia, and 103 eos/hpf in patients with confirmed EoE. The pathology re-review of slides confirmed that the SNOMED coding for esophageal eosinophilia was correct in the vast majority of cases, with 90% meeting criteria for histologic EoE (≥ 15 eos/hpf) (Figure 1).

Characteristics of the validation set

There were 49 patients with esophageal eosinophilia identified in Northern Denmark from 2008-2009, and 24 (49%) had a confirmed diagnosis of EoE (Table 1). This group comprised the reference standard for the validation set. Patients with esophageal eosinophilia had a mean age of 48 years, 73% were male, and 39% had dysphagia. While the EoE patients had a similar proportion of males (83%), they were also somewhat younger (42 years) and 67% had dysphagia. The maximum eosinophil count was 68 eos/hpf in all patients with esophageal eosinophilia, and 77 eos/hpf in patients with confirmed EoE. Overall, the clinical characteristics of the EoE cases in the development and validation sets were similar, and pathology re-review in the validation set also confirmed correct SNOMED coding in 90% of cases (Figure 1).

EoE case definition algorithms and operating characteristics

The iterative methodology for case definition development and validation yielded two registry-based case diagnostic algorithms, one that was highly sensitive and one that was highly specific. The highly sensitive case definition maximized the number of cases of EoE that were identified in the database search, while the highly specific definition accepted a lower sensitivity (i.e., not identifying all EoE cases) in exchange for ensuring that all cases were true EoE cases (i.e., minimizing false positives).

The final case definition algorithms are presented in Table 2. The "sensitive" algorithm has three steps. The

Table 2 Case definition algorithm

For both the sensitive and the specific case definitions:
(1) Include all patients with systematized nomenclature of medicine M47150 (finding of tissue eosinophil) and T62xxx (esophageal biopsy)
(2) Exclude patients if they have one of the following International Classification of Diseases-10 codes occurring at any time:
B20.x - human immunodeficiency virus
C15.x - malignant neoplasm of esophagus
C16.0 - malignant neoplasm of cardia
C92.x - myeloid leukemia
C94.x - other leukemias of specialized cell type
C96.x - other and unspecified lymph/heme malignancies
K22.0 - achalasia
K50.x - Crohn's disease
K52.8 - eosinophilic gastritis/duodenitis
T28.1 - burn of esophagus
T28.6 - corrosion of esophagus
(3) Include patients if one of the following International Classification of Diseases-10 codes is present at or before the date of the procedure:
K22.2 - esophageal obstruction
K30.9 - dyspepsia
P92.x - feeding problems of the newborn
R07.x - pain in throat and chest
R10.x - abdominal pain
R11.x - nausea/vomiting
R12.x - heartburn
R13.x - dysphagia
T18.0 - foreign body in mouth
T18.1 - foreign body in esophagus
T18.2 - foreign body in stomach
T18.9 - foreign body in genitourinary tract unspecified
T98.0 - sequelae of foreign body entering through a natural orifice
Additional requirement for the specific case definition:
(4) Include patients if there is a proton-pump inhibitor prescription (A02BCxx) or H2RA prescription (A02BAxx) within 2 mo of esophageal biopsy date, up to and including 2 d prior to the esophagogastroduodenoscopy

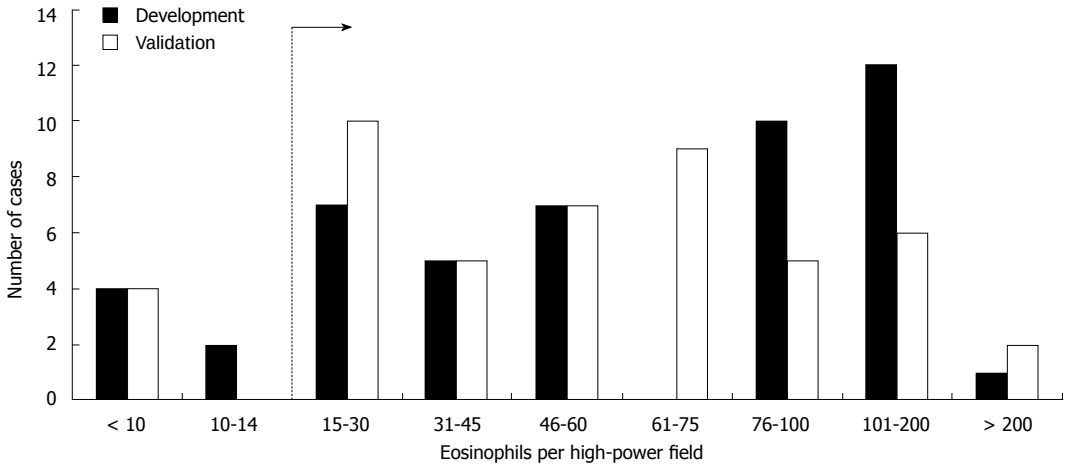


Figure 1 Distribution of levels of esophageal eosinophilia in the development and validation sets of candidate cases of eosinophilic esophagitis identified in the Denmark medical registries. Ranges of esophageal eosinophilia [in eosinophils per high-power field (eos/hpf)] determined after pathology re-review of archived slides are on the x axis. The typical histologic threshold for considering a diagnosis of eosinophilic esophagitis in the appropriate clinical context of 15 eos/hpf is indicated with a dotted line.

first uses SNOMED codes to include all patients with esophageal eosinophilia. The second excludes patients if they have any one of eleven ICD-10 codes that could cause non-EoE related esophageal eosinophilia or esophageal injury that could mimic EoE clinically. The third requires patients to have at least one of 13 ICD-10 codes for symptoms of esophageal dysfunction. The "specific" algorithm has an additional step to exclude false positives which requires a documented proton-pump inhibitor (PPI) or histamine-2 receptor antagonist prescription within 2 mo of the esophageal biopsy date.

When these algorithms were applied to the development set, the clinical characteristics of the cases identified for both the sensitive definition (n = 31; mean age 40 years; 71% male; 90% dysphagia; 84 eos/hpf) and the specific definition (n = 8; mean age 38 years; 78% male; 89% dysphagia; 93 eos/hpf) were similar to those for the reference standard EoE cases (Table 1).

Table 3 Sensitivity and specificity of the case definitions[1] *n* (%)

	Development set		Validation set	
	Sensitive definition	Specific definition	Sensitive definition	Specific definition
Sensitivity	90 (90)	38 (38)	88 (88)	21 (21)
Specificity	60 (60)	97 (97)	80 (80)	96 (96)

[1]Sensitivity: Number of true positive eosinophilic esophagitis (EoE) cases identified by the case definition divided by the total number of reference standard EoE cases; Specificity: Number of true negative subjects identified by the case definition divided by the total number of subjects without EoE.

When these algorithms were applied to the validation set, the clinical features for the sensitive definition (n = 26; mean age 49 years; 81% male; 69% dysphagia; 60 eos/hpf) and specific definition (n = 6; mean age 49 years; 67% male; 50% dysphagia; 43 eos/hpf) were also similar to those for the reference standard for EoE cases (Table 1).

In the development set, the "sensitive" algorithm had a sensitivity and specificity of 90% and 60%, and the "specific" algorithm had values of 38% and 97%, respectively (Table 3). When these algorithms were applied to the validation set, the operating characteristics were essentially unchanged, with a sensitivity and specificity of 88% and 80%, for the "sensitive" algorithm, and 21% and 96% for the "specific" algorithms.

DISCUSSION

The epidemiology of EoE has been rapidly evolving over the past two decades, with a marked increase in incidence and prevalence. The study of EoE in large national or administrative databases, however, has been hampered by the lack of a validated case definition of EoE and the difficulty of developing such a definition due to the clinicopathologic nature of the disorder. This study takes advantage of the fact that the health registries in Denmark can link pathologic data to diagnostic and prescription coding data, thus providing all of the needed components to identify cases of EoE.

There are two main results for this study. The first is that the confirmed EoE cases identified in Denmark *via* the health registries had similar features to those reported both in Denmark as well as elsewhere in the world[1,7-17,32-35]. The second is that two registry-based case definitions, one highly sensitive and one highly specific, were successfully developed and validated for use in the Danish national health databases. This is the first study to do so, and this result makes future large-scale population-based studies feasible in that country. This is important because the majority of investigations studying the epidemiology of EoE are either from selected counties within a country or region, from single centers but are not population-based, or from larger databases that have limitations.

For example, in the two counties studied in northern Sweden during the Kalixanda study, Ronkainen *et al*[36] found that 11 of 1000 (1.1%) subjects had prevalent esophageal eosinophilia ⩾ 15 eos/hpf. While this is the only published study with a true population-based sampling strategy, these individuals would not necessarily meet current EoE diagnostic guidelines because not all were symptomatic and competing causes of esophageal eosinophilia were not excluded[1,28]. In Olten County, Switzerland, a well-defined geographic region with a stable population and practitioners who are expert in EoE diagnosis, Hruz *et al*[14] have recently updated their estimates of the incidence (7/100 000) and prevalence (43/100 000) of EoE[12]. Prasad *et al*[9] reported similar estimates from Olmstead County, Minnesota (incidence 9/100 000; prevalence 55/100 000) in patients identified retrospectively, and Spergel *et al*[37] derived a similar prevalence estimate (52/100 000) from physician surveys. In all of these studies, however, the included patients and providers were not sampled in population-based frames. Additional retrospective single center studies, while providing important data, are subject to similar limitations[7,8,10,11,13,38,39]. Three prospective studies examining the prevalence of EoE in patients undergoing upper endoscopy for any symptom[17], or for dysphagia provide equally important data[15,16], but are also from single centers and enrolled patients who were actively undergoing evaluation and so cannot necessarily be generalized to an entire population. In a series of studies that utilized a large pathology database in the United States, the national distribution of esophageal eosinophilia and EoE has been confirmed, but the data are also not population based[40-42]. Most recently, abstract data from the national health system in the Netherlands has been presented showing a rapid rise in the incidence of esophageal eosinophilia, but this study did not employ a validated case definition of EoE[43]. The current study attempts to address the challenges encountered in the definition of EoE when using county- or national-level database or registry data, and to set the stage for conducting true population-level EoE research.

There are both limitations and strengths of this study that should be acknowledged. The first issue is whether there could be misclassification of cases of EoE in the reference standard groups. This appears to be unlikely given that histology, symptom coding, and prescription data were used to apply EoE consensus diagnostic guidelines to the study population. Nevertheless, because this is a retrospective analysis, we cannot completely exclude the possibility of overlap between gastroesophageal reflux disease and EoE, and are unable to fully address the issue of PPI-responsive esophageal eosinophilia (PPI-REE). It is interesting to observe, however that only 41% and 49% of subjects with esophageal eosinophilia were confirmed to have EoE in the development and validation sets, respectively, suggesting that half or more of subjects with esophageal eosinophilia do not have EoE. The proportion of subjects with esophageal eosinophilia who did not have EoE in our study is similar to the reported proportion of subjects with esophageal eosinophilia who have PPI-REE, ranging in various studies from approximately 30%-75%[44-48]. The poor specificity

of the presence of esophageal eosinophilia for the diagnosis of EoE, coupled with the high proportion of subjects with esophageal eosinophilia who have PPI-REE, emphasize that esophageal eosinophilia alone is not adequate for case-finding of EoE in pathology databases, and cannot be used in isolation to diagnose EoE[1,28,49,50]. A related point is that while it is a strength of this study to have obtained and re-reviewed the original pathology slides, it is important to acknowledge that the biopsies were originally taken for clinical purposes at the discretion of individual endoscopists, and the location and number of biopsies could vary.

It is a strength of the study that two case definitions, one sensitive and one specific, were developed. In instances where overlap or misclassification may be a concern, the "specific" algorithm could be used to ensure the most homogenous EoE population is generated. The two case definitions also allow for bounding and performing sensitivity analyses around epidemiologic estimates. Moreover, the use of the linked Denmark databases is another strength of this study, as there are relatively few data sources that are as rich and contain patient-level pathology information linked to administrative coding. This is illustrated by our ability to obtain and re-review the original glass pathology slides in order to validate the SNOMED codes for esophageal eosinophilia.

The case definitions developed in this study are derived from the Denmark databases and it is unknown whether these would be valid in other settings. However, the methodology presented here could be readily replicated in different databases to validate EoE case definitions, and our proposed algorithm could be used as a starting point in an iterative process to develop case definitions in other databases.

In conclusion, this study of the linked Danish national health registries successfully identified and confirmed cases of EoE in Denmark. Two administrative registry-based case definitions, one highly sensitive to maximize the number of cases of EoE identified, and one highly specific to minimize the number of false positive EoE cases included, were developed and validated. The operating characteristics of the case algorithms are sufficient to support future population-based studies of the epidemiology of EoE in Denmark, and may serve as a template for developing similar definitions in other databases.

COMMENTS

Background

Population-based data on eosinophilic esophagitis (EoE) are lacking. The national medical registries in Denmark offer a unique opportunity to systematically study EoE because these resources link pathology, clinical, and pharmacological data at the patient level. However, no validated registry-based case definitions of EoE exist, and these must be developed prior to conducting large-scale studies.

Research frontiers

EoE is a newly emerging chronic immune-mediated disorder characterized by symptoms of esophageal dysfunction and infiltration of the esophageal mucosa by eosinophils. The epidemiology of EoE is increasingly understood, but much of the data are from single referral centers, and there are few population-based studies published.

Innovations and breakthroughs

This is the first study to systematically develop and validate an administrative case definition of EoE in the Danish databases. The authors present two registry-based definitions, one highly sensitive and one highly specific for EoE.

Applications

These definitions can now be utilized in future population-based studies of EoE in Denmark.

Peer review

This is a well designed study about development and validation of a registry based case definition of EoE in Northern Denmark. It has to be pointed out that in the re-reviewed slides of pathology represents an advantage in methodology.

REFERENCES

1 **Liacouras CA**, Furuta GT, Hirano I, Atkins D, Attwood SE, Bonis PA, Burks AW, Chehade M, Collins MH, Dellon ES, Dohil R, Falk GW, Gonsalves N, Gupta SK, Katzka DA, Lucendo AJ, Markowitz JE, Noel RJ, Odze RD, Putnam PE, Richter JE, Romero Y, Ruchelli E, Sampson HA, Schoepfer A, Shaheen NJ, Sicherer SH, Spechler S, Spergel JM, Straumann A, Wershil BK, Rothenberg ME, Aceves SS. Eosinophilic esophagitis: updated consensus recommendations for children and adults. *J Allergy Clin Immunol* 2011; **128**: 3-20.e6; quiz 21-22 [PMID: 21477849 DOI: 10.1016/j.jaci.2011.02.040]

2 **Landres RT**, Kuster GG, Strum WB. Eosinophilic esophagitis in a patient with vigorous achalasia. *Gastroenterology* 1978; **74**: 1298-1301 [PMID: 648822]

3 **Attwood SE**, Smyrk TC, Demeester TR, Jones JB. Esophageal eosinophilia with dysphagia. A distinct clinicopathologic syndrome. *Dig Dis Sci* 1993; **38**: 109-116 [PMID: 8420741]

4 **Straumann A**, Spichtin HP, Bernoulli R, Loosli J, Vögtlin J. [Idiopathic eosinophilic esophagitis: a frequently overlooked disease with typical clinical aspects and discrete endoscopic findings]. *Schweiz Med Wochenschr* 1994; **124**: 1419-1429 [PMID: 7939509]

5 **Kelly KJ**, Lazenby AJ, Rowe PC, Yardley JH, Perman JA, Sampson HA. Eosinophilic esophagitis attributed to gastroesophageal reflux: improvement with an amino acid-based formula. *Gastroenterology* 1995; **109**: 1503-1512 [PMID: 7557132]

6 **Sealock RJ**, Rendon G, El-Serag HB. Systematic review: the epidemiology of eosinophilic oesophagitis in adults. *Aliment Pharmacol Ther* 2010; **32**: 712-719 [PMID: 20662785 DOI: 10.1111/j.1365-2036.2010.04411.x]

7 **Spergel JM**, Brown-Whitehorn TF, Beausoleil JL, Franciosi J, Shuker M, Verma R, Liacouras CA. 14 years of eosinophilic esophagitis: clinical features and prognosis. *J Pediatr Gastroenterol Nutr* 2009; **48**: 30-36 [PMID: 19172120]

8 **Dellon ES,** Gibbs WB, Fritchie KJ, Rubinas TC, Wilson LA, Woosley JT, Shaheen NJ. Clinical, endoscopic, and histologic findings distinguish eosinophilic esophagitis from gastroesophageal reflux disease. *Clin Gastroenterol Hepatol* 2009; **7:** 1305-1313; quiz 1261 [PMID: 19733260]

9 **Prasad GA**, Alexander JA, Schleck CD, Zinsmeister AR, Smyrk TC, Elias RM, Locke GR, Talley NJ. Epidemiology of eosinophilic esophagitis over three decades in Olmsted County, Minnesota. *Clin Gastroenterol Hepatol* 2009; **7**: 1055-1061 [PMID: 19577011]

10 **Gill R**, Durst P, Rewalt M, Elitsur Y. Eosinophilic esophagitis disease in children from West Virginia: a review of the last decade (1995-2004). *Am J Gastroenterol* 2007; **102**: 2281-2285 [PMID: 17573789]

11 **Noel RJ**, Putnam PE, Rothenberg ME. Eosinophilic esophagitis. *N Engl J Med* 2004; **351**: 940-941 [PMID: 15329438]

12 **Straumann A**, Simon HU. Eosinophilic esophagitis: escalating epidemiology? *J Allergy Clin Immunol* 2005; **115**: 418-419 [PMID: 15696105]

13 **Cherian S**, Smith NM, Forbes DA. Rapidly increasing prevalence of eosinophilic oesophagitis in Western Australia. *Arch Dis Child* 2006; **91**: 1000-1004 [PMID: 16877474]

14 **Hruz P**, Straumann A, Bussmann C, Heer P, Simon HU, Zwahlen M, Beglinger C, Schoepfer AM. Escalating incidence of eosinophilic esophagitis: a 20-year prospective, population-based study in Olten County, Switzerland. *J Allergy Clin Immunol* 2011; **128**: 1349-1350.e5 [PMID: 22019091 DOI: 10.1016/j.jaci.2011.09.013]

15 **Prasad GA**, Talley NJ, Romero Y, Arora AS, Kryzer LA, Smyrk TC, Alexander JA. Prevalence and predictive factors of eosinophilic esophagitis in patients presenting with dysphagia: a prospective study. *Am J Gastroenterol* 2007; **102**: 2627-2632 [PMID: 17764492]

16 **Mackenzie SH**, Go M, Chadwick B, Thomas K, Fang J, Kuwada S, Lamphier S, Hilden K, Peterson K. Eosinophilic oesophagitis in patients presenting with dysphagia--a prospective analysis. *Aliment Pharmacol Ther* 2008; **28**: 1140-1146 [PMID: 18624788]

17 **Veerappan GR**, Perry JL, Duncan TJ, Baker TP, Maydonovitch C, Lake JM, Wong RK, Osgard EM. Prevalence of eosinophilic esophagitis in an adult population undergoing upper endoscopy: a prospective study. *Clin Gastroenterol Hepatol* 2009; **7**: 420-426, 426.e1-2 [PMID: 19162236]

18 **Desai TK**, Stecevic V, Chang CH, Goldstein NS, Badizadegan K, Furuta GT. Association of eosinophilic inflammation with esophageal food impaction in adults. *Gastrointest Endosc* 2005; **61**: 795-801 [PMID: 15933677]

19 **Kerlin P**, Jones D, Remedios M, Campbell C. Prevalence of eosinophilic esophagitis in adults with food bolus obstruction of the esophagus. *J Clin Gastroenterol* 2007; **41**: 356-361 [PMID: 17413601]

20 **Sperry SL**, Crockett SD, Miller CB, Shaheen NJ, Dellon ES. Esophageal foreign-body impactions: epidemiology, time trends, and the impact of the increasing prevalence of eosinophilic esophagitis. *Gastrointest Endosc* 2011; **74**: 985-991 [PMID: 21889135 DOI: 10.1016/j.gie.2011.06.029]

21 **Frank L**. Epidemiology. When an entire country is a cohort. *Science* 2000; **287**: 2398-2399 [PMID: 10766613]

22 **Frank L**. Epidemiology. The epidemiologist's dream: Denmark. *Science* 2003; **301**: 163 [PMID: 12855788]

23 **Sorensen HT,** Christensen T, Schlosser HK, Pedersen L. Use of Medical Databases in Clinical Epidemiology. Aarhus, Denmark: Department of Clinical Epidemiology, Aarhus University Hospital, 2008

24 **Erichsen R,** Lash TL, Hamilton-Dutoit SJ, Bjerregaard B, Vyberg M, Pedersen L. Existing data sources for clinical epidemiology: the Danish National Pathology Registry and Data Bank. *Clin Epidemiol* 2010; **2:** 51-56 [PMID: 20865103]

25 **Andersen TF**, Madsen M, Jørgensen J, Mellemkjoer L, Olsen JH. The Danish National Hospital Register. A valuable source of data for modern health sciences. *Dan Med Bull* 1999; **46**: 263-268 [PMID: 10421985]

26 **Ehrenstein V**, Antonsen S, Pedersen L. Existing data sources for clinical epidemiology: Aarhus University Prescription Database. *Clin Epidemiol* 2010; **2**: 273-279 [PMID: 21152254 DOI: 10.2147/clep.s13458]

27 **Dellon ES**, Fritchie KJ, Rubinas TC, Woosley JT, Shaheen NJ. Inter- and intraobserver reliability and validation of a new method for determination of eosinophil counts in patients with esophageal eosinophilia. *Dig Dis Sci* 2010; **55**: 1940-1949 [PMID: 19830560 DOI: 10.1007/s10620-009-1005-z]

28 **Furuta GT**, Liacouras CA, Collins MH, Gupta SK, Justinich C, Putnam PE, Bonis P, Hassall E, Straumann A, Rothenberg ME. Eosinophilic esophagitis in children and adults: a systematic review and consensus recommendations for diagnosis and treatment. *Gastroenterology* 2007; **133**: 1342-1363 [PMID: 17919504]

29 **Fonager K**, Sørensen HT, Rasmussen SN, Møller-Petersen J, Vyberg M. Assessment of the diagnoses of Crohn's disease and ulcerative colitis in a Danish hospital information system. *Scand J Gastroenterol* 1996; **31**: 154-159 [PMID: 8658038]

30 **Herrinton LJ**, Liu L, Lewis JD, Griffin PM, Allison J. Incidence and prevalence of inflammatory bowel disease in a Northern California managed care organization, 1996-2002. *Am J Gastroenterol* 2008; **103**: 1998-2006 [PMID: 18796097]

31 **Liu L**, Allison JE, Herrinton LJ. Validity of computerized diagnoses, procedures, and drugs for inflammatory bowel disease in a northern California managed care organization. *Pharmacoepidemiol Drug Saf* 2009; **18**: 1086-1093 [PMID: 19672855]

32 **Dalby K**, Nielsen RG, Kruse-Andersen S, Fenger C, Bindslev-Jensen C, Ljungberg S, Larsen K, Walsted AM, Husby S. Eosinophilic oesophagitis in infants and children in the region of southern Denmark: a prospective study of prevalence and clinical presentation. *J Pediatr Gastroenterol Nutr* 2010; **51**: 280-282 [PMID: 20512060 DOI: 10.1097/MPG.0b013e3181d1b107]

33 **Dalby K**, Nielsen RG, Kruse-Andersen S, Fenger C, Durup J, Husby S. Gastroesophageal reflux disease and eosinophilic esophagitis in infants and children. A study of esophageal pH, multiple intraluminal impedance and endoscopic ultrasound. *Scand J Gastroenterol* 2010; **45**: 1029-1035 [PMID: 20504244 DOI: 10.3109/00365521.2010.487917]

34 **Dellon ES,** Aderoju A, Woosley JT, Sandler RS, Shaheen NJ. Variability in diagnostic criteria for eosinophilic esophagitis: a systematic review. *Am J Gastroenterol* 2007; **102:** 2300-2313 [PMID: 17617209]

35 **Gonsalves N**, Policarpio-Nicolas M, Zhang Q, Rao MS, Hirano I. Histopathologic variability and endoscopic correlates in adults with eosinophilic esophagitis. *Gastrointest Endosc* 2006; **64**: 313-319 [PMID: 16923475]

36 **Ronkainen J**, Talley NJ, Aro P, Storskrubb T, Johansson SE, Lind T, Bolling-Sternevald E, Vieth M, Stolte M, Walker MM, Agréus L. Prevalence of oesophageal eosinophils and eosinophilic oesophagitis in adults: the population-based Kalixanda study. *Gut* 2007; **56**: 615-620 [PMID: 17135307]

37 **Spergel JM**, Book WM, Mays E, Song L, Shah SS, Talley NJ, Bonis PA. Variation in prevalence, diagnostic criteria, and initial management options for eosinophilic gastrointestinal diseases in the United States. *J Pediatr Gastroenterol Nutr* 2011; **52**: 300-306 [PMID: 21057327 DOI: 10.1097/MPG.0b013e3181eb5a9f]

38 **Franciosi JP**, Tam V, Liacouras CA, Spergel JM. A case-control study of sociodemographic and geographic characteristics of 335 children with eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2009; **7**: 415-419 [PMID: 19118642]

39 **DeBrosse CW**, Collins MH, Buckmeier Butz BK, Allen CL, King EC, Assa'ad AH, Abonia JP, Putnam PE, Rothenberg ME, Franciosi JP. Identification, epidemiology, and chronicity of pediatric esophageal eosinophilia, 1982-1999. *J Allergy Clin Immunol* 2010; **126**: 112-119 [PMID: 20620567 DOI: 10.1016/j.jaci.2010.05.027]

40 **Kapel RC**, Miller JK, Torres C, Aksoy S, Lash R, Katzka DA. Eosinophilic esophagitis: a prevalent disease in the United States that affects all age groups. *Gastroenterology* 2008; **134**: 1316-1321 [PMID: 18471509]

41 **Dellon ES**, Peery AF, Shaheen NJ, Morgan DR, Hurrell JM, Lash RH, Genta RM. Inverse association of esophageal eosinophilia with Helicobacter pylori based on analysis of a US pathology database. *Gastroenterology* 2011; **141**: 1586-1592 [PMID: 21762663 DOI: 10.1053/j.gastro.2011.06.081]

42 Hurrell JM, Genta RM, Dellon ES. Prevalence of esophageal eosinophilia varies by climate zone in the United States. *Am J Gastroenterol* 2012; **107:** 698-706 [PMID: 22310220]

43 **van Rhijn BD,** Verheij J, Smout AJ, Bredenoord AJ. Rapidly increasing incidence of eosinophilic esophagitis in a large cohort. *Neurogastroenterol Motil* 2013; **25:** 47-e5 [PMID: 22963642]

44 **Molina-Infante J**, Ferrando-Lamana L, Ripoll C, Hernandez-Alonso M, Mateos JM, Fernandez-Bermejo M, Dueñas C, Fernandez-Gonzalez N, Quintana EM, Gonzalez-Nuñez MA. Esophageal eosinophilic infiltration responds to proton

pump inhibition in most adults. *Clin Gastroenterol Hepatol* 2011; **9**: 110-117 [PMID: 20920599 DOI: 10.1016/j.cgh.2010.09.019]

45 **Sayej WN**, Patel R, Baker RD, Tron E, Baker SS. Treatment with high-dose proton pump inhibitors helps distinguish eosinophilic esophagitis from noneosinophilic esophagitis. *J Pediatr Gastroenterol Nutr* 2009; **49**: 393-399 [PMID: 19633574]

46 **Dranove JE**, Horn DS, Davis MA, Kernek KM, Gupta SK. Predictors of response to proton pump inhibitor therapy among children with significant esophageal eosinophilia. *J Pediatr* 2009; **154**: 96-100 [PMID: 18783791 DOI: 10.1016/j.jpeds.2008.07.042]

47 **Peterson KA**, Thomas KL, Hilden K, Emerson LL, Wills JC, Fang JC. Comparison of esomeprazole to aerosolized, swallowed fluticasone for eosinophilic esophagitis. *Dig Dis Sci* 2010; **55**: 1313-1319 [PMID: 19533356]

48 **Moawad FJ,** Dias JA, Veerappan GR, Baker T, Maydonovitch CL. Comparison of aerosolized swallowed fluticasone to esomeprazole for the treatment of eosinophilic esophagitis. *Am J Gastroenterol* 2011; **106** (Suppl 2): S12

49 **Rodrigo S**, Abboud G, Oh D, DeMeester SR, Hagen J, Lipham J, DeMeester TR, Chandrasoma P. High intraepithelial eosinophil counts in esophageal squamous epithelium are not specific for eosinophilic esophagitis in adults. *Am J Gastroenterol* 2008; **103**: 435-442 [PMID: 18289205]

50 **Dellon ES**. Diagnosis and management of eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2012; **10**: 1066-1078 [PMID: 22728382]

P- Reviewer von Arnim U **S- Editor** Wen LL
L- Editor A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i4.511

World J Gastroenterol 2013 January 28; 19(4): 511-515
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Endoscopic ultrasound evaluation in the surgical treatment of duodenal and peri-ampullary adenomas

Lilian C Azih, Brett L Broussard, Milind A Phadnis, Martin J Heslin, Mohamad A Eloubeidi, Shayam Varadarajulu, Juan Pablo Arnoletti

Lilian C Azih, Brett L Broussard, Department of General Surgery, University of Alabama Birmingham, Birmingham, AL 35233, United States
Milind A Phadnis, Department of Biostatistics, Kansas University Medical Center, Kansas City, KS 66160, United States
Martin J Heslin, Department of Surgical Oncology, University of Alabama Birmingham, Birmingham, AL 35233, United States
Mohamad A Eloubeidi, Department of Gastroenterology, American University of Beirut, Beirut 1107 2020, Lebanon
Shayam Varadarajulu, Department of Gastroenterology, the Center for Specialized Surgery at Florida Hospital, Orlando, FL 32804, United States
Juan Pablo Arnoletti, Department of Surgical Oncology, The Center for Specialized Surgery at Florida Hospital, Orlando, FL 32804, United States
Author contributions: Azih LC and Arnoletti JP designed research; Azih LC, Arnoletti JP, Broussard BL, Phadnis MA and Varadarajulu S analyzed data; Azih LC, Arnoletti JP, Heslin MJ, Eloubeidi MA and Varadarajulu S contributed new ideas to analyzing data for clinical application to research; Azih LC and Arnoletti JP wrote the paper.
Correspondence to: Lilian C Azih, MD, Department of General Surgery, General Surgery Resident, University of Alabama Birmingham, 1922 3rd Avenue South KB 217, Birmingham, AL 35233, United States. lazih@uab.edu
Telephone: +1-404-3746085 Fax: +1-407-3037305
Received: June 11, 2012 Revised: September 28, 2012
Accepted: October 22, 2012
Published online: January 28, 2013

Abstract

AIM: To investigate endoscopic ultrasound (EUS) for predicting depth of mucosal invasion and to analyze outcomes following endoscopic and transduodenal resection.

METHODS: Records of 111 patients seen at our institution from November 1999 to July 2011 with the post-operative pathological diagnosis of benign ampullary and duodenal adenomas were reviewed. Records of patients who underwent preoperative EUS for diagnostic purposes were identified. The accuracy of EUS in predicting the absence of muscular invasion was assessed by comparing EUS reports to the final surgical pathological results. In addition, the incidence of the post-operative complications over a period of 30 d and the subsequent long-term outcome (recurrence) over a period of 30 mo associated with endoscopic and transduodenal surgical resection was recorded, compared and analyzed.

RESULTS: Among 111 patients with benign ampullary and duodenal adenomas, 47 underwent preoperative EUS for 29 peri-ampullary lesions and 18 duodenal lesions. In addition, computed tomography was performed in 18 patients, endoscopic retrograde cholangio-pancreatography in 10 patients and esophagogastroduodenoscopy in 22 patients. There were 43 patients with sporadic adenomas and 4 patients with familial adenomatous polyposis (FAP)/other polyposis syndromes. In 38 (81%, $P < 0.05$) patients, EUS reliably identified absence of submucosal and muscularis invasion. In 4 cases, EUS underestimated submucosal invasion that was proven by pathology. In the other 5 patients, EUS predicted muscularis invasion which could not be demonstrated in the resected specimen. EUS predicted tumor muscularis invasion with a specificity of 88% and negative predictive value of 90% ($P < 0.05$). Types of resection performed included endoscopic resection in 22 cases, partial duodenectomy in 9 cases, transduodenal ampullectomy with sphincteroplasty in 10 cases and pancreaticoduodenectomy in 6 cases. The main post-operative final pathological results included villous adenoma ($n = 5$), adenoma ($n = 8$), tubulovillous adenoma ($n = 10$), tubular adenoma ($n = 20$) and hyperplastic polyp ($n = 2$). Among the 47 patients who underwent resection, 8 (17%, 5 of which corresponded to surgical resection) developed post-procedural complications which included retroperitoneal hematoma,

intra-abdominal abscess, wound infection, delayed gastric emptying and prolonged ileus. After median follow-up of 20 mo there were 6 local recurrences (13%, median follow-up = 20 mo) 4 of which were in patients with FAP.

CONCLUSION: EUS accurately predicts the depth of mucosal invasion in suspected benign ampullary and duodenal adenomas. These patients can safely undergo endoscopic or local resection.

© 2013 Baishideng. All rights reserved.

Key words: Endoscopic ultrasound; Duodenal periampullary adenoma; Esophagogastroduodenoscopy; Cholangio-pancreatography

Azih LC, Broussard BL, Phadnis MA, Heslin MJ, Eloubeidi MA, Varadarajulu S, Arnoletti JP. Endoscopic ultrasound evaluation in the surgical treatment of duodenal and peri-ampullary adenomas. *World J Gastroenterol* 2013; 19(4): 511-515 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i4/511.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i4.511

INTRODUCTION

Duodenal tumors often pose diagnostic and therapeutic challenges in their management as they arise in close proximity to biliary and pancreatic structures[1]. Villous tumors of the duodenum was first described by Perry in 1893 as a broad-based cauliflower-like mass that he referred to as a duodenal papilloma and in a 1981 review, only 73 cases had been reported by Komorowski *et al*[2]. Although tumors that arise in the duodenum are not typically common (only 1% of gastrointestinal tumors) a significant fraction of them (at least 25%) are adenomas at the time of presentation. Treatment of ampullary adenoma is complicated by difficult preoperative staging, malignant potential and a high recurrence rate[3]. Various techniques are advocated for their management ranging from simple excision to the ampullary tumor and the contiguous duodenal mucosa to wide resection of the mass including the papilla and adjacent duodenal, ductal and pancreatic tissue[4]. Despite their benign nature peri-ampullary and duodenal adenomas remain a therapeutic challenge because of their potential for malignant transformation. Surgical resection of duodenal tumors can be particularly difficult because of their location in the retroperitoneal space in contact with normal pancreatic gland entailing a high risk of postoperative morbidity and mortality[5]. The complex anatomy of the ampullary region and the difficulty of accurately diagnosing and staging *via* imaging and endoscopy makes the management of these tumors controversial and require multidisciplinary teams[6]. While these tumors can typically arise anywhere along the duodenum, they predominately appear in the first or second portion with tumors in the 3rd and 4th portions being less frequent but posing more difficulty in endoscopic detection[1]. Accurate preoperative histological diagnosis and staging of these tumors is therefore often difficult and inconclusive leading to controversy over the ideal management of treating these lesions. Transduodenal local excision (TDE), endoscopic snare excision or pancreatoduodenectomy (PD) are valid options for resection of these tumors[6]. Halstead first reported TDE of an ampullary mass in 1899[7]. In 1935 Whipple was the first to perform *en bloc* removal of the entire duodenum with the head of the pancreas in 1935 a procedure later refined to a one-stage procedure called PD in 1940 by Whipple[8]. Some studies have reported that the incidence of malignancy occurring in duodenal or ampullary tumors vary widely with some reports ranging from 35% to 60% and report that while the region of the ampulla can be accessible for endoscopic biopsy procedure, that there is a high incidence of false negative results for carcinoma, ranging from 25% to 60%[7]. In 1990, a collective series of 78 Japanese patients reported that the biopsy diagnosis of adenoma does not rule out the possibility of deeper carcinoma in ampullary tumors[9]. Another case report in 1992 found EUS as an emerging useful adjuvant to the preoperative evaluation of patients for potential local resection due to its ability to accurately diagnose duodenal adenomas and therefore usefulness in treatment planning[10]. Based on these earlier reported high incidence of malignancy and unreliability of preoperative endoscopic diagnosis, optimal operative management of these tumors remains controversial[7]. We performed a retrospective review of patients with suspected benign duodenal tumors who underwent preoperative EUS to determine the accuracy of this technique in predicting the absence of muscular invasion and also to analyze outcomes associated with endoscopic and transduodenal surgical resection at our institution.

MATERIALS AND METHODS

Patients

Records of 111 patients evaluated and treated at the University of Alabama Birmingham from November 1999 to July 2011 with the post-operative pathological diagnosis of benign ampullary and duodenal adenomas were identified and reviewed retrospectively. There were 43 patients with sporadic adenomas and 4 patients with familial adenomatous polyposis (FAP)/other polyposis syndromes. Patients were 55% women and average age was 63 years old (Table 1.)

Imaging diagnostic modality used

Among 111 patients examined, 47 underwent preoperative EUS for diagnostic purposes (Figure 1). In addition, computed tomography (CT) was performed in 18 patients, endoscopic retrograde cholangiopancreatography in 10 patients and esophagogastroduodenoscopy in 22 patients.

The accuracy of EUS in predicting duodenal adenoma absence of muscular invasion was assessed by com-

Table 1 Summary of clinical data of patients with benign duodenal and peri-ampullary adenomas who underwent endoscopic ultrasound pre-operatively

Age, yr, mean ± SD (range)	63.4 ± 12.63 (25-90)
Sex	45% male; 55% female
Types of resection	Endoscopic (22); pancreaticoduodenectomy (6); transduodenal (10); partial duodenectomy (9)
Tumor location	Periampullary (29); duodenal (18)
Post operative final path	Villous adenoma (5); tubulovillous adenoma (10); tubular adenoma (20); hyperplastic polyp (2); chronic inflammation (1); adenoma (8); Reactive epithelial changes of normal small bowel mucosa (1)
EUS prediction	Absence of muscularis invasion (n = 5, P < 0.05); underestimated submucosal invasion (n = 4, P < 0.05); Accurately predicted depth of tumor invasion (n = 38, P < 0.05)

EUS: Endoscopic ultrasound.

Table 2 2 × 2 Contingency table

	Absence of muscularis involvement path	
	Yes[1]	No
Muscularis involvement EUS		
No	36	4
Yes[1]	5	2

[1]Positive predictive value: 28%; Negative predictive value: 90%; Sensitivity: 33%; Specificity: 87.8% (P < 0.05). EUS: Endoscopic ultrasound.

paring EUS reports and comparing it to final surgical pathological results. In addition, the incidence of post-operative complications over a period of 30 d and subsequent long-term outcome (recurrence) over a period of 30 mo associated with endoscopic and transduodenal surgical resection was recorded, compared and analyzed.

RESULTS

Location of tumors

Twenty-nine patients had peri-ampullary tumors defined by immediate proximity (within 2 cm) to the major duodenal papilla. The remaining 18 patients had adenomas elsewhere in the duodenum as described in Table 1.

Type of resection performed

Resection of the analyzed adenomas was performed endoscopically in 22 cases. Partial duodenectomy was performed in 9 cases, TDE with ampullectomy with sphincteroplasty in 10 cases and pancreaticoduodenectomy in 6 cases.

Final pathologic results

Postoperative final pathology results included villous adenoma (n = 5), adenoma (n = 8), tubulovillous adenoma (n = 10), tubular adenoma (n = 20) and hyperplastic polyp (n = 2), chronic inflammation (n = 1), reactive changes of normal small bowel mucosa (n = 1).

Long-term follow-up

After median follow-up of 20 mo, there were 6 local recurrences (13%) 4 of which developed in patients with FAP (Table 2). Among the 47 patients who underwent resection, 8 (17%, 5 of which corresponded to surgical resection) developed post-procedural complications, which included retroperitoneal hematoma, intra-abdominal abscess, wound infection, delayed gastric emptying and prolonged ileus. EUS reliably identified absence of submucosal and muscularis invasion in 38 (81%) (P < 0.05) patients. There were 4 patients in whom EUS under-estimated invasion of the deep layers which was subsequently demonstrated in the final pathological analysis. In 5 cases (3 of which underwent pancreaticoduodenectomy, 1 underwent endoscopic resection, and 1 underwent transduodenal resection) EUS over-estimated submucosal invasion as this feature could not be proven in the resected specimen. Overall analysis therefore demonstrated that EUS predicted tumor muscularis invasion with a specificity of 88% and negative predictive value (NPV) of 90% (P < 0.05).

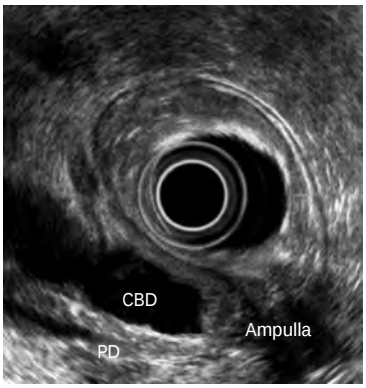


Figure 1 Endoscopic ultrasound showing absence of muscularis invasion in this lesion and as such amendable to local resection. PD: Pancreatoduodenectomy; CBD: Common bile duct.

DISCUSSION

The characterization of benign duodenal and peri-ampullary tumors, offers a diagnostic challenge to reliably distinguish adenomas from malignant lesions and render the possibility of transduodenal resection. While duodenal tumors are not common accounting for less than 1% of total gastrointestinal tumors and 25% of them are benign, the precise characterization continues to present a diagnostic challenge secondary to the complexity of the involved anatomical region. The surgical management of duodenal tumors is also challenging largely due

to their close relationship with the pancreatic and biliary ducts and their deep location[5].

The incidence of malignancy occurring in duodenal or ampullary tumors varies widely with false negative results for carcinoma as high as 60%[7] in the peri-ampullary region.

PD is the treatment of choice for invasive malignancies arising in the ampullary region and duodenum but its indication for treatment of benign peri-ampullary lesions is less clear. Several studies indicate that TDE exhibits less mortality and morbidity than PD[11]. Ampullectomy has been recommended as the procedure of choice to resect benign lesions smaller than 3 cm. TDE is therefore an organ-preserving operation with low morbidity but careful attention to the complex nature of the anatomy of the peri-ampullary region must be given to maximize its chances of success[11]. EUS is reportedly helpful in identifying non-invasive lesions suitable for local resection, but no preoperative test has been proven accurate enough to substitute for clinical judgment and intraoperative pathological confirmation[12]. In a study of local resection for ampullary tumors, the findings recommend local ampullary resection as an acceptable treatment in benign and selected premalignant and malignant ampullary lesions with a low threshold for conversion to PD when appropriate[13]. Another study of 63 patients proposed PD for even benign lesions because two patients in their series had to undergo repeat operations (PD) 4 and 22 years later for stage Ⅳ disease[14]. Local resection has been shown to be a viable alternative to PD in patients with benign tumors or as a palliative procedure in malignant cases with severe co morbidities where radical resection carries unacceptable surgical risk[15]. A review of 19 cases of villous tumors of the duodenum, suggests that some small benign ampullary villous adenomas or those with carcinoma in situ can be excised locally but prefer PD in the fit patient for better local control both of extensive benign lesions and cancers without distant metastases[16]. Other proponents of local resection confirm that benign duodenal villous tumors can be managed successfully by local submucosal excision[17]. In selected patients, endoscopic mucosal resection of superficial neoplastic lesions is associated with low morbidity when compared to surgery[18]. Pre-operative evaluation of these tumors to assess tumor depth is therefore paramount when planning the optimal therapeutic approach for resection of benign duodenal and peri-ampullary tumors. EUS has emerged as a useful technique in assessing the depth of invasion and is often employed when planning therapeutic approach. EUS images of tumors of the duodenal papilla correspond well to the final histological findings and report EUS as a reliable procedure for determining the extent of tumors in this location[19]. Comparison of EUS with other imaging modalities such as conventional sonography, CT, and angiography proved it to be the most effective method for local staging of pancreatic and ampullary cancers[20]. EUS can readily detect the presence of an ampullary or duodenal tumor in 96% of cases and reliably characterizes malignant lesions[21,22].

Consistent with prior publications, 16 of the 18 duodenal tumors in our study had tumors in the first or second portion of the duodenum (Table 1). Considering the above stated advantages, EUS is often employed at our institution when planning therapeutic approach to these tumors (Figure 1). Multiple studies have shown that EUS is superior to CT, magnetic resonance imaging, and transabdominal US in local peri-ampullary staging[23,24]. Previous studies evaluating the role of endoscopic resection of ampullary adenomas have presented it as a reasonable alternative to transduodenal surgical excision but long term follow-up data are needed to evaluate pre-operative staging accuracy and recurrence rates[25,26].

Our retrospective review shows that patients with peri-ampullary tumors and EUS showing absence of muscularis invasion can safely undergo transduodenal ampullectomy with sphincteroplasty and that this procedure results in satisfactory long-term outcomes. Muscularis invasion and pancreatic duct dilatation are features of malignant neoplasms that can be safely ruled out by EUS. Our findings further support endoscopic resection and TDE as safe treatment modalities for benign duodenal adenomas that avoid morbidity associated with PD and are associated with satisfactory long-term outcomes.

We conclude that EUS can accurately predict depth of mucosal invasion in the preoperative evaluation of suspected peri-ampullary and duodenal adenomas. These patients can safely undergo endoscopic or local resection with acceptable local control rates sparing the need for more extensive operations.

COMMENTS

Background

Management of benign duodenal and peri-ampullary adenomas is controversial as to the best management strategy. Some proponents of a more radical approach advocate a more aggressive pancreaticoduodenectomy while other proponents are in favor of a less invasive local resection.

Research frontiers

Endoscopic ultrasound (EUS) has emerged as a useful modality in the preoperative evaluation of these lesions and is used at our institution to guide management planning.

Innovations and breakthroughs

Duodenal tumors often pose diagnostic and therapeutic challenges in their management as they arise in close proximity to biliary and pancreatic structures and EUS has been shown to be a useful means of preoperative evaluation of these lesions to guide with the management planning. In one study, the sensitivity of EUS in the diagnosis of benign tumors was reported as 92%. The management of these benign tumors continues to be controversial posing a challenge to the surgeon, endoscopist and patients.

Applications

Based on their results, EUS can accurately predict depth of mucosal invasion in 81% ($P < 0.05$) of benign ampullary and duodenal adenomas with a specificity of 88% ($P < 0.05$) and negative predictive value of 90% ($P < 0.05$). These patients can safely undergo endoscopic or local resection with acceptable local control rates sparing the need for more extensive operations.

Terminology

Endoscopic ultrasound is an imaging modality that is used mostly in the upper digestive tract and in the respiratory system. It involves the insertion of a probe into a hollow organ and using ultrasound it is able to obtain images of internal organs that are in the chest, and abdomen and aids in visualizing these structures as well as blood vessels adjacent to them using the doppler imaging.

Gastroenterologists with advanced training typically perform the procedure. Transduodenal ampullectomy with sphincteroplasty is a local surgical excision procedure that is less invasive than the standard pancreaticoduodenectomy which is a radical resection typically used for more invasive lesions.

Peer review

This is a unique collection of cases establishing the value of endoscopic ultrasound in the management of duodenal and peri-ampullary adenomas and also reviewing the suitability and the results of the various possible operative procedures. With attention to minor details, it is an important paper and well worth publishing.

REFERENCES

1 **Yan JQ**, Peng CH, Yang WP, Ding JZ, Zhou GW, Ma D, Li HW. Surgical management of benign duodenal tumours. *ANZ J Surg* 2010; **80**: 526-530 [PMID: 20795967 DOI: 10.1111/j.1445-2197.2010.05378.x]

2 **Chappuis CW**, Divincenti FC, Cohn I. Villous tumors of the duodenum. *Ann Surg* 1989; **209**: 593-598; discussion 593-598 [PMID: 2650645 DOI: 10.1364/OE.18.010088]

3 **Cahen DL**, Fockens P, de Wit LT, Offerhaus GJ, Obertop H, Gouma DJ. Local resection or pancreaticoduodenectomy for villous adenoma of the ampulla of Vater diagnosed before operation. *Br J Surg* 1997; **84**: 948-951 [PMID: 9240132 DOI: 10.1002/bjs.1800840711]

4 **Treitschke F**, Beger HG. Local resection of benign periampullary tumors. *Ann Oncol* 1999; **10** Suppl 4: 212-214 [PMID: 10436825]

5 **Cavallini M**, Cavaniglia D, Felicioni F, Vitale V, Pilozzi E, Ziparo V. Large periampullary villous tumor of the duodenum. *J Hepatobiliary Pancreat Surg* 2007; **14**: 526-528 [PMID: 17909726 DOI: 10.1007/s00534-006-1206-1]

6 **Paramythiotis D**, Kleeff J, Wirtz M, Friess H, Büchler MW. Still any role for transduodenal local excision in tumors of the papilla of Vater? *J Hepatobiliary Pancreat Surg* 2004; **11**: 239-244 [PMID: 15368107 DOI: 10.1007/s00534-004-0896-5]

7 **Posner S**, Colletti L, Knol J, Mulholland M, Eckhauser F. Safety and long-term efficacy of transduodenal excision for tumors of the ampulla of Vater. *Surgery* 2000; **128**: 694-701 [PMID: 11015104 DOI: 10.1067/msy.2000.108218]

8 **Howe JR**, Klimstra DS, Moccia RD, Conlon KC, Brennan MF. Factors predictive of survival in ampullary carcinoma. *Ann Surg* 1998; **228**: 87-94 [PMID: 9671071 DOI: 10.1097/00000658-199807000-00013]

9 **Yamaguchi K**, Enjoji M, Kitamura K. Endoscopic biopsy has limited accuracy in diagnosis of ampullary tumors. *Gastrointest Endosc* 1990; **36**: 588-592 [PMID: 2279648]

10 **Tio TL**, Sie LH, Verbeek PC, Dé Wit LT, Tytgat GN. Endosonography in diagnosing and staging duodenal villous adenoma. *Gut* 1992; **33**: 567-568 [PMID: 1582606 DOI: 10.1136/gut.33.4.567]

11 **Sakorafas GH**, Sarr MG. Local excision of periampullary villous tumours of the duodenum. *Eur J Surg Oncol* 1999; **25**: 90-93 [PMID: 10188863 DOI: 10.1053/ejso.1998.0607]

12 **Rattner DW**, Fernandez-del Castillo C, Brugge WR, Warshaw AL. Defining the criteria for local resection of ampullary neoplasms. *Arch Surg* 1996; **131**: 366-371 [PMID: 8615720 DOI: 10.1007/s00464-008-0076-9]

13 **Asbun HJ**, Rossi RL, Munson JL. Local resection for ampullary tumors. Is there a place for it? *Arch Surg* 1993; **128**: 515-520 [PMID: 8098205]

14 **Chareton B**, Coiffic J, Landen S, Bardaxoglou E, Campion JP, Launois B. Diagnosis and therapy for ampullary tumors: 63 cases. *World J Surg* 1996; **20**: 707-712 [PMID: 8662157 DOI: 10.1007/s002689900108]

15 **Farouk M**, Niotis M, Branum GD, Cotton PB, Meyers WC. Indications for and the technique of local resection of tumors of the papilla of Vater. *Arch Surg* 1991; **126**: 650-652 [PMID: 2021350]

16 **Ryan DP**, Schapiro RH, Warshaw AL. Villous tumors of the duodenum. *Ann Surg* 1986; **203**: 301-306 [PMID: 3954483]

17 **Bjork KJ**, Davis CJ, Nagorney DM, Mucha P. Duodenal villous tumors. *Arch Surg* 1990; **125**: 961-965 [PMID: 2378560 DOI: 10.1001/archsurg.1990.01410200019001]

18 **Ahmad NA**, Kochman ML, Long WB, Furth EE, Ginsberg GG. Efficacy, safety, and clinical outcomes of endoscopic mucosal resection: a study of 101 cases. *Gastrointest Endosc* 2002; **55**: 390-396 [PMID: 11868015 DOI: 10.1067/mge.2002.121881]

19 **Yasuda K**, Mukai H, Cho E, Nakajima M, Kawai K. The use of endoscopic ultrasonography in the diagnosis and staging of carcinoma of the papilla of Vater. *Endoscopy* 1988; **20** Suppl 1: 218-222 [PMID: 3049060]

20 **Rösch T**, Braig C, Gain T, Feuerbach S, Siewert JR, Schusdziarra V, Classen M. Staging of pancreatic and ampullary carcinoma by endoscopic ultrasonography. Comparison with conventional sonography, computed tomography, and angiography. *Gastroenterology* 1992; **102**: 188-199 [PMID: 1727753]

21 **Will U**, Bosseckert H, Meyer F. Correlation of endoscopic ultrasonography (EUS) for differential diagnostics between inflammatory and neoplastic lesions of the papilla of Vater and the peripapillary region with results of histologic investigation. *Ultraschall Med* 2008; **29**: 275-280 [PMID: 18491258 DOI: 10.1055/s-2008-1027327]

22 **Kalantzis N**, Laoudi F, Kallimanis G, Gabriel P, Farmakis N. The role of endoscopic ultrasonography in diagnosis of benign lesions of the upper GI tract. *Eur J Surg Oncol* 1993; **19**: 449-454 [PMID: 8405481]

23 **Chini P**, Draganov PV. Diagnosis and management of ampullary adenoma: The expanding role of endoscopy. *World J Gastrointest Endosc* 2011; **3**: 241-247 [PMID: 22195233]

24 **Chen CH**, Tseng LJ, Yang CC, Yeh YH. Preoperative evaluation of periampullary tumors by endoscopic sonography, transabdominal sonography, and computed tomography. *J Clin Ultrasound* 2001; **29**: 313-321 [PMID: 11424095]

25 **Hernandez LV**, Catalano MF. Endoscopic papillectomy. *Curr Opin Gastroenterol* 2008; **24**: 617-622 [PMID: 19122504 DOI: 10.1097/MOG.0b013e3283088e12]

26 **Bohra AK**, McKie L, Diamond T. Transduodenal excision of ampullary tumours. *Ulster Med J* 2002; **71**: 121-127 [PMID: 12513008]

P- Reviewer Tovey FI **S- Editor** Gou SX
L- Editor A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i4.516

World J Gastroenterol 2013 January 28; 19(4): 516-522
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Effect of alcohol consumption on liver stiffness measured by transient elastography

Edouard Bardou-Jacquet, Ludivine Legros, Draman Soro, Marianne Latournerie, Anne Guillygomarc'h, Caroline Le Lan, Pierre Brissot, Dominique Guyader, Romain Moirand

Edouard Bardou-Jacquet, Ludivine Legros, Draman Soro, Marianne Latournerie, Guillygomarc'h A, Caroline Le Lan, Pierre Brissot, Dominique Guyader, Romain Moirand, Hepatology and Addictology Department, University Hospital Pontchaillou, F35000 Rennes, France

Edouard Bardou-Jacquet, Pierre Brissot, Romain Moirand, Institut National de la Santé et de la Recherche Médicale U991, Institut Fédératif de Recherche 140, University of Rennes 1, F35000 Rennes, France

Author contributions: Bardou-Jacquet E, Moirand R and Legros L designed the research, analyzed the data and wrote the paper; Soro D, Latournerie M, Guillygomarc'h A, Le Lan C, Brissot P and Guyader D gathered and analyzed the data.

Correspondence to: Edouard Bardou-Jacquet, MD, Hepatology and Addictology Department, University Hospital Pontchaillou, CHU Pontchaillou, 2 rue Henri le Guilloux, F35000 Rennes, France. edouard.bardou-jacquet@chu-rennes.fr
Telephone: +33-299-284297 Fax: +33-299-284112

Received: August 26, 2012 Revised: September 12, 2012
Accepted: October 30, 2012
Published online: January 28, 2013

Abstract

AIM: To determine the evolution of transient elastography (TE) in patients with alcoholic liver disease according to alcohol cessation or continuation.

METHODS: We retrospectively selected in our local database all patients who had two TE between June 2005 and November 2010 with chronic alcohol excessive consumption and excluded those with associated cause of liver disease. TE was performed at least one week apart by senior operator. TE examinations with less than ten successful measures or with an interquartile range above 30% were excluded. We retrospectively reviewed file of all patients to include only patient followed up by trained addictologist and for which definite information on alcohol consumption was available. Concomitant biological parameters [aspartate amino transferase (AST), alanine amino transferase and gamma-glutamyl transpeptidase (GGT)] within 4 wk of initial and final TE were recorded. Putative fibrosis score according to initial and final TE were determined with available cut-off for alcoholic liver disease and hepatitis C. Initial and final putative fibrosis score were compared according to alcohol consumption during follow-up.

RESULTS: During the study period 572 patients had TE examination for alcoholic liver disease and 79 of them had at least two examinations. Thirty-seven patients met our criteria with a median follow-up of 32.5 wk. At the end of the study, 13 (35%) were abstinent, and 24 (65%) relapsers. Eight patients had liver biopsy during follow-up. TE decreased significantly during follow-up in 85% of abstinent patients [median (range): -4.9 (-6.1,-1.9)], leading to a modification of the putative fibrosis stage in 28%-71% of patient according to different cut-off value. In relapsers TE increased in 45% and decreased in 54% of patient. There was no statistical difference between initial and final TE in relapsers. In the overall population, using 22.6 kPa as cut-off for cirrhosis, 4 patients had cirrhosis at initial TE and 3 patients had cirrhosis at final TE. Using 19.5 kPa as cut-off for cirrhosis, 7 patients had cirrhosis at initial TE and 5 patients had cirrhosis at final TE. Using 12.5 kPa as cut-off for cirrhosis, 16 patients had cirrhosis at initial TE and 15 patients had cirrhosis at final TE. Evolution of biological data was in accordance with the relapse or abstinent status: abstinence ratio (duration of abstinence/duration follow-up) was correlated with AST ratio (r = -0.465, P = 0.007) and GGT ratio (r = -0.662, P < 0.0001). GGT was correlated with initial (r = 0.488, P = 0.002) and final TE (r = 0.49, P < 0.005). Final TE was correlated with AST (r = 0.362, P < 0.05). Correlation between TE ratio and AST ratio (r = 0.44, P = 0.01) revealed that TE varied proportionally to AST for all patients irrespective of their alcohol status. The same relationship was observed between TE ratio and

GGT ratio (r = 0.65, P < 0.0001). Evolution of TE was significantly correlated with the ratio of time of abstinence to observation time (r = -0.387, P = 0.016) and the evolution of liver enzymes.

CONCLUSION: TE significantly decreased with abstinence. Results of TE in alcoholic liver disease cannot be interpreted without taking into account alcohol consumption and liver enzymes.

© 2013 Baishideng. All rights reserved.

Key words: Alcohol; Transient elastography; Cirrhosis; Fibrosis; Liver biopsy; Liver stiffness

Bardou-Jacquet E, Legros L, Soro D, Latournerie M, Guillygomarc'h A, Le Lan C, Brissot P, Guyader D, Moirand R. Effect of alcohol consumption on liver stiffness measured by transient elastography. *World J Gastroenterol* 2013; 19(4): 516-522 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i4/516.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i4.516

INTRODUCTION

Alcohol excessive consumption is a major public health issue[1,2] as it may lead to liver fibrosis and cirrhosis with life threatening complications such as hepatocellular carcinoma[3], liver failure and death[4]. Early diagnosis of cirrhosis is an important goal as it may promote change in alcohol consumption by increasing motivation in patients and lead for specific screening of esophageal varices[5] or hepatocellular carcinoma[6]. Diagnosis of cirrhosis can be easy when patients have clinical[7] or biological signs of portal hypertension or liver failure. But at the early stage, these signs are for the most part absent, and liver biopsy is the only way to assess fibrosis[8]. Cost and complications of liver biopsy[9-11], leading to significant morbidity or mortality, urge the search for non invasive tools using clinical, biological or morphological finding to establish a probability of fibrosis[12-15].

Transient elastography (TE) is a reproducible and non-invasive test to assess liver fibrosis in chronic liver disease[16]. Initial studies in hepatitis C[17-19] had shown an accurate identification of patients with mild fibrosis or cirrhosis and TE is now part of the European Association for the Study of Liver (EASL) clinical guidelines for hepatitis C management[20].

Studies have also suggested correlation between TE and fibrosis in other chronic liver disease such as hepatitis B[21,22], primary biliary cirrhosis[23], primary sclerosing cholangitis[24], and non alcoholic steatohepatitis[25,26], but it then appears that disease-specific cut-off values for significant fibrosis or cirrhosis should be used[27,28].

Concerning alcoholic liver disease, two studies have shown that TE is correlated with fibrosis[27,28], but diagnosis of severe fibrosis or cirrhosis need the use of higher cut-off values than in other diseases, moreover there are some discrepancies between proposed cut-off values. Explanations offered are different spatial distribution of fibrosis and, that acute liver damage increases liver stiffness by different ways such as hepatocyte swelling, cholestasis, inflammation, and hepatocyte necrosis[29]. Thus excessive alcohol intake, with or without acute alcoholic hepatitis, could increase TE and lead to a false positive diagnosis of fibrosis. Of note, no information regarding patient alcohol consumption at time of fibrosis evaluation was available in the studies which proposed those cut-off values. Our hypothesis is that the alcohol consumption greatly influences TE and thus explains the difficulty to validate TE in alcoholic liver disease, but in the other hand it could make TE a useful tool in the follow-up of patient as an indicator of alcohol consumption beyond the sole fibrosis evaluation.

The aim of this study was to assess the evolution of TE during follow up of patients with alcohol excessive intake history in regards to alcohol withdrawal or continuation. Secondary objective was to determine correlation between biological parameters and TE modification.

MATERIALS AND METHODS

Patients and data

This is a retrospective study conducted in the department of Hepatology and Addictology, University Hospital, Rennes, France. In this unit, a measure of TE is routinely performed in patients hospitalized for alcohol withdrawal or compensated alcoholic liver disease. TE is measured with the Fibroscan® (Echosens, Paris) by a senior operator.

Internal memory of our Fibroscan® was screened for patients who had two TE between June 2005 and November 2010 for alcoholic liver disease. The two different TE examinations had to be performed at an interval of more than one week, ten successful measures were required with an interquartile range ⩽ 30% of the median value. Then we reviewed the patients files to collect information on their history, duration of alcohol withdrawal, and alanine aminotransferase (ALT), aspartate aminotransferase (AST) and gamma glutamyltranspeptidase (GGT) at the time (or up to 4 wk before or after) of the two TE.

Patients were excluded if (1) they presented other causes of chronic liver disease (hepatitis B or C, extrahepatic cholestasis, hemochromatosis); (2) information about alcohol consumption between the two TE were missing, or (3) liver tests at the time of the two TE were missing.

Patients' alcohol consumption during follow-up was assessed by self-declaration and corroborated by biochemistry evolution. Only patients followed by trained addictologists with definite information regarding alcohol consumption were included. We defined abstinence ratio as the duration of abstinence/duration of observation. In this study, patients were considered as abstinent if they were totally sober at least 90% of the time between the two TE.

TE initial (TEi) corresponds to the first TE, TE final

Table 1 Initial and final biological parameters and transient elastography and of patients according to their alcohol consumption status

	Abstinent (n = 13)		Relapser (n = 24)	
	Initial	Final	Initial	Final
Normal AST	8%	75%	8%	15%
AST (IU/L)	130 (39-374)	42 (15-118)	140 (16-472)	119 (17-749)
ALT (IU/L)	109 (34-387)	44 (13-163)	88 (18-266)	76 (9-329)
GGT (IU/L)	1414 (121-3405)	301 (17-1040)	874 (48-5749)	536 (53-1858)
GGT (kPa)	15.5 (10.9-24.3)	11.7 (4.8-22.5)	11.7 (4-34.8)	13.5 (4.2-26.4)
Age, yr	48 ± 9		44 ± 10	
Sex (M/F)	11/2		4/20	

Data are expressed by median (quartile range) and mean ± SD. ALT: Alanine amino transferase; AST: Aspartate aminotransferase; GGT: Gamma glutamyltranspeptidase; M: Male; F: Female.

Table 2 Transient elastography and histological results of patient with liver biopsy

Patient	TEi (kPa)	EASL stage	Liver biopsy initial	TEf (kPa)	EASL stage	Liver biopsy final	Alcohol status
1	33.8	F4		26.4	F4	F4	Relapser
2	34.8	F4		75	F4	F4	Relapser
3	10.2	F3	F4	20.6	F4		Relapser
4	21.3	F4		14	F4	F3	Relapser
5	9.9	F3	F1	4.6	F ≤ 2		Relapser
6	5.3	F ≤ 2		16.9	F4	F3	Relapser
7	20.3	F4		14.3	F4	F4	Abstinent
8	11.7	F3		13.6	F4	F4	Abstinent

Data are shown in chronological order. EASL: European Association for the Study of Liver; TEi: Transient elastography initial; TEf: Transient elastography final.

(TEf) to the second TE and ΔTE to the difference between TEf and TEi. To determine variation of TE, we also defined TE ratio as (TEf-TEi)/TEi.

The liver fibrosis stages of the patients were calculated according to TE using cut-off values from different publications or guidelines: EASL recommendations for hepatitis C: 8 kPa for F ≥ 3 and 12.5 kPa for F = 4; Nahon[6]: 12.9 kPa for F ≥ 3 and 22.6 kPa for F = 4; and Nguyen[7]: 5.9 kPa for F ≥ 1, 7.8 kPa for F ≥ 2, 11 kPa for F ≥ 3 and 19.5 kPa for F = 4. Liver fibrosis stage referred to Metavir classification[9]: stage 0 corresponds to the absence of fibrosis, 1 to the presence of periportal fibrotic extension, 2 to periportal septa; severe fibrosis corresponds to stages 3 (porto-central septa) and 4 (cirrhosis). We defined Δstage as the difference between the fibrosis stages calculated at the first and the second TE, according to the different publications.

When liver biopsy had been performed, fibrosis was classified according to Metavir score[30].

We defined ASTi and GGTi as biological data related to the first TE, ASTf and GGTf to the second TE. The AST ratio [(ASTf-ASTi)/ASTi] and the GGT ratio [(GGTf-GGTi)/GGTi] were calculated to determine variation of biological markers.

Statistical analysis

Demographic data were expressed as median and inter quartile range. Qualitative variables were compared using the χ^2 test or Fischer exact test, when appropriate. Quantitative variables were compared using the Mann Whitney U test or Wilcoxon test for paired values. Correlation between variables was assessed using Spearman's rank correlation coefficient.

All tests were two sided with a significance of 5%. Tests were performed using JMP® software version 9.0 SAS, Cary, NC.

RESULTS

Characteristics of patients

During the period of the study, 6160 TE examinations were performed, of which 572 for patients with alcohol liver disease and 79 of them had 2 examinations. Forty-two patients were excluded because of missing data (lack of biological data or definite information on alcohol intake). Thus 37 patients corresponded to our criteria and were considered for analysis.

There were 7 women and 30 men with a median age of 44 years (39-53.5 years). Median follow up time was 32.5 wk (15-85 wk). At the end of the observation time, 13 (35%) were considered as abstinent, and 24 as relapsers.

Results of TE measures and biochemistry are shown in Table 1. Patients had marked increase in liver enzymes. TEi was significantly correlated with GGTi (r = 0.488, P = 0.002), and TEf with ASTf (r = 0.362, P < 0.05) and GGTf (r = 0.49, P < 0.05).

Repartition of patient according to putative liver fibrosis stage was different depending on TE cut-off used (EASL[2], Nahon[6] or Nguyen[7]). Results are shown in Figure 1. Overall, 16 patients had a TE indicating liver cirrhosis according to EASL, 7 according to Nguyen and 4 according Nahon cut-off values during initial evaluation.

Liver biopsy was performed in 8 patients. Results are shown in Table 2. According to EASL cut-off, 2 relapsers were misclassified. Patient 5 had stage 1 fibrosis, with TEi indicating severe fibrosis and normal TEf; and patient 6 had stage 3 fibrosis with TEf indicating severe fibrosis and normal TEi.

Evolution of TE according to alcohol consumption during observation period

During the observation period, 85% of abstinent patients had a decrease of TE, whereas among relapsers 45% had an increase and 54% a decrease of TE (P = 0.05, χ^2 test).

In the abstinent group, TE decreased significantly (Wilcoxon test for paired values, P = 0.0085) [median ΔTE = -4.9 kPa (-6.1, -1.9)] whereas TEf and TEi were not significantly different in relapsers [median ΔTE = -0.4 kPa (-4.8, +4)]. Δ TE were significantly different between abstinent and relapsers patients (P < 0.05).

There were variations in the putative staging of fibro-

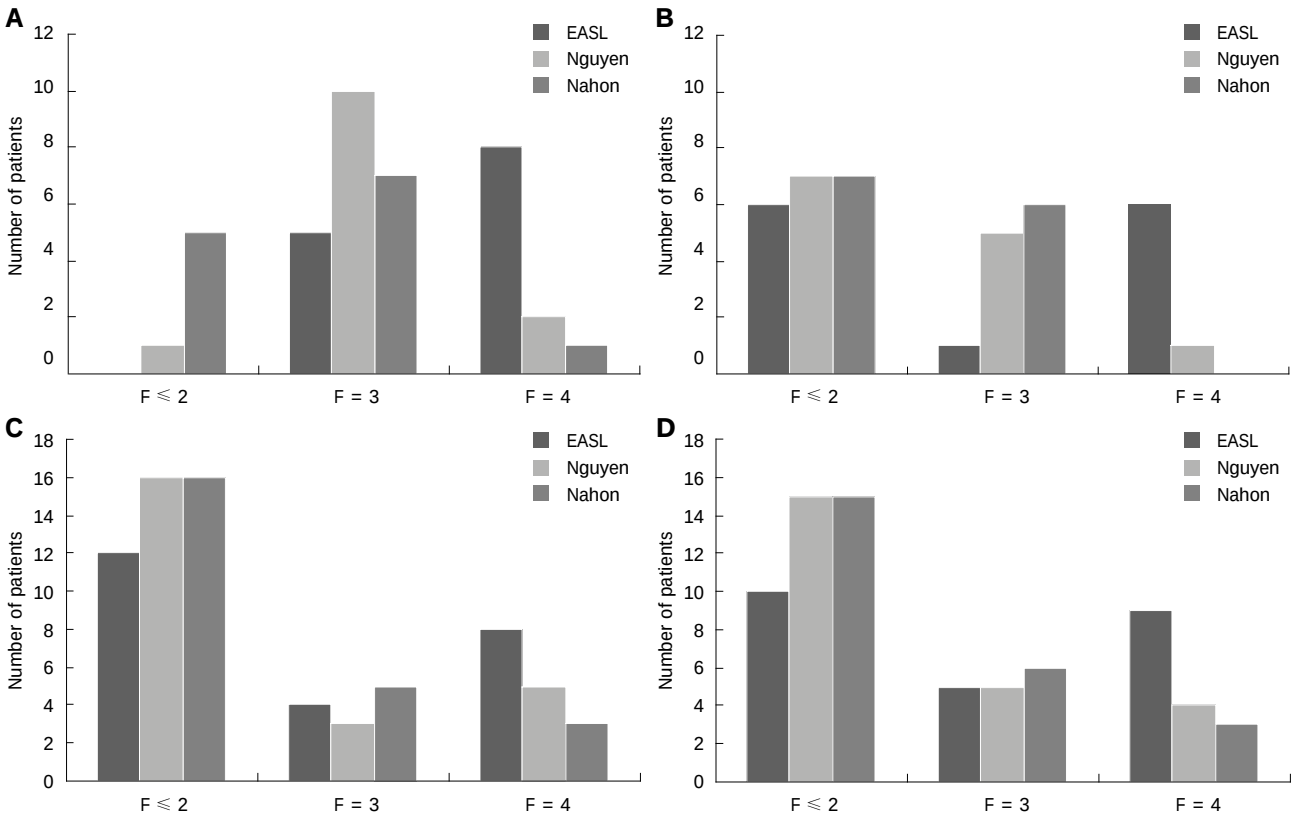


Figure 1 Initial and final repartition of liver fibrosis stage according to different cut-offs values of transient elastography (European Association for the Study of Liver, Nguyen and Nahon recommendations). A: Abstinent group [transient elastography initial (TEi)]; B: Abstinent group [transient elastography final (TEf)]; C: Relapser group (TEi); D: Relapser group (TEf). EASL: European Association for the Study of Liver.

sis according to TE during the observation time (Table 3). Putative fibrosis stage decreased in abstinent patients (in 28%-71% according to the cut-off used). In relapsers, stability, increase or decrease was observed. Δ stage according to EASL and Nguyen cut-offs were significantly different between relapsers and abstinent patients (P = 0.015 and P = 0.012 respectively).

TE decreased proportionally to abstinence, as demonstrated by the correlation between abstinence ratio and TE ratio (r = -0.47, P = 0.0029).

Variation of TE according to biological data

As expected abstinence ratio was correlated with AST ratio (r = -0.465, P = 0.007) and GGT ratio (r = -0.662, P < 0.0001).

Correlation between TE ratio and AST ratio (r = 0.44, P = 0.01) revealed that TE varied proportionally to AST for all patients irrespective of their alcohol status. The same relationship was observed between TE ratio and GGT ratio (r = 0.65, P < 0.0001).

DISCUSSION

TE is a useful tool which has been extensively validated in hepatitis C, but despite the incomparable more important frequency of alcoholic liver disease, few studies have been performed to assess TE in alcoholics. Moreover, despite of this active research, few studies evaluated TE regarding the current alcohol consumption of patients and only one studied the evolution of TE after alcohol detoxification. Our study is the first to assess TE evolution with a medium term follow up according to the alcohol consumption of patients.

Our results confirm the important variation of TE with alcohol withdrawal: 85% of abstinent patients showed a significant decrease of TE during a median follow up period of 32.5 wk. Moreover we showed a likely risk of overestimation of the fibrosis stage according to the different cut off values using TEi. TE evolution was correlated to AST and GGT evolution, which are usually used as marker of alcoholic hepatitis.

The results of our study may suffer from the retrospective data collection and the low number of patient. This is in part induced by the stringent inclusion criteria regarding missing data about alcohol consumption. Indeed, to avoid bias induced by poor quality of data and follow up bias we excluded all patients that were not followed by an addictologist and who lack definite information on alcohol consumption. Moreover, albeit alcoholic liver disease is an extremely frequent disease, patients are seldom compliant with follow up in the absence of severe disease, making rare those completing our inclusion criteria as shown by the number of TE performed for alcoholic liver disease (572) contrasting with the low number of patient who had a second TE (79).

In chronic hepatitis C, TE cut off associated with

Table 3 Percent of abstinent and relapser patient presenting a variation of Metavir score at the end of follow-up according to transient elastography using different cut off values

	Cut-off value	Increase in one or more stage of liver fibrosis (%)	Stable (%)	Decrease in one or more stage of liver fibrosis (%)
Abstinents	EASL	7	36	57
	Nguyen	7	22	71
	Nahon	7	65	28
Relapsers	EASL	29	55	16
	Nguyen	33	30	37
	Nahon	16	72	12

Results are expressed as percent of patient for each class. EASL: European Association for the Study of Liver.

fibrosis greater than stage 3 ranges from 8 to 9 kPa, and cirrhosis can be diagnosed with cut-off ranging from 14 to 15 kPa. Studies performed to assess TE diagnostic accuracy in other diseases than hepatitis C found higher cut off values for significant fibrosis or cirrhosis. Ganne-Carrié *et al*[31] studied 122 patients with non alcoholic or alcoholic steatohepatitis and found a cut off value for cirrhosis of 21.5 kPa. Nahon *et al*[27] studied 147 patients with alcoholic liver disease. Seventy-five percent of them had at least significant fibrosis, which is far more important than expected in alcoholic patients. They found a cut off value for significant fibrosis of 12.9 kPa and 22.6 kPa for cirrhosis. All the patients without cirrhosis and misclassified by TE had histological alcoholic hepatitis. In both studies, the patients were included if liver biopsy was indicated for chronic liver disease and there was no information regarding current alcohol consumption at the time of evaluation. Those data stressed the potential impact of alcohol induced liver modification beyond fibrosis on TE.

Coco *et al*[32] had reported that liver stiffness assessed by TE increased 1.3-to-3 fold during ALT flares in patients with viral hepatitis exacerbation. Studying 195 patients who had both liver biopsy and TE in acute liver damage, Fraquelli *et al*[33] showed that liver stiffness is increased in the acute phase with a correlation between TE and necroinflammatory activity. Recently Mueller *et al*[34] have showed that decrease of TE is correlated with the decrease of AST. Studying liver biopsies for alcoholic liver disease, they found that TE was constant if AST $\leqslant$ 100 UI/L and that accuracy of cirrhosis diagnosis by TE was improved in patient with AST $\leqslant$ 100 UI/L. Those data suggest that TE should be assessed differently regarding the current alcohol consumption and the presence or absence of acute liver modification or biochemical activity. In our study we used biochemical data gathered up to 4 wk from the date of TE, this is a long period which could have induced bias, however the rate of decrease or increase of TE in regard to the biochemical evolution remain to be determined in prospective study.

To date only one study assessed the evolution of TE with alcohol detoxification. Gelsi *et al*[35] studied in a population from an addictology unit the evolution of TE after alcohol weaning over a period of 60 d, and compared this evolution between relapser and abstinent patients. They found a rapid decrease of TE (-21% ± 27% at day 8) with detoxification in an increase proportion of patients if abstinence was sustained: 41% of patient had a decreased at day 8 and 66.7% at day 60. Relapsers were found to have a new increase in TE during follow up after alcohol relapse. As fibrosis is not likely to evolve during that short period of time, and albeit no liver biopsies were performed, one can assume that first TE measurement could have lead to overestimation of fibrosis.

Our results confirm those data, during a much longer follow up period (median 32.5 wk) with a precise addictologic follow up. TE decrease after alcohol cessation over a long period of time, and this was of particular importance in TEi ranging from 8 to 16 kPa, which could indicate significant fibrosis or cirrhosis in chronic hepatitis C, but should be interpreted with caution in alcoholic liver disease. However due to the retrospective design of the study which induced a variable timespan between TE examination, we could not assess the optimal time to perform TE after alcohol cessation.

Relapsers were found to have either an increase or a decrease of TE during follow up, this could be due to the level of alcohol consumption after relapse, as relapsers could have a lower alcohol consumption during follow up which could lead to a decrease of TE, as suggested by the correlation between Δ TE and GGT ratio. This point should be assessed in a prospective study with precise alcohol consumption amount, if confirmed TE could thus be a useful tool to monitor adherence during follow up and fluctuation in alcohol consumption.

TE was correlated with AST and GGT, and TE ratio was correlated with AST and GGT ratio, indicating that TE could be corrected by a calculated modifying factor based on liver enzymes value in order to increase the precision of this test in the diagnosis of fibrosis in alcoholic liver disease. This remains to be proven on large scale prospective studies of TE during alcohol withdrawal with liver biopsy as a gold standard. Such a study should also determine the optimal duration of alcohol cessation before initial increased TE could be controlled.

In conclusion our results show that TE decreased significantly after alcohol cessation over a long period of follow up. Thus TE in alcoholic liver disease should be interpreted with caution and assessed in regard to the current alcohol consumption. Variation of TE is correlated to AST and GGT suggesting that fibrosis could be more likely overestimated in patients with high biological perturbations. Large-scale prospective studies should be performed to determine the different optimal cut off values according to alcohol consumption and more data are required to determine the best delay of alcohol cessation for TE evaluation. TE examination could be a useful tool during the follow-up of alcoholic liver disease to assess actual alcohol consumption and maybe used as a prognosis tool as in chronic hepatitis C[36].

COMMENTS

Background

Excessive alcohol consumption is a major public health issue. Alcoholic liver disease can lead to liver fibrosis and cirrhosis with a risk of developing liver cancer or liver failure. Assessing the severity of liver fibrosis is thus an important landmark in alcoholic liver disease. Until recently the unique way to assess liver fibrosis was liver biopsy which is an invasive procedure with some risk of severe complication. Recently transient elastography (TE) was shown to be efficient in assessing liver fibrosis in chronic viral hepatitis C, it is now frequently used and acknowledged as a validated method by international hepatology society. Some studies have shown that TE could be used in alcoholic liver disease but with method differing from those in hepatitis C.

Research frontiers

TE (Fibroscan®) is an innovative non invasive method, measuring liver stiffness which is correlated to the severity of liver fibrosis. It has been widely validated in hepatitis C, but many studies have shown that results cannot be transposed "as this" to other type of liver disease because liver stiffness can be modified by other liver anomaly than fibrosis. In the area of alcoholic liver disease and liver fibrosis assessment, the research hotspot is how to use liver stiffness measured by TE and what are the parameter influencing liver stiffness.

Innovations and breakthroughs

This study showed that if performed in patient with alcoholic liver disease but without biological inflammation, performance of TE to assess fibrosis was good, which led the auhors to study the evolution of liver stiffness in patient with alcoholic liver disease in regard to their alcohol consumption.They compared the evolution of liver stiffness and biological data in patient with continued or stopped alcohol consumption. They showed that liver stiffness significantly decreases in patient after alcohol cessation and that this decrease is proportional to the severity of the biological inflammation.

Applications

The study results suggest that transient elastrography could be used in alcoholic liver disease by interpreting stiffness values in regard to the ongoing or not alcohol consumption. This hypothesis needs prospective confirmation before clinical application.

Terminology

Liver fibrosis is a "scar" that is developed in liver secondary to toxic (alcohol, iron overload) or viral (hepatitis C) damages. With increasing amount of fibrosis, cirrhosis appear thus leading to liver loss of function and an increased risk of liver cancer.

Peer review

The enclosed manuscript aims to the effect of alcohol consumption on liver stiffness measured by TE. The aim of the manuscript is sound.This is an interesting study.

REFERENCES

1 **Li TK**. Quantifying the risk for alcohol-use and alcohol-attributable health disorders: present findings and future research needs. *J Gastroenterol Hepatol* 2008; **23** Suppl 1: S2-S8 [PMID: 18336658 DOI: 10.1111/j.1440-1746.2007.05298.x]

2 **Cargiulo T**. Understanding the health impact of alcohol dependence. *Am J Health Syst Pharm* 2007; **64**: S5-11 [PMID: 17322182 DOI: 10.2146/ajhp060647]

3 **Seitz HK**, Stickel F. Risk factors and mechanisms of hepatocarcinogenesis with special emphasis on alcohol and oxidative stress. *Biol Chem* 2006; **387**: 349-360 [PMID: 16606331 DOI: 10.1515/bc.2006.047]

4 **Williams R**. The pervading influence of alcoholic liver disease in hepatology. *Alcohol Alcohol* 2008; **43**: 393-397 [PMID: 18385413 DOI: 10.1093/alcalc/agn013]

5 **Garcia-Tsao G**, Sanyal AJ, Grace ND, Carey W. Prevention and management of gastroesophageal varices and variceal hemorrhage in cirrhosis. *Hepatology* 2007; **46**: 922-938 [PMID: 17879356 DOI: 10.1002/hep.21907]

6 **European Association For The Study Of The Liver; European Organisation For Research And Treatment Of Cancer.** EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: 22424438 DOI: 10.1016/j.jhep.2011.12.001]

7 **McCormick PA**, Nolan N. Palpable epigastric liver as a physical sign of cirrhosis: a prospective study. *Eur J Gastroenterol Hepatol* 2004; **16**: 1331-1334 [PMID: 15618841]

8 **Persico M**, Palmentieri B, Vecchione R, Torella R, de SI. Diagnosis of chronic liver disease: reproducibility and validation of liver biopsy. *Am J Gastroenterol* 2002; **97**: 491-492 [PMID: 11866299 DOI: 10.1111/j.1572-0241.2002.05507.x]

9 **Castéra L**, Nègre I, Samii K, Buffet C. Pain experienced during percutaneous liver biopsy. *Hepatology* 1999; **30**: 1529-1530 [PMID: 10610352 DOI: 10.1002/hep.510300624]

10 **Piccinino F**, Sagnelli E, Pasquale G, Giusti G. Complications following percutaneous liver biopsy. A multicentre retrospective study on 68,276 biopsies. *J Hepatol* 1986; **2**: 165-173 [PMID: 3958472]

11 **Bravo AA**, Sheth SG, Chopra S. Liver biopsy. *N Engl J Med* 2001; **344**: 495-500 [PMID: 11172192 DOI: 10.1056/nejm200102153440706]

12 **Sporea I**, Sirli R, Deleanu A, Popescu A, Cornianu M. Liver stiffness measurement by transient elastography in clinical practice. *J Gastrointestin Liver Dis* 2008; **17**: 395-399 [PMID: 19104699]

13 **Smith JO**, Sterling RK. Systematic review: non-invasive methods of fibrosis analysis in chronic hepatitis C. *Aliment Pharmacol Ther* 2009; **30**: 557-576 [PMID: 19519733 DOI: 10.1111/j.1365-2036.2009.04062.x]

14 **Castera L**, Pinzani M. Biopsy and non-invasive methods for the diagnosis of liver fibrosis: does it take two to tango? *Gut* 2010; **59**: 861-866 [PMID: 20581229 DOI: 10.1136/gut.2010.214650]

15 **Bedossa P**. Assessment of hepatitis C: non-invasive fibrosis markers and/or liver biopsy. *Liver Int* 2009; **29** Suppl 1: 19-22 [PMID: 19207962 DOI: 10.1111/j.1478-3231.2008.01940.x]

16 **Castera L**. Transient elastography and other noninvasive tests to assess hepatic fibrosis in patients with viral hepatitis. *J Viral Hepat* 2009; **16**: 300-314 [PMID: 19254351 DOI: 10.1111/j.1365-2893.2009.01087.x]

17 **Ziol M**, Handra-Luca A, Kettaneh A, Christidis C, Mal F, Kazemi F, de Lédinghen V, Marcellin P, Dhumeaux D, Trinchet JC, Beaugrand M. Noninvasive assessment of liver fibrosis by measurement of stiffness in patients with chronic hepatitis C. *Hepatology* 2005; **41**: 48-54 [PMID: 15690481 DOI: 10.1002/hep.20506]

18 **Foucher J**, Chanteloup E, Vergniol J, Castéra L, Le Bail B, Adhoute X, Bertet J, Couzigou P, de Lédinghen V. Diagnosis of cirrhosis by transient elastography (FibroScan): a prospective study. *Gut* 2006; **55**: 403-408 [PMID: 16020491 DOI: 10.1136/gut.2005.069153]

19 **Gómez-Domínguez E**, Mendoza J, Rubio S, Moreno-Monteagudo JA, García-Buey L, Moreno-Otero R. Transient elastography: a valid alternative to biopsy in patients with chronic liver disease. *Aliment Pharmacol Ther* 2006; **24**: 513-518 [PMID: 16886917 DOI: 10.1111/j.1365-2036.2006.02999.x]

20 **European Association for the Study of the Liver.** EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2011; **55**: 245-264 [PMID: 21371579 DOI: 10.1016/j.jhep.2011.02.023]

21 **Marcellin P**, Ziol M, Bedossa P, Douvin C, Poupon R, de Lédinghen V, Beaugrand M. Non-invasive assessment of liver fibrosis by stiffness measurement in patients with chronic hepatitis B. *Liver Int* 2009; **29**: 242-247 [PMID: 18637064 DOI: 10.1111/j.1478-3231.2008.01802.x]

22 **Viganò M**, Paggi S, Lampertico P, Fraquelli M, Massironi S, Ronchi G, Rigamonti C, Conte D, Colombo M. Dual cut-off transient elastography to assess liver fibrosis in chronic hepatitis B: a cohort study with internal validation. *Aliment Pharmacol Ther* 2011; **34**: 353-362 [PMID: 21631559 DOI: 10.1111/j.1365-2036.2011.04722.x]

23 **Gómez-Dominguez E**, Mendoza J, García-Buey L, Trapero M, Gisbert JP, Jones EA, Moreno-Otero R. Transient elastography to assess hepatic fibrosis in primary biliary cirrhosis. *Aliment Pharmacol Ther* 2008; **27**: 441-447 [PMID: 18081731 DOI: 10.1111/j.1365-2036.2007.03585.x]

24 **Corpechot C**, El Naggar A, Poujol-Robert A, Ziol M, Wendum D, Chazouillères O, de Lédinghen V, Dhumeaux D, Marcellin P, Beaugrand M, Poupon R. Assessment of biliary fibrosis by transient elastography in patients with PBC and PSC. *Hepatology* 2006; **43**: 1118-1124 [PMID: 16628644 DOI: 10.1002/hep.21151]

25 **Wong VW**, Vergniol J, Wong GL, Foucher J, Chan HL, Le Bail B, Choi PC, Kowo M, Chan AW, Merrouche W, Sung JJ, de Lédinghen V. Diagnosis of fibrosis and cirrhosis using liver stiffness measurement in nonalcoholic fatty liver disease. *Hepatology* 2010; **51**: 454-462 [PMID: 20101745 DOI: 10.1002/hep.23312]

26 **Abenavoli L**, Beaugrand M. Transient elastography in non-alcoholic fatty liver disease. *Ann Hepatol* 2012; **11**: 172-178 [PMID: 22345333]

27 **Nahon P**, Kettaneh A, Tengher-Barna I, Ziol M, de Lédinghen V, Douvin C, Marcellin P, Ganne-Carrié N, Trinchet JC, Beaugrand M. Assessment of liver fibrosis using transient elastography in patients with alcoholic liver disease. *J Hepatol* 2008; **49**: 1062-1068 [PMID: 18930329 DOI: 10.1016/j.jhep.2008.08.011]

28 **Nguyen-Khac E**, Chatelain D, Tramier B, Decrombecque C, Robert B, Joly JP, Brevet M, Grignon P, Lion S, Le Page L, Dupas JL. Assessment of asymptomatic liver fibrosis in alcoholic patients using fibroscan: prospective comparison with seven non-invasive laboratory tests. *Aliment Pharmacol Ther* 2008; **28**: 1188-1198 [PMID: 18705692 DOI: 10.1111/j.1365-2036.2008.03831.x]

29 **Sagir A**, Erhardt A, Schmitt M, Häussinger D. Transient elastography is unreliable for detection of cirrhosis in patients with acute liver damage. *Hepatology* 2008; **47**: 592-595 [PMID: 18098325 DOI: 10.1002/hep.22056]

30 **Bedossa P**, Poynard T. An algorithm for the grading of activity in chronic hepatitis C. The METAVIR Cooperative Study Group. *Hepatology* 1996; **24**: 289-293 [PMID: 8690394 DOI: 10.1002/hep.510240201]

31 **Ganne-Carrié N**, Ziol M, de Ledinghen V, Douvin C, Marcellin P, Castera L, Dhumeaux D, Trinchet JC, Beaugrand M. Accuracy of liver stiffness measurement for the diagnosis of cirrhosis in patients with chronic liver diseases. *Hepatology* 2006; **44**: 1511-1517 [PMID: 17133503 DOI: 10.1002/hep.21420]

32 **Coco B**, Oliveri F, Maina AM, Ciccorossi P, Sacco R, Colombatto P, Bonino F, Brunetto MR. Transient elastography: a new surrogate marker of liver fibrosis influenced by major changes of transaminases. *J Viral Hepat* 2007; **14**: 360-369 [PMID: 17439526 DOI: 10.1111/j.1365-2893.2006.00811.x]

33 **Fraquelli M**, Rigamonti C, Casazza G, Conte D, Donato MF, Ronchi G, Colombo M. Reproducibility of transient elastography in the evaluation of liver fibrosis in patients with chronic liver disease. *Gut* 2007; **56**: 968-973 [PMID: 17255218 DOI: 10.1136/gut.2006.111302]

34 **Mueller S**, Millonig G, Sarovska L, Friedrich S, Reimann FM, Pritsch M, Eisele S, Stickel F, Longerich T, Schirmacher P, Seitz HK. Increased liver stiffness in alcoholic liver disease: differentiating fibrosis from steatohepatitis. *World J Gastroenterol* 2010; **16**: 966-972 [PMID: 20180235]

35 **Gelsi E**, Dainese R, Truchi R, Mariné-Barjoan E, Anty R, Autuori M, Burroni S, Vanbiervliet G, Evesque L, Cherikh F, Tran A. Effect of detoxification on liver stiffness assessed by Fibroscan® in alcoholic patients. *Alcohol Clin Exp Res* 2011; **35**: 566-570 [PMID: 21143253 DOI: 10.1111/j.1530-0277.2010.01374.x]

36 **Vergniol J**, Foucher J, Terrebonne E, Bernard PH, le Bail B, Merrouche W, Couzigou P, de Ledinghen V. Noninvasive tests for fibrosis and liver stiffness predict 5-year outcomes of patients with chronic hepatitis C. *Gastroenterology* 2011; **140**: 1970-1979, 1979.e1-3 [PMID: 21376047 DOI: 10.1053/j.gastro.2011.02.058]

P- Reviewers Badea R, Sandahl TD
S- Editor Jiang L **L- Editor** A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i4.523

World J Gastroenterol 2013 January 28; 19(4): 523-527
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Role of Ki-67 as a prognostic factor in gastrointestinal stromal tumors

Borislav Belev, Iva Brčić, Juraj Prejac, Zrna Antunac Golubić, Damir Vrbanec, Jadranka Božikov, Ivan Alerić, Marko Boban, Jasminka Jakić Razumović

Borislav Belev, Zrna Antunac Golubić, Damir Vrbanec, Department of Medical Oncology, Clinic of Oncology, Clinical Hospital Center Zagreb, 10000 Zagreb, Croatia
Iva Brčić, Jasminka Jakić Razumović, Department of Pathology, Clinical Hospital Center Zagreb, 10000 Zagreb, Croatia
Juraj Prejac, Department of Gastrointestinal Oncology, Clinic of Oncology, Clinical Hospital Center Zagreb, 10000 Zagreb, Croatia
Jadranka Božikov, Institute for Public Health, Dr. Andrija Štampar, Medical School of Zagreb, 10000 Zagreb, Croatia
Ivan Alerić, Clinical Hospital for Lung Diseases Jordanovac, Clinical Hospital Center Zagreb, 10000 Zagreb, Croatia
Marko Boban, Department of Gastroenterology, Clinical Hospital Center, Sisters of Mercy, 10000 Zagreb, Croatia
Author contributions: Belev B designed the study and wrote the manuscript; Brčić I made the concept of the data, was involved in drafting the manuscript and its editinig as well as their interpretation; Prejac J and Golubić ZA were involved in data collection, especially clinical data; Vrbanec D coordinated the collection of the materials as well as concept of statistical analysis; Razumović JJ provided immunohistochemistry for the tumor specimen as well as pathohistological analysis; Božikov J and Alerić I provided statistical analysis of the data; Boban M was involved in collecting patient data by providing data on clinical basis (patients' data).
Correspondence to: Borislav Belev, MD PhD, Department of Medical Oncology, Clinic of Oncology, Clinical Hospital Center Zagreb, Medical School of Zagreb, Kišpatićeva 12, 10000 Zagreb, Croatia. borislavbelev@gmail.com
Telephone: +385-1-2388725 Fax: +385-1-2376300
Received: July 24, 2012 Revised: October 4, 2012
Accepted: December 25, 2012
Published online: January 28, 2013

Abstract

AIM: To investigate primarily the prognostic value of Ki-67, as well as other parameters, in gastrointestinal stromal tumors (GISTs).

METHODS: Ki-67, c-KIT, platelet-derived growth factor receptor-alpha (PDGFRα), smooth muscle actin (SMA), CD34, S100 were stained for immunohistochemistry which was performed on formalin-fixed, paraffin-embeded sections on representative block from each case. Proliferation index counted by Ki-67 antibody was calculated as a number of positive nuclear reaction over 100 cells. Immunoreactivity for c-KIT and PDGFRα was evaluated semiquantitatively (weak, intermediate, strong) and for c-KIT type of reactivity was analyzed (cytoplasmic, membrane and "dot-like" staining). Immunoreactivity for SMA, CD34 and S100 were was evaluated as positive or negative antigen expression. Pathologic parameters investigated in this study included tumor size, cell type (pure spindle, pured epitheloid mixed spindle and epitheloid), mitotic count, hemorrhage, necrosis, mucosal ulceration. Clinical data included age, gender, primary tumor location and spread of disease. χ^2 test and Student's *t*-test were used for comparisons of baseline characteristics. The Cox's proportional hazard model was used for univariable and multivariable analyses. Survival rates were calculated by Kaplan-Meier method and statistical significance was determined by the log-rank test.

RESULTS: According to the stage of disease, there were 36 patients with localized disease, 29 patients with initially localized disease but with its recurrence in the period of follow up, and finally, 35 patients had metastatic disease from the very beginning of disease. Tumor originated most commonly in the stomach (41%), small intestine was the second most common location (36%). The mean size of primary tumors was 6.5 cm. The mean duration of follow-up was 60 mo. Multiple parameters were analyzed for their effect on overall survival, but no one reached statistical significance (P = 0.06). Analysis of time to progression/relapse in initially localized disease (univariate analysis), tumor size, mitotic count, Ki-67 and type of d-KIT distribution (cytoplasmic *vs* membrane/"dot-like") showed statistically significant correlation. In multivariate analysis in the group of patients with localized disease, there were only 2 parameters that have impact on relapse, Ki-67 and SMA (P < 0.0001 and P < 0.034, respectively).

Furthermore, Ki-67 was analyzed in localized disease *vs* localized with recurrence and metastatic disease. It was shown that there is a strict difference between these 2 groups of patients (median value was 2.5 for localized disease *vs* 10.0 for recurrent/metastatic disease, $P < 0.0001$). It was also shown that the cut-off value which is still statistically significant in terms of relapse on the level of 6%. The curves for survival on that cut-off level are significantly different ($P < 0.04$, Cox F).

CONCLUSION: Ki-67 presents a significant prognostic factor for GIST recurrence which could be of great importance in evaluating malignant potential of disease.

© 2013 Baishideng. All rights reserved.

Key words: Gastrointestinal stromal tumors; Prognostic factor; Ki-67; Recurrence

Belev B, Brčić I, Prejac J, Golubić ZA, Vrbanec D, Božikov J, Alerić I, Boban M, Razumović JJ. Role of Ki-67 as a prognostic factor in gastrointestinal stromal tumors. *World J Gastroenterol* 2013; 19(4): 523-527 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i4/523.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i4.523

INTRODUCTION

Gastrointestinal stromal tumors (GIST) present a wide spectrum of tumors with variable malignant potential[1-3]. Although this entity has been defined rather recently, retrospectively it has been shown to be the most frequent mesenchymal neoplasm arising in the gastrointestinal tract[4]. Namely, in the last few decades, GISTs were sometimes wrongly diagnosed as leiomyoma, leiomyosarcoma and schwannoma. However, immunohistochemial staining and electron microscopic studies suggested difference comparing to other smooth muscle or Schwann cells. Finally, Hirota *et al*[5] demonstrated in 1998. mutation of c-KIT proto-oncogen as a paradigm of single-mutation tumorogenesis. It has also been shown that GIST originates from interstitial cells of Cajal[6].

Today we also know that both c-KIT and platelet-derived growth factor receptor-alpha (PDGFRα) oncoproteins are growth factor receptors that are normally activated by specific ligand stem cell factor. Their mutations lead to an independent activation of the receptor and constitutive activation and proliferation of cells[5]. Along with KIT-expression, very often is expressed CD34 and sometimes smooth muscle actin (SMA; focal of diffuse)[7].

The prediction of GIST behavior still remains controversial after all these years and, in many cases, non-conclusive. Many prognostic factors have been suggested and investigated in previous studies[8]. It is well known and established that the size of tumor (> 5 cm), mitotic rate > 5/50 HPF and site of the tumor play an important role. Nevertheless, it is not quite possible to predict the behavior of all GISTs. According to guidelines for the assessment of likely behavior, GISTs have been traditionally divided into probably benign, malignant and uncertain/low malignant potential (Fletcher)[9].

Imatinib mesylate, a selective tyrosine kinase inhibitor, has been known to have activity against such tumors. However, no patient had a complete response to the treatment so delineating prognostic factors for patients with GIST may be important even in post imatinib era[10]. It is not clear as well whether there is a role of adjuvant treatment in some GIST patients, although some current trials are still on going[11].

Ki-67 is a nuclear proliferation associated antigen. It is expressed in the growth and synthesis phases of the cell cycle but not in G0-phase (resting phase)[12]. It is a rather reliable marker of cell proliferation although there are differences between studies of Ki-67 labeling index, ranging from 4% to 22%[13,14].

The aim of this study was to investigate the role of Ki-67 among other parameters, as a prognostic factor of clinical behavior and prognosis, along with morphological and immunohistochemical profile in 100 GIST patients.

MATERIALS AND METHODS

All mesenchymal tumors of the gastrointestinal tract were retrieved from the files of the University Hospital Center Zagreb, in the period from 1997-2007. Since the immunohistochemistry has been developed on routine basis from the 2001, all specimens obtained before that time were retrospectively analyzed for c-KIT positivity. Inclusion criteria for the study were appropriate morphology and c-KIT positivity. Clinical data were retrieved from the files of the Clinic of Oncology and Surgical Oncology. Follow-up information was obtained from patients records or interviews. Clinical data included age, gender, tumor location (esophagus, stomach, small intestine, large bowel, retroperitoneum, mesentery) and spread of the disease. Spread of disease was classified into three groups: localized disease, localized disease with recurrence and metastatic disease. Pathological parameters that were assesed included tumor size, mitotic count, cell type (pure spindle, mixed spindle and epithelioid, pure epithelioid), necrosis, hemorrhagic areas and mucosal ulceration (invasion of lamina propria). Tumor size was measured in three dimensions and the largest dimension was taken into account. The mitoses were counted on 50 HPFx (400 ×). Immunohistochemistry was performed on formalin-fixed, paraffin-embedded sections on representative block from each case. Following antibodies were used: CD-117 (Dako, Glostrup, Denmark), CD34 (DAKO), PDGFRα (Novocastra, England), SMA (SMA-1, DAKO), S-100 (DAKO), Ki-67 (MIB-5, DAKO). Positive controls were included. All samples were evaluated by two experienced pathologists. Immunoreactivity for c-KIT and PDGFRα was evaluated semiquantitatively (weak, intermediate and strong), and c-KIT type of reactivity was analyzed (cytoplasmic, membrane or "dot-like" staining). Proliferating index counted by Ki-67 antibody was calculated as a number of positive nuclear reaction over 100 cells. Immunoreactivity for SMA, S100 and CD34 was evaluated as positive or negative antigen expression.

Table 1 Patient distribution according to sex and age in three different groups

Groups		Sex		Total
		Male	Female	
Localized disease	Number	19	17	36
	Average age (yr)	59.5	62.9	61.1
Recurrent disease	Number	17	12	29
	Average age (yr)	55.5	58.4	56.7
Metastatic disease	Number	20	15	35
	Average	60.7	48.7	55.5
Total	Number	56	44	100
	Average age (yr)	58.7	56.8	57.9

Table 2 Multivariate analysis of different parameters for survival in all analyzed patients (n = 100)

Parameter	Beta	Standard error	t value	P value
Tumor size	0.04	0.03	1.12	0.26
Mitosis	-0.01	0.01	-0.91	0.35
Necrosis	0.76	0.73	1.04	0.29
Haemorrhage	-0.63	0.68	-0.93	0.35
Ki-67	0.05	0.02	1.83	0.06
c-KIT	1.44	0.79	1.83	0.06
SMA	0.19	0.51	0.38	0.70
S100	-0.33	0.67	-0.49	0.62
CD34	-0.34	0.52	-0.66	0.50
Cell morphology	-0.19	0.52	-0.37	0.70

SMA: Smooth muscle actin.

Table 3 Multivariate analysis of different parameters for the disease free interval in patients with initially localized disease (n = 65)

Parameter	Beta	Standard error	t value	Exponent beta	P value
Tumor size	0.052	0.031	1.643	1.053	0.100
Number of mitosis	0.011	0.014	0.797	1.011	0.424
Necrosis	0.525	0.633	0.830	1.691	0.406
Haemorrhage	0.865	0.673	1.285	2.377	0.198
Ki-67	0.113	0.030	3.778	1.120	0.0001
c-KIT	0.592	0.536	1.102	1.807	0.270
SMA	-1.213	0.575	-2.110	0.297	0.034
S100	-0.237	0.771	-0.308	0.788	0.757
CD34	-1.088	0.562	-1.935	0.336	0.052

SMA: Smooth muscle actin.

Statistical analysis

χ^2 test or Student's t-test was used for statistical comparisons of baseline characteristics. In statistical analysis of our results we also used appropriate descriptive methods (median, range, minimum and maximum). The Cox's proportional hazard model was used in univariable and multivariable analyses. Survival rates were calculated by the Kaplan-Meier method, and statistical significance was determined by the log-rank test. The observed differences were assumed to be statistically significant if the probability of chance occurence was $P < 0.05$. All statistical analysis were performed by the statistical package statistica.

RESULTS

Our study comprised 100 GIST patients. Mean patients' age was 60.5 (range 20-78) years; 56% of patients were men. Patient distribution in three groups according to sex and age is shown in Table 1. There were 36 patients presenting initially with localized disease, 29 had localized disease further with recurrence and 35 had metastatic disease from the very beginning. Tumors originated most commonly in the stomach (41%), the small intestine was the second most common location (36%), in 8% colon and rectum and in 5% retroperitoneum was involved. In 10% of cases primary site of GIST was not clearly determined, because of the wide spread of the disease. The mean size of primary tumors (in patients without metastases) was 6.5 cm and 35 patients had distant metastases in the time of diagnosis. Metastases were most often localized in the liver, all other sites were rarely involved. The mean duration of follow-up was 60 (range 28-110) mo. Survival curve for all patients included in our study is shown in Figure 1. Further on, multiple parameters were analyzed for their effect on overall survival in all patients (Table 2). Most of them showed no effect, more precisely, only 2 parameters are close to statistically significant prediction of outcome and biological behavior, on the level of $P = 0.06$. These are Ki-67 and type of distribution of c-KIT. On the contrary, when we analyzed time to progression/relapse in localized disease, in univariate analysis tumor size, mitotic rate, Ki-67 and type of c-KIT distribution (cytoplasmic *vs* membrane/"dot-like") showed statistically significant correlation. In multivariate analysis in the group of patients with localized disease, there were only 2 parameters that have impact on relapse, Ki-67 and SMA expression (Table 3). Furthermore, when we compared Ki-67 in three different patients group it was obvious that there is a strict difference between mean value of Ki-67 in localized disease *vs* recurrent and metastatic disease together (median in localized disease was 2.5 *vs* 10.0 in recurrent and metastatic disease, $P < 0.0001$). It was shown that the cut-off value which is still statistically significant in terms of relapse on the level of 6% (Figure 2). Also, it has been shown that the curves for survival on that cut-off level are significantly different ($P = 0.04$, Cox F-test; Figure 3).

DISCUSSION

According to data obtained from our study, like age, sex distribution and primary localization, they are comparable to previous data in literature[8,15,16]. GISTs are rather rare tumors with very interesting biological behavior and sometimes unpredictable clinical course[17-19]. Although many reports indicated that the size and mitotic count are the most important and reliable prognostic factors[7], it has become obvious that the primary tumor localization was also very important issue[17]. Some 10 years ago, DeMatteo *et al*[20] showed that only tumor size is reliable predictor of survival in multivariate analysis. Some other

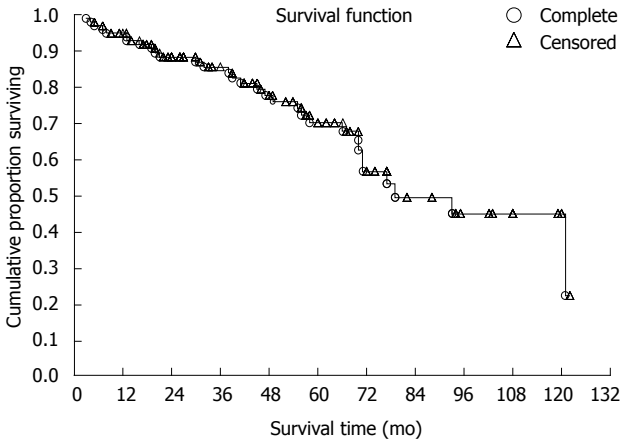


Figure 1 Survival curve for all patients with gastrointestinal stromal tumor (Cox regression analysis).

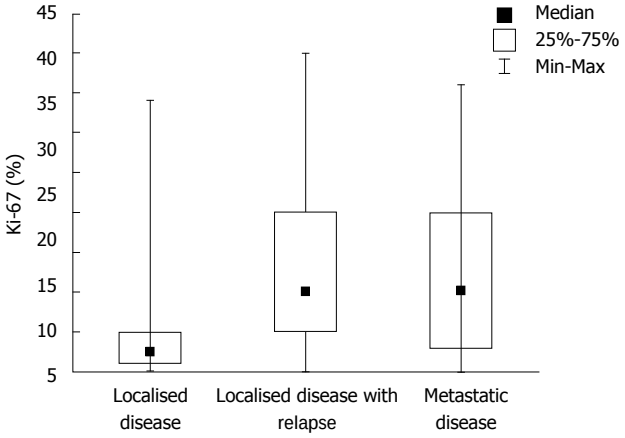


Figure 2 Value of Ki-67 in three different groups of patients.

authors found tumor size > 5 cm a poor prognostic factors[21]. However, there are only few reports of Ki-67 as a good indicator of the risk of metastases and as a prognostic factor[22]. Among other parameters, it was used for distinguishing leiomyoma and leiomyosarcoma[23]. Carrillo *et al*[13] stated that Ki-67 is one of the most accurate predictor of clinical behavior in GIST although it was not confirmed always and some other authors reported about Ki-67 with mitotic index as a prognostic factors in stomach GISTs[24]. In addition, we showed in our study, that there is no statistically significant difference in mean Ki-67 value between stomach- and small intestine-localization of GIST (52 patients together, 29 *vs* 23) (data not shown). That might support an idea of Ki-67 as a non-location-specific prognostic factor, what could be its advantage comparing to mitotic indeks which shows strong correlation to anatomic site of tumor.

One of the most important fact is observation that the level of Ki-67 is a prognostic factor for the relapse of initially localized disease ($P < 0.0001$), and the cut-off value is on 6%. Even in the survival setting this distinction remains statistically significant, no matter how these patients were treated ($P = 0.04$, Cox *F*-test). The expression of Ki-67 changes widely from one to another study, probably by the variety of cut-offs of various authors.

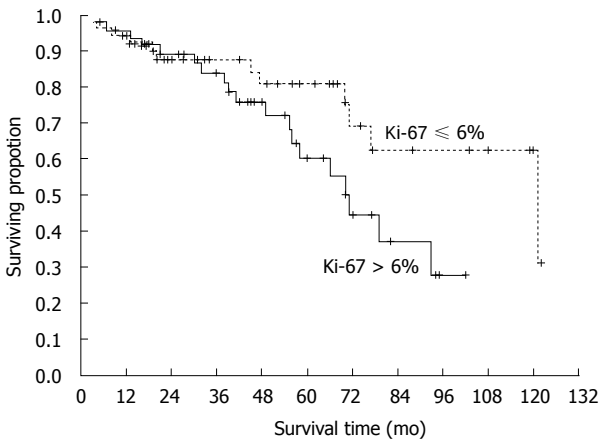


Figure 3 Survival curves with different values of Ki-67 (cut-off on 6%).

Toquet *et al*[25] and Nakamura *et al*[26] described cut-off of 10%, some others on the level of 5%. In that sense, our finding is important as additional indicator of importance of Ki-67 no matter the origin of GIST, and as well this might suggest, among other factors, which patient might have a greater risk of disease relaps. Maybe it might indicate the patients suitable for adjuvant treatment after surgical resection of localized GIST, in addition to contemporary concept of high-risk disease.

Considering other analyzed parameters, no one showed statistical power and impact on survival, as we expected. Just to mention that the meaning of statistical significance of SMA in DFI ($P < 0.034$) should be cautiously interpreted. It might be based on the fact that we assumed SMA-positive tumors generally better differentiated ones, thus it is to expect less aggressive course of disease which means fewer recurrences.

In conclusion, although there are some differences in numerical values of Ki-67 in various studies , that may be caused by different methodology in the assessment. But Ki-67 stays very important parameter of GIST-prognosis and should not be neglected when assessing malignant potential of the GIST.

COMMENTS

Background

Gastrointestinal stromal tumors (GIST) have a specific tumor biology with a wide spectrum of clinical behaviors. Thus, it is of great importance to define prognostic factors which could indicate risk of recurrence or spread of disease. Ki-67 is potentially good prognostic factor.

Research frontiers

So far, some of prognostic factors were rather well documented as the prognostic factors of further tumor behavior, for example mitotic count and tumor size, along with tumor primary localization. Ki-67 was unequivocally addressed as a prognostic factor.

Innovations and breakthroughs

This is the first study showing so clearly the significance of Ki-67 as a prognostic factor. Of importance is, that the patients included in the study were in different stage of disease, and also that comparison to other parameters have been done. It was shown that the cut-off value of Ki-67 of 6%, present the borderline value for recurrence of initially localized disease.

Applications

By understanding the meaning of Ki-67, it could be used as a parameter predicting tumor recurrence and suggest adjuvant treatment after surgery of localized disease.

Terminology

Ki-67 or MKI-67 is a protein that is in humans encoded by *MKI-67* gene. Ki-67 antigen is associated, and probably necessary, for cellular proliferation, it is associated with ribosomal RNA transcription. It is to assume that higher Ki-67 reveals tumor cell activity and thus predicts further behavior.

Peer review

This study is a contribution to the knowledge of GIST. It has a great value in better understanding of the biology of the disease, since some other parameters have already been recognized important as prognostic factors.

REFERENCES

1 **Goss GA,** Merriam P, Manola J Singer S, Fletcher C, Demetri G. Clinical and pathological characteristics of gastrointestinal stromal tumors (GIST). *Prog Proc Am Soc Clin Oncol* 2000; **19:** 2000

2 **Franquemont DW**. Differentiation and risk assessment of gastrointestinal stromal tumors. *Am J Clin Pathol* 1995; **103**: 41-47 [PMID: 7817943]

3 **Clary BM**, DeMatteo RP, Lewis JJ, Leung D, Brennan MF. Gastrointestinal stromal tumors and leiomyosarcoma of the abdomen and retroperitoneum: a clinical comparison. *Ann Surg Oncol* 2001; **8**: 290-299 [PMID: 11352301 DOI: 10.1245/aso.2001.8.4.290]

4 **Nishida T**, Hirota S. Biological and clinical review of stromal tumors in the gastrointestinal tract. *Histol Histopathol* 2000; **15**: 1293-1301 [PMID: 11005253]

5 **Hirota S**, Isozaki K, Moriyama Y, Hashimoto K, Nishida T, Ishiguro S, Kawano K, Hanada M, Kurata A, Takeda M, Muhammad Tunio G, Matsuzawa Y, Kanakura Y, Shinomura Y, Kitamura Y. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. *Science* 1998; **279**: 577-580 [PMID: 9438854 DOI: 10.1126/science.279.5350.577]

6 **Kindblom LG**, Remotti HE, Aldenborg F, Meis-Kindblom JM. Gastrointestinal pacemaker cell tumor (GIPACT): gastrointestinal stromal tumors show phenotypic characteristics of the interstitial cells of Cajal. *Am J Pathol* 1998; **152**: 1259-1269 [PMID: 9588894]

7 **Saul SH**, Rast ML, Brooks JJ. The immunohistochemistry of gastrointestinal stromal tumors. Evidence supporting an origin from smooth muscle. *Am J Surg Pathol* 1987; **11**: 464-473 [PMID: 3035954 DOI: 10.1097/00000478-198706000-00007]

8 **Nilsson B**, Bümming P, Meis-Kindblom JM, Odén A, Dortok A, Gustavsson B, Sablinska K, Kindblom LG. Gastrointestinal stromal tumors: the incidence, prevalence, clinical course, and prognostication in the preimatinib mesylate era-a population-based study in western Sweden. *Cancer* 2005; **103**: 821-829 [PMID: 15648083 DOI: 10.1002/cncr.20862]

9 **Fletcher CD**, Berman JJ, Corless C, Gorstein F, Lasota J, Longley BJ, Miettinen M, O'Leary TJ, Remotti H, Rubin BP, Shmookler B, Sobin LH, Weiss SW. Diagnosis of gastrointestinal stromal tumors: A consensus approach. *Hum Pathol* 2002; **33**: 459-465 [PMID: 12094370 DOI: 10.1053/hupa.2002.123545]

10 **Joensuu H**, Roberts PJ, Sarlomo-Rikala M, Andersson LC, Tervahartiala P, Tuveson D, Silberman S, Capdeville R, Dimitrijevic S, Druker B, Demetri GD. Effect of the tyrosine kinase inhibitor STI571 in a patient with a metastatic gastrointestinal stromal tumor. *N Engl J Med* 2001; **344**: 1052-1056 [PMID: 11287975 DOI: 10.1056/NEJM200104053441404]

11 **Nilsson B**, Sjölund K, Kindblom LG, Meis-Kindblom JM, Bümming P, Nilsson O, Andersson J, Ahlman H. Adjuvant imatinib treatment improves recurrence-free survival in patients with high-risk gastrointestinal stromal tumours (GIST). *Br J Cancer* 2007; **96**: 1656-1658 [PMID: 17533389 DOI: 10.1038/sj.bjc.6603797]

12 **Seidal T**, Edvardsson H. Expression of c-kit (CD117) and Ki67 provides information about the possible cell of origin and clinical course of gastrointestinal stromal tumours. *Histopathology* 1999; **34**: 416-424 [PMID: 10231416 DOI: 10.1046/j.1365-2559.1999.00643.x]

13 **Carrillo R**, Candia A, Rodriguez-Peralto JL, Caz V. Prognostic significance of DNA ploidy and proliferative index (MIB-1 index) in gastrointestinal stromal tumors. *Hum Pathol* 1997; **28**: 160-165 [PMID: 9023396 DOI: 10.1016/S0046-8177(97)90100-3]

14 **Rudolph P**, Gloeckner K, Parwaresch R, Harms D, Schmidt D. Immunophenotype, proliferation, DNA ploidy, and biological behavior of gastrointestinal stromal tumors: a multivariate clinicopathologic study. *Hum Pathol* 1998; **29**: 791-800 [PMID: 9712419 DOI: 10.1016/S0046-8177(98)90447-6]

15 **Saund MS**, Demetri GD, Ashley SW. Gastrointestinal stromal tumors (GISTs). *Curr Opin Gastroenterol* 2004; **20**: 89-94 [PMID: 15703627 DOI: 10.1097/00001574-200403000-00007]

16 **Kindblom LG,** Meis-Kindblom J, Bumming P, Dimitrijevic S, Miret M, Dortok A. Incidence, prevalence, phenotype and biologic spectrum of gastrointestinal stromal tumors (GIST) - A population-based study of 600 cases. *Ann Oncol* 2003: **13** (Suppl 5): 157

17 **Strickland L**, Letson GD, Muro-Cacho CA. Gastrointestinal stromal tumors. *Cancer Control* 2008; **8**: 252-261 [PMID: 11378651]

18 **Gupta P**, Tewari M, Shukla HS. Gastrointestinal stromal tumor. *Surg Oncol* 2008; **17**: 129-138 [PMID: 18234489 DOI: 10.1016/j.suronc.2007.12.002]

19 **Miettinen M**, Lasota J. Gastrointestinal stromal tumors--definition, clinical, histological, immunohistochemical, and molecular genetic features and differential diagnosis. *Virchows Arch* 2001; **438**: 1-12 [PMID: 11213830 DOI: 10.1007/s004280000338]

20 **Dematteo RP,** Gold JS, Saran L, Gönen M, Liau KH, Maki RG, Singer S, Besmer P, Brennan MF, Antonescu CR. Tumor mitotic rate, size, and location independently predict recurrence after resection of primary gastrointestinal stromal tumor (GIST). *Cancer* 2008; **112:** 608-615 [PMID: 18076015 DOI: 10.1002/cncr.23199]

21 **Kwon SJ**. Surgery and prognostic factors for gastric stromal tumor. *World J Surg* 2001; **25**: 290-295 [PMID: 11343178 DOI: 10.1007/s002680020040]

22 **Ohdaira H,** Ohyama S, Yamaguchi T, Yanagisawa A, Kato Y, Urashima M. Ki67 and tumor size as prognostic factors of gastrointestinal stromal tumors. *JMAJ* 2005; **48:** 586-592

23 **Matsuda K,** Watanabe H, Nishikura K, Endoh Y, Ajioka Y, Hitomi J. Gastric myogenic tumors Its Ki-67 mitotic count, myogenic markers, histogenesis of sarcoma and differentiation from gastric stromal tumor. *Stomach and Intestine* (Tokyo) 1995; **30:** 1109-1124

24 **Nagasako Y**, Misawa K, Kohashi S, Hasegawa K, Okawa Y, Sano H, Takada A, Sato H. Evaluation of malignancy using Ki-67 labeling index for gastric stromal tumor. *Gastric Cancer* 2003; **6**: 168-172 [PMID: 14520530 DOI: 10.1007/s10120-003-0246-3]

25 **Toquet C,** Le Néel JC, Guillou L, Renaudin K, Hamy A, Heymann MF, Simon-Valla S, Le Borgne J, Maugard C, Fiche M. Elevated (& gt; or = 10%) MIB-1 proliferative index correlates with poor outcome in gastric stromal tumor patients: a study of 35 cases. *Dig Dis Sci* 2002; **47:** 2247-2253 [PMID: 12395897]

26 **Nakamura N,** Yamamoto H, Yao T, Oda Y, Nishiyama K, Imamura M, Yamada T, Nawata H, Tsuneyoshi M. Prognostic significance of expressions of cell-cycle regulatory proteins in gastrointestinal stromal tumor and the relevance of the risk grade. *Hum Pathol* 2005; **36:** 828-837 [PMID: 16084954]

P- Reviewers Chen P, Linhares E **S- Editor** Wen LL
L- Editor A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i4.528

World J Gastroenterol 2013 January 28; 19(4): 528-535
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Can endoscopic submucosal dissection be safely performed in a smaller specialized clinic?

Naondo Sohara, Satoshi Hagiwara, Riki Arai, Haruhisa Iizuka, Yasuhiro Onozato, Satoru Kakizaki

Naondo Sohara, Satoshi Hagiwara, Riki Arai, Haruhisa Iizuka, Yasuhiro Onozato, Department of Endoscopy and Endoscopic Surgery, Shirakawa Clinic, Maebashi, Gunma 371-0051, Japan
Satoru Kakizaki, Department of Medicine and Molecular Science, Gunma University Graduate School of Medicine, Maebashi, Gunma 371-8511, Japan
Author contributions: Sohara N, Hagiwara S, Arai R, Iizuka H and Onozato Y performed the majority of therapeutic procedures and acquisition of data; Kakizaki S designed the study and wrote the manuscript.
Correspondence to: Satoru Kakizaki, MD, Department of Medicine and Molecular Science, Gunma University Graduate School of Medicine, 3-39-15 Showa-machi, Maebashi, Gunma 371-8511, Japan. kakizaki@showa.gunma-u.ac.jp
Telephone: +81-27-2208127 Fax: +81-27-2208136
Received: July 21, 2012 Revised: August 6, 2012
Accepted: November 6, 2012
Published online: January 28, 2013

Abstract

AIM: To investigate whether endoscopic submucosal dissection (ESD) can be safely performed at small clinics, such as the Shirakawa Clinic.

METHODS: One thousand forty-seven ESDs to treat gastrointestinal tumors were performed at the Shirakawa Clinic from April 2006 to March 2011. The efficacy, technical feasibility and associated complications of the procedures were assessed. The ESD procedures were performed by five endoscopists. Sedation was induced with propofol for esophagogastorduodenal ESD.

RESULTS: One thousand forty-seven ESDs were performed to treat 64 patients with esophageal cancer (E), 850 patients with gastric tumors (G: 764 patients with cancer, 82 patients with adenomas and four others), four patients with duodenal cancer (D) and 129 patients with colorectal tumors (C: 94 patients with cancer, 21 patients with adenomas and 14 others). The *en bloc* resection rate was 94.3% (E: 96.9%, G: 95.8%, D: 100%, C: 79.8%). The median operation time was 46 min (range: 4-360 min) and the mean size of the resected specimens was 18 mm (range: 2-150 mm). No mortal complications were observed in association with the ESD procedures. Perforation occurred in 12 cases (1.1%, E: 1 case, G: 9 cases, D: 1 case, C: 1 case) and postoperative bleeding occurred in 53 cases (5.1%, G: 51 cases, D: 1 case, C: 1 case); however, no case required either emergency surgery or blood transfusion. All of the perforations and postperative bleedings were resolved by endoscopic clipping or hemostasis. The other problematic complication observed was pneumonia, which was treated with conservative therapy.

CONCLUSION: ESD can be safely performed in a clinic with established therapeutic methods and medical services to address potential complications.

© 2013 Baishideng. All rights reserved.

Key words: Endoscopic submucosal dissection; Complication; Perforation; Clinic

Sohara N, Hagiwara S, Arai R, Iizuka H, Onozato Y, Kakizaki S. Can endoscopic submucosal dissection be safely performed in a smaller specialized clinic? *World J Gastroenterol* 2013; 19(4): 528-535 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i4/528.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i4.528

INTRODUCTION

One-piece resection is considered the gold standard for endoscopic mucosal resection (EMR) because it provides an accurate histological assessment and reduces the risk of recurrence[1-5]. However, it is difficult to resect large and ulcerative lesions *en bloc* using conventional EMR techniques. Therefore, a new technique, endoscopic submu-

cosal dissection (ESD), has been developed[6-8]. ESD is a minimally invasive treatment for gastrointestinal cancer. It is usually performed in general hospitals because of the high frequency of complications and the need for a high level of technical skill[9-12]. The occurrence of complications negatively affects the quality life of patients and sometimes requires surgical treatment. Bleeding is one of the most common complications of ESD[13]. The use of hemostatic devices or endoscopic clipping prevents the occurrence of bleeding after ESD procedures[13]. Therefore, cases that require surgical treatment for bleeding after therapeutic procedures are quite rare. Perforation is also one of the most common complications of ESD[13]. Surgical treatment for iatrogenic perforation should be avoided at local clinics because most of these clinics do not have appropriate equipment for such surgeries.

The Shirakawa Clinic, a 19 bed inpatient clinic, was founded as a special clinic for endoscopic surgery. The main procedures performed at this clinic include endoscopy and endoscopic treatments. This clinic does not have surgeons or appropriate equipment for surgery. When surgical treatment is required, patients are transferred to a related tertiary hospital.

This study investigated whether ESD can be safely performed at small clinics such as the Shirakawa Clinic. The efficacy, technical feasibility and associated complications of ESD were assessed in the Shirakawa Clinic.

MATERIALS AND METHODS

Patients

We reviewed all cases of endoscopy and ESD to treat gastrointestinal tumors performed at the Shirakawa Clinic from April 2006 to March 2011. ESD was performed not only for treatment purposes, but also to make accurate histological diagnoses. Indications for ESD primarily included mucosal types of gastrointestinal cancers, excepting poorly differentiated cancers. A detailed description of the indications has been previously reported[9-12]. Depending on the procedure, we followed the guidelines of either the Japan Esophageal Society[14], the Japanese Gastric Cancer Association[15] or the Japanese Society for Cancer of the Colon and Rectum[16].

Clinic and ESD procedure

The Shirakawa Clinic includes 19 inpatient beds, 5 endoscopists and 5 endoscopical technicians certified by the Japan Gastroenterological Endoscopy Society. The endoscopic treatment room and treatment procedures are shown in Figure 1. The treatment teams included two doctors (one operator and one doctor who administered propofol sedation), two nurses and two endoscopical technicians. The years of experience of the endoscopists who performed the endoscopic treatment, including EMR, were as follows: 24 (Onozato Y), 20 (Sohara N), 16 (Hagiwara S), 14 (Iizuka H), and 11 (Arai R) years. The number of years of experience with ESD treatment were 10 (Onozato Y), 5 (Sohara N), 4 (Hagiwara S), 8 (Iizuka H), and 2 (Arai R) years. The numbers of ESD treatments performed were about 1000 (Onozato Y), 250 (Sohara N), 150 (Hagiwara S), 300 (Iizuka H), and 50 (Arai R) cases, respectively. All ESD procedures were supervised by Onozato Y, who had most experience performing ESD among the five endoscopists. As a result, all ESD cases were similarly treated because of the careful supervision.

The intravenous administration of pethidine hydrochloride and propofol was used for sedation, except in colorectal ESDs. The tumors were treated using standard ESD procedures as previously described[9-12]. For each patient, a data collection sheet was used to obtain relevant clinical information about the patient, tumor, procedure and complications. The data sheets were reviewed retrospectively. Findings from abdominal X-P, physical examinations and blood tests were assessed using a clinical pathway as a basis. Patients without complications were permitted to eat soft foods two days after ESD and then were discharged after endoscopic follow-up on the seventh day. The efficacy and complications of ESD were assessed.

One-piece resection was defined as *en bloc* resection. The resections were considered to be curative when tumor-free vertical and horizontal margins were achieved, *en bloc* resection was performed and resection criteria were met[14-16]. The operation time was calculated from the data of digital versatile disc recording systems for endoscopic procedures. Time from the start of the mucosal incision to the completion of the dissection was defined as the operation time. The time for marking or hemostasis after dissection was excluded from the operation time.

Procedure-related bleeding was subdivided into intra- and postoperative groups. Intraoperative bleeding was defined as large amounts of bleeding during the procedure and difficult cases of hemostasis. Postoperative bleeding was defined as clinical evidence of bleeding as evidenced by hematemesis or melena at 0-30 d after ESD and requiring endoscopic treatment. Furthermore, postoperative bleeding was subdivided into early postoperative (within three days of ESD) and delayed postoperative (more than three days after ESD) groups. We also recorded the incidence of perforation during endoscopy and after the procedures based on clinical evidence.

Endoscopic follow-up examinations were performed routinely one week, three months, nine months and every six months after ESD. To exclude distant metastases, abdominal ultrasonography (and/or computed tomography) was performed before ESD and three months and every six months after ESD on the same day as endoscopy.

Statistical analysis

The continuous variables are presented as the mean ± SD. The data were analyzed using the Fisher's exact probability test, and Mann-Whitney's *U*-test. A P value < 0.05 was considered to be significant.

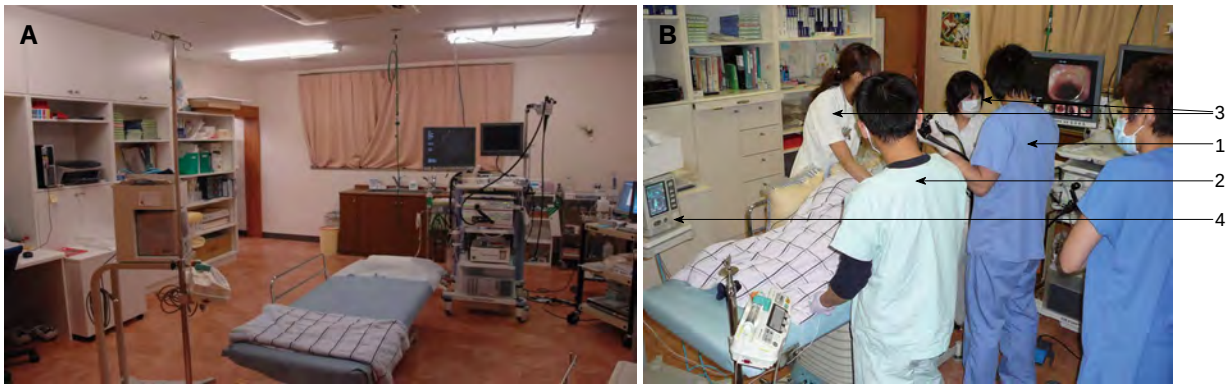


Figure 1 Endoscopic treatment room (A) and treatment procedures (B) of the Shirakawa Clinic. The treatment team included two doctors [one operator (1) and one doctor to administer propofol sedation (2)], two nurses who care for the patients (3) and two endoscopical technicians (not in photo). 4: Vital sign monitor.

Table 1 Clinical characteristics and major complications of the endoscopic submucosal dissection patients

	Total	Esophagus	Stomach	Duodenum	Colorectum
Patients, *n*	873	59	681	4	129
Age (yr), mean (range)	69.9 (44-91)	68.0 (46-81)	70.9 (45-91)	65.5 (54-77)	65.9 (44-80)
Sex (male:female), *n*	549:324	52:7	408:273	2:2	87:42
Lesions, *n*	1047	64	850	4	129
Tumor size (mm), mean (range)	22.2 (2-150)	23 (4-46)	20.8 (2-150)	16.8 (9-31)	31.6 (2-92)
Tumor diagnosis and depth, *n*					
Benign tumor	121	0	86	0	35
Cancer (m)	808	56	668	4	80
Cancer (sm1)	61	4	48	0	9
Cancer (sm2 or deeper)	57	4	48	0	5
En bloc resection rate, %	94.3	96.9	95.8	100	79.8
Operation time (min), mean (range)	46 (4-360)	62 (48-206)	42 (4-360)	90 (50-114)	60 (7-300)
Complications associated with ESD, *n* (%)					
Perforation	12 (1.1)	1 (1.7)	9 (1.1)	1 (25.0)	1 (0.8)
Postoperative bleeding	53 (5.1)	0 (0.0)	51 (6.0)	1 (25.0)	1 (0.8)

ESD: Endoscopic submucosal dissection.

RESULTS

Total patients

From April 2006 to March 2011, a total of 35 540 endoscopies were performed at the Shirakawa Clinic. A total of 1047 lesions in 873 patients were resected with ESD (Table 1). Sixty-four patients with esophageal cancer (E), 850 patients with gastric tumors (G: 764 patients with cancer, 82 patients with adenomas and four others), four patients with duodenal cancer (D) and 129 patients with colorectal tumors (C: 94 patients with cancer, 21 patients with adenomas and 14 others) were treated. The *en bloc* resection rate was 94.3% (E: 96.9%, G: 95.8%, D: 100%, C: 79.8%). The median operation time was 46 min (range: 4-360 min) and the mean size of the resected specimens was 18 mm (range: 2-150 mm). No mortal complications were observed in association with the ESD procedures. Perforation occurred in 12 cases (1.1%, E: 1 case, G: 9 cases, D: 1 case, C: 1 case) and postoperative bleeding occurred in 53 cases (5.1%, G: 51 cases, D: 1 case, C: 1 case); however, no cases required emergency surgery or blood transfusion. All of the perforations were resolved using endoscopic clipping or hemostasis. The other problematic complication observed was pneumonia, which was treated with conservative therapy.

Esophageal cancers

The follow-up results of the 64 patients with esophageal cancer are shown in Figure 2A. The median follow-up period was 24 mo (range: 12-62 mo). Forty-six patients met the absolute indication criteria of the guidelines issued by the Japan Esophageal Society in 2007[14]. Twelve patients with lesions did not meet the conventional absolute criteria and instead met expanded indication criteria. Four patients did not meet the indication criteria. The *en bloc* resection rate was 96.9%. One of the 12 patients who met the expanded indication criteria and two of the four patients who did not meet the indication criteria received additional treatment. No local recurrences were observed in the patients who met the indication criteria.

Gastric cancers and adenomas

The follow-up results of the 850 patients with gastric tumors (764 patients with cancer, 82 patients with adenomas and 4 others) are shown in Figure 2B. The median follow-up period was 26 mo (range: 12-62 mo). Four

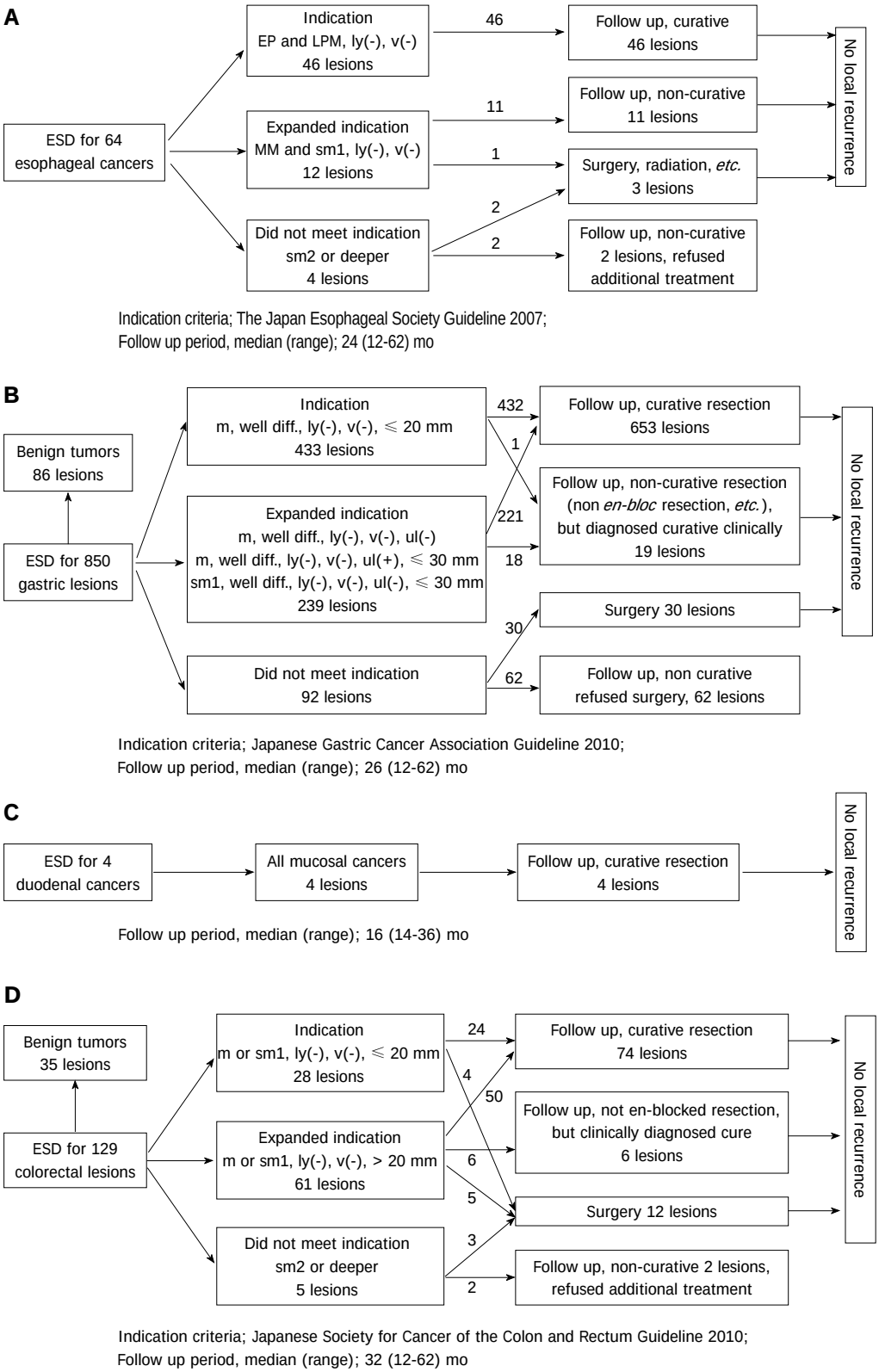


Figure 2 The clinical courses after the endoscopic submucosal dissection of esophageal cancers (A), gastric lesions (B), duodenal cancers (C) and colorectal lesions (D). ESD: Endoscopic submucosal dissection; EP: Epithelium; MM: Muscularis mucosae; LPM: Lamina propria mucosae; Well diff.: Well differentiated adenocarcinoma.

hundred and thirty-three of the 764 patients with gastric cancer met the absolute indication criteria of the guidelines issued by the Japanese Gastric Cancer Association in 2010[15]. Two hundred and thirty-nine patients with lesions did not meet the conventional absolute indication criteria and instead met expanded indication criteria. Ninety-two patients with lesions exceeded the indication criteria. The *en bloc* resection rate was 95.8%. Curative

Table 2 Operation times and tumor sizes in the cases with and those without complications (mean ± SD)

	Number of lesions	Size of treated lesions (mm)	Operation time (min)
Esophagus			
All lesions	64	23.0 ± 11.0	76.4 ± 45.4
No complication	58	23.0 ± 11.1	76.4 ± 45.7
Complication			
Perforation	1	24	57
Postoperative bleeding	0	ND	ND
Stomach			
All lesions	850	20.2 ± 13.4	63.2 ± 47.2
No complication	791	19.9 ± 13.2	62.1 ± 46.9
Complication			
Perforation	8	31.7 ± 14.7[a]	108.9 ± 45.7[a]
Postoperative bleeding	51	23.5 ± 15.5	73.1 ± 47.9[a]
Duodenum			
All lesions	4	16.8 ± 10.1	94.5 ± 29.9
No complication	2	20.0 ± 15.6	112.0 ± 2.8
Complication			
Perforation	1	10	104
Postoperative bleeding	1	17	50
Colorectum			
All lesions	129	31.6 ± 16.5	86.6 ± 66.4
No complication	127	31.3 ± 16.4	84.5 ± 64.5
Complication			
Perforation	1	35	135
Postoperative bleeding	1	65	300

[a]$P < 0.05$ *vs* no complication group. ND: No data.

resection was achieved in 653 of the 764 patients with gastric cancer (85.5%). Nineteen patients with lesions underwent non-curative resection (non *en bloc* resection was performed, *etc*.) but were diagnosed curative clinically. No local recurrences were observed in the patients who met the indication criteria or in those who underwent clinically curative resection.

Duodenal cancers

The follow-up results of the four patients with duodenal cancer are shown in Figure 2C. The median follow-up period was 16 mo (range: 14-36 mo). All four patients underwent curative resection, and the *en bloc* resection rate was 100%.

Colorectal cancers and adenomas

The follow-up results of the 129 patients with colorectal tumors (C: 94 patients with cancer, 21 patients with adenomas and 14 others) are shown in Figure 2D. The median follow-up period was 32 mo (range: 12-62 mo). Twenty-eight of the 94 patients with colorectal cancer met the absolute indication criteria of the guidelines issued by the Japanese Society for Cancer of the Colon and Rectum in 2010[16]. Sixty-one patients with lesions did not meet the conventional absolute indication criteria and instead met expanded indication criteria. Five patients with lesions exceeded the indication criteria. The *en bloc* resection rate was 79.8%. Curative resection was achieved in 74 of the 94 patients with colorectal cancer (78.7%). Six patients with lesions underwent non-curative resection (because non *en bloc* resection was performed, *etc*.) but were diagnosed curative clinically. No local recurrences were observed in the patients who met the indication criteria or in those who underwent clinically curative resection.

Complications and endoscopic treatment

No mortal complications were observed in association with the ESD procedures. Perforations occurred in 12 cases (1.1%, E: 1 case, G: 9 cases, D: 1 case, C: 1 case) and postoperative bleeding occurred in 53 cases (5.1%, G: 51 cases, D: 1 case, C: 1 case) (Table 1). No problematic complications related to sedation were observed. One patient who experienced postoperative pneumonia was treated with an infusion of antibiotics and recovered in one week.

Operation times and tumor size for the cases with and without complications are shown in Table 2. Because the number of esophageal, duodenal and colorectal cases complicated by perforation or bleeding was small, it was not possible to make a statistical comparison between a no complication group and a complication group. It was possible to analyze the cases of gastric lesions statistically. Tumor size and operation times in the gastric cases with perforations were significantly larger than those observed in the cases without complications ($P < 0.05$).

All perforations were managed endoscopically using endoclips, a patch of omentum or conservative therapy. None of the patients with perforations required further surgery, and each of these patients were hospitalized for an additional zero to two days. A representative case of perforation is shown in Figure 3. Briefly, after detecting a perforation hole, the air was removed using an endoscopic fiber. Next, endoscopic clipping was performed. When the omentum became visible through the perforation hole, we attempted to aspirate the omentum through the perforation hole and clip the omentum to the hole.

All cases of intraoperative bleeding were controlled with endoscopic procedures such as soft coagulation with hemostatic forceps (Coagrasper; FD-410LR or FD-410-QR, Olympus, Tokyo). No case of intraoperative bleeding required surgical intervention or blood transfusion. Postoperative bleeding occurred in 53 cases. Early postoperative bleeding (within three days of ESD) occurred in 25/53 cases and delayed postoperative bleeding (more than three days after ESD) occurred in 28/53 lesions. All cases of postoperative bleeding were controlled with endoscopic treatment (clipping and/or electrocoagulation). No case of postoperative bleeding required surgical intervention or blood transfusion. A representative case of bleeding treated endoscopically is shown in Figure 3. Briefly, bleeding from a post-ESD ulcer was detected. After removing the coagulation, the bleeding point was detected. Clips were used to achieve hemostasis endoscopically.

DISCUSSION

ESD allows for the treatment of large and ulcerative

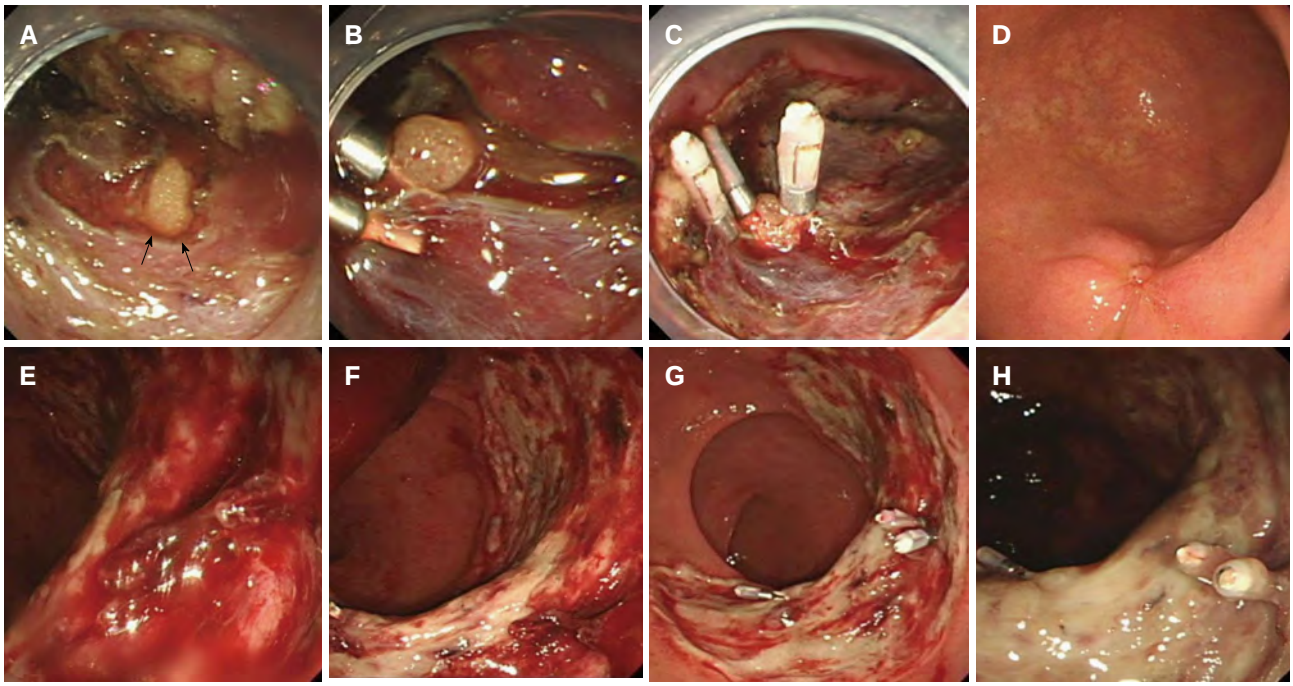


Figure 3 Representative cases of perforation and bleeding treated endoscopically. A: A perforation was detected (arrows); B: When the omentum became visible through the perforation hole, we attempted to aspirate the omentum through the perforation hole, then clipped the gastric wall and omentum together; C: Several clips were used to seal the hole completely; D: The appearance three months after clipping; E: Bleeding from a post-endoscopic submucosal dissection ulcer was detected; F: After removing the coagulation, the bleeding point was detected; G: Clips were used to achieve hemostasis endoscopically; H: The appearance 1 wk after clipping.

lesions and has revolutionized the management of gastrointestinal tumors[6,7,17,18]. ESD is a minimally invasive treatment for gastrointestinal cancer. However, ESD requires a high level of expertise and experience[19,20]. It is usually performed in general hospitals because of the high frequency of complications and the need for a high level of technical skill. Hemostatic devices and/or endoscopic clipping prevents the occurrence of complications after ESD. In this study, all complications of ESD were resolved using non-surgical therapies. Therefore, ESD can be safely performed in clinics with established therapeutic methods and medical services to address potential complications. This study showed that ESD can be safely performed in small clinics.

Since ESD is an endoscopic surgical procedure, the risk of complications is unavoidable[13]. Bleeding and perforation are most common complications of ESD procedures[13]. The incidence of complications[13] associated with ESD are reported to be: 0% for bleeding in the esophagus, 4.0%-6.0% for perforation of the esophagus[21,22], 1.8%-15.6% for bleeding in the stomach, 1.2%-4.7% for perforation of the stomach[23-26], 0.3%-1.5% for bleeding in the colon and 2.2%-5.5% for perforation of the colon[27-29]. Except in cases of esophageal ESD, bleeding is a common complication. It can be treated endoscopically using soft coagulation with hemostatic forceps or hemoclips[13]. In most reports, the incidence of bleeding is less than 10%[13]. In this study, the incidence of complications was 0% for bleeding in the esophagus, 1.7% for perforation of the esophagus, 6.0% for bleeding in the stomach, 1.1% for perforation of the stomach, 0.8% for bleeding in the colon and 0.8% for perforation of the colon. These complication rates are acceptable in comparison to those seen at other institutes[13].

Perforation is another common complication of ESD. Since mediastinitis and peritonitis occuring consequent to esophageal perforation and colon perforation, respectively, have high mortality rates, surgery was previously the method of choice for treating gastrointestinal perforations. Currently, conservative or endoscopic treatment tends to be selected to treat smaller perforations when the lesions are clean[30,31]. Most perforations resulting from ESD are smaller and tend to be treated in a non-surgical manner[32,33]. It is very important to recognize the occurrence of a perforation immediately. Endoscopic closure should be considered if the lesion is clean (i.e. without feces and so on). Matsui *et al*[13] have suggested the indications for endoscopic clipping of perforations to include (1) a small defect size (less than 10 mm); (2) the ability to prepare the bowel adequately; and (3) a stable patient condition immediate following perforation. After successful endoscopic closure is completed, the patient should be fasted and treated with antibiotics. Formal guidelines for fasting periods have not been established; however, one report regarding colonic perforations indicates a mean fasting period of 4.2 d and a mean duration of intravenous antibiotics of 5.5 d[34]. Surgical treatment should be considered when the perforated lesion cannot be closed with endoclips, abdominal pain has exacerbated or severe peritonitis is suspected. Of course, if there is any concern regarding the patient's condition, the patient should be transferred to surgery without hesitation. In

this study, all perforations were managed endoscopically using endoclips, patches of the omentum or conservative therapy. No patient required additional surgery due to perforation in this study.

Because ESD requires a high level of technical skill, an endoscopist's skill level[9-12], rather than the size of a clinic or hospital, may be more strongly related to the results of ESD, such as the success of *en bloc* resections, the length of operations and the rate of complications. In this study, all five endoscopists had more than 10 years of experience with endoscopic treatment, including EMR. Furthermore, all ESD procedures were supervised by the most experienced endoscopist in our clinic. As a result, all ESD cases received similar levels of treatment. This is one of the important reasons why ESD can be safely performed in small clinics. The patient volume may also strongly affect the ESD results. In this study, a total of 1047 lesions in 873 patients were treated during the five-year period, averaging approximately 210 ESDs each year. This is considered to be a moderate to high patient volume. Many experiences with cases and a relatively high patient volume are also needed for the improvement of ESD skills and were found to be important for safely performing ESD.

Two patients with cancer of the esophagus, 62 patients with cancer of the stomach and two patients with cancer of the colon refused additional treatment/surgery in spite of a non-curative resection in this study. The reasons for refusing additional treatment/surgery were varied, including advanced age, complicated diseases, *etc*. The long-term outcomes for such non-curative ESD patients who refused additional treatment/surgery may be of interest. It also may be important data for deciding on the indications for the treatment of elderly patients. However, we did not analyze the overall data in terms of the long-term outcomes for such non-curative ESD patients in this study, so a future study will be needed to clarify these issues.

In conclusion, ESD is associated with a few problematic complications. However, in this study, all complications were resolved using non-surgical therapies such as endoscopic clipping. ESD can be safely performed in clinics with established therapeutic methods and medical services to address potential complications.

COMMENTS

Background

Endoscopic submucosal dissection (ESD) is a minimally invasive treatment for gastrointestinal cancer. It is usually performed in general hospitals because of the high frequency of complications and the need for a high level of technical skill. The aim of this study was to determine whether ESD can be safely performed at small clinics. The efficacy, technical feasibility and associated complications of the procedures were assessed.

Research frontiers

ESD is usually performed in general hospitals. Treatment data and outcome of ESD at general hospitals were reported. However, such reports from clinics were rare. The efficacy, technical feasibility and associated complications of the ESD procedures at a local clinic were assessed.

Innovations and breakthroughs

This study showed that ESD can be safely performed in clinics with established therapeutic methods and medical services to address potential complications. ESD is associated with a few problematic complications. However, in this study, all complications were resolved using non-surgical therapies such as endoscopic clipping.

Applications

ESD can be safely performed in clinics with established therapeutic methods and medical services to address potential complications.

Terminology

ESD is a minimally invasive treatment for gastrointestinal cancer. One-piece resection is considered the gold standard for endoscopic treatment because it provides an accurate histological assessment and reduces the risk of recurrence. ESD enable us to resect large and ulcerative lesions *en bloc*.

Peer review

This study investigated whether ESD can be safely performed at small clinics. The authors indicated ESD can be safely performed in their small 19-bed clinic with similar ESD results as previously published from general hospitals. This is an interesting topic.

REFERENCES

1 **Ono H**, Kondo H, Gotoda T, Shirao K, Yamaguchi H, Saito D, Hosokawa K, Shimoda T, Yoshida S. Endoscopic mucosal resection for treatment of early gastric cancer. *Gut* 2001; **48**: 225-229 [PMID: 11156645 DOI: 10.1136/gut.48.2.225]

2 **Ahmad NA**, Kochman ML, Long WB, Furth EE, Ginsberg GG. Efficacy, safety, and clinical outcomes of endoscopic mucosal resection: a study of 101 cases. *Gastrointest Endosc* 2002; **55**: 390-396 [PMID: 11868015 DOI: 10.1067/mge.2002.121881]

3 **Hirao M**, Masuda K, Asanuma T, Naka H, Noda K, Matsuura K, Yamaguchi O, Ueda N. Endoscopic resection of early gastric cancer and other tumors with local injection of hypertonic saline-epinephrine. *Gastrointest Endosc* 1998; **34**: 264-269 [PMID: 3391382 DOI: 10.1016/S0016-5107(88)71327-9]

4 **Tada M**, Murakami A, Karita M, Yanai H, Okita K. Endoscopic resection of early gastric cancer. *Endoscopy* 1993; **25**: 445-450 [PMID: 8261986 DOI: 10.1055/s-2007-1010365]

5 **Kojima T**, Parra-Blanco A, Takahashi H, Fujita R. Outcome of endoscopic mucosal resection for early gastric cancer: review of the Japanese literature. *Gastrointest Endosc* 1998; **48**: 550-554; discussion 554-555 [PMID: 9831855 DOI: 10.1016/S0016-5107(98)70108-7]

6 **Gotoda T**, Kondo H, Ono H, Saito Y, Yamaguchi H, Saito D, Yokota T. A new endoscopic mucosal resection procedure using an insulation-tipped electrosurgical knife for rectal flat lesions: report of two cases. *Gastrointest Endosc* 1999; **50**: 560-563 [PMID: 10502182 DOI: 10.1016/S0016-5107(99)70084-2]

7 **Yamamoto H**, Kawata H, Sunada K, Sasaki A, Nakazawa K, Miyata T, Sekine Y, Yano T, Satoh K, Ido K, Sugano K. Successful en-bloc resection of large superficial tumors in the stomach and colon using sodium hyaluronate and small-caliber-tip transparent hood. *Endoscopy* 2003; **35**: 690-694 [PMID: 12929067 DOI: 10.1055/s-2003-41516]

8 **Oda I,** Gotoda T, Hamanaka H, Eguchi T, Saito Y, Matsuda T, Bhandari P, Emura F, Saito D, Ono H. Endscopic submucosal dissection for early gastric cancer: Technical feasibility, operation time and complications from a large consecutive series. *Dig Endosc* 2005; **17:** 54-58 [DOI: 10.1111/j.1443-1661.2005.00459.x]

9 **Onozato Y**, Ishihara H, Iizuka H, Sohara N, Kakizaki S, Okamura S, Mori M. Endoscopic submucosal dissection for early gastric cancers and large flat adenomas. *Endoscopy* 2006; **38**: 980-986 [PMID: 17058161 DOI: 10.1055/s-2006-944809]

10 **Onozato Y**, Kakizaki S, Ishihara H, Iizuka H, Sohara N, Okamura S, Mori M, Itoh H. Endoscopic submucosal dissection for rectal tumors. *Endoscopy* 2007; **39**: 423-427 [PMID: 17354181 DOI: 10.1055/s-2007-966237]

11 **Iizuka H**, Okamura S, Onozato Y, Ishihara H, Kakizaki S, Mori M. Endoscopic submucosal dissection for colorectal tumors. *Gastroenterol Clin Biol* 2009; **33**: 1004-1011 [PMID:

19762190 DOI: 10.1016/j.gcb.2009.02.039]
12 **Onozato Y,** Kakizaki S, Ishihara H, Iizuka H, Sohara N, Sohara N, Okamura S, Mori M. Feasibility of endoscopic submucosal dissection for elderly patients with early gastric cancers and adenomas. *Dig Endosc* 2008; **20:** 12-16 [DOI: 10.1111/j.1443-1661.2007.00763.x]
13 **Matsui N**, Akahoshi K, Nakamura K, Ihara E, Kita H. Endoscopic submucosal dissection for removal of superficial gastrointestinal neoplasms: A technical review. *World J Gastrointest Endosc* 2012; **4**: 123-136 [PMID: 22523613 DOI: 10.4253/wjge.v4.i4.123]
14 **The Japan Esophageal Society.** Guidelines for the Diagnosis and Treatment of Esophageal Cancer. Tokyo: Kanehara & Co. Ltd, 2007
15 **Japanese Gastric Cancer Association.** Japanese gastric cancer treatment guidelines 2010 (ver. 3). *Gastric Cancer* 2011; **14**: 113-123 [PMID: 21573742 DOI: 10.1007/s10120-011-0042-4]
16 **Watanabe T**, Itabashi M, Shimada Y, Tanaka S, Ito Y, Ajioka Y, Hamaguchi T, Hyodo I, Igarashi M, Ishida H, Ishiguro M, Kanemitsu Y, Kokudo N, Muro K, Ochiai A, Oguchi M, Ohkura Y, Saito Y, Sakai Y, Ueno H, Yoshino T, Fujimori T, Koinuma N, Morita T, Nishimura G, Sakata Y, Takahashi K, Takiuchi H, Tsuruta O, Yamaguchi T, Yoshida M, Yamaguchi N, Kotake K, Sugihara K. Japanese Society for Cancer of the Colon and Rectum (JSCCR) guidelines 2010 for the treatment of colorectal cancer. *Int J Clin Oncol* 2012; **17**: 1-29 [PMID: 22002491 DOI: 10.1007/s10147-011-0315-2]
17 **Yamamoto H**. Endoscopic submucosal dissection of early cancers and large flat adenomas. *Clin Gastroenterol Hepatol* 2005; **3**: S74-S76 [PMID: 16013004 DOI: 10.1016/S1542-3565(05)00254-5]
18 **Hirasawa K**, Kokawa A, Oka H, Yahara S, Sasaki T, Nozawa A, Tanaka K. Superficial adenocarcinoma of the esophagogastric junction: long-term results of endoscopic submucosal dissection. *Gastrointest Endosc* 2010; **72**: 960-966 [PMID: 21034897 DOI: 10.1016/j.gie.2010.07.030]
19 **Ohkuwa M**, Hosokawa K, Boku N, Ohtu A, Tajiri H, Yoshida S. New endoscopic treatment for intramucosal gastric tumors using an insulated-tip diathermic knife. *Endoscopy* 2001; **33**: 221-226 [PMID: 11293753 DOI: 10.1055/s-2001-12805]
20 **Rösch T**, Sarbia M, Schumacher B, Deinert K, Frimberger E, Toermer T, Stolte M, Neuhaus H. Attempted endoscopic en bloc resection of mucosal and submucosal tumors using insulated-tip knives: a pilot series. *Endoscopy* 2004; **36**: 788-801 [PMID: 15326574 DOI: 10.1055/s-2004-825838]
21 **Oyama T**, Tomori A, Hotta K, Morita S, Kominato K, Tanaka M, Miyata Y. Endoscopic submucosal dissection of early esophageal cancer. *Clin Gastroenterol Hepatol* 2005; **3**: S67-S70 [PMID: 16013002 DOI: 10.1016/S1542-3565(05)00291-0]
22 **Ono S**, Fujishiro M, Niimi K, Goto O, Kodashima S, Yamamichi N, Omata M. Long-term outcomes of endoscopic submucosal dissection for superficial esophageal squamous cell neoplasms. *Gastrointest Endosc* 2009; **70**: 860-866 [PMID: 19577748 DOI: 10.1016/j.gie.2009.04.044]
23 **Kato M**, Nishida T, Tsutsui S, Komori M, Michida T, Yamamoto K, Kawai N, Kitamura S, Zushi S, Nishihara A, Nakanishi F, Kinoshita K, Yamada T, Iijima H, Tsujii M, Hayashi N. Endoscopic submucosal dissection as a treatment for gastric noninvasive neoplasia: a multicenter study by Osaka University ESD Study Group. *J Gastroenterol* 2011; **46**: 325-331 [PMID: 21107615 DOI: 10.1007/s00535-010-0350-1]
24 **Mannen K**, Tsunada S, Hara M, Yamaguchi K, Sakata Y, Fujise T, Noda T, Shimoda R, Sakata H, Ogata S, Iwakiri R, Fujimoto K. Risk factors for complications of endoscopic submucosal dissection in gastric tumors: analysis of 478 lesions. *J Gastroenterol* 2010; **45**: 30-36 [PMID: 19760133 DOI: 10.1007/s00535-009-0137-4]
25 **Isomoto H**, Shikuwa S, Yamaguchi N, Fukuda E, Ikeda K, Nishiyama H, Ohnita K, Mizuta Y, Shiozawa J, Kohno S. Endoscopic submucosal dissection for early gastric cancer: a large-scale feasibility study. *Gut* 2009; **58**: 331-336 [PMID: 19001058 DOI: 10.1136/gut.2008.165381]
26 **Chung IK**, Lee JH, Lee SH, Kim SJ, Cho JY, Cho WY, Hwangbo Y, Keum BR, Park JJ, Chun HJ, Kim HJ, Kim JJ, Ji SR, Seol SY. Therapeutic outcomes in 1000 cases of endoscopic submucosal dissection for early gastric neoplasms: Korean ESD Study Group multicenter study. *Gastrointest Endosc* 2009; **69**: 1228-1235 [PMID: 19249769 DOI: 10.1016/j.gie.2008.09.027]
27 **Saito Y**, Uraoka T, Yamaguchi Y, Hotta K, Sakamoto N, Ikematsu H, Fukuzawa M, Kobayashi N, Nasu J, Michida T, Yoshida S, Ikehara H, Otake Y, Nakajima T, Matsuda T, Saito D. A prospective, multicenter study of 1111 colorectal endoscopic submucosal dissections (with video). *Gastrointest Endosc* 2010; **72**: 1217-1225 [PMID: 21030017 DOI: 10.1016/j.gie.2010.08.004]
28 **Fujishiro M**, Yahagi N, Kakushima N, Kodashima S, Muraki Y, Ono S, Yamamichi N, Tateishi A, Oka M, Ogura K, Kawabe T, Ichinose M, Omata M. Outcomes of endoscopic submucosal dissection for colorectal epithelial neoplasms in 200 consecutive cases. *Clin Gastroenterol Hepatol* 2007; **5**: 678-683; quiz 645 [PMID: 17466600 DOI: 10.1016/j.cgh.2007.01.006]
29 **Toyonaga T**, Man-i M, Fujita T, East JE, Nishino E, Ono W, Morita Y, Sanuki T, Yoshida M, Kutsumi H, Inokuchi H, Azuma T. Retrospective study of technical aspects and complications of endoscopic submucosal dissection for laterally spreading tumors of the colorectum. *Endoscopy* 2010; **42**: 714-722 [PMID: 20806155 DOI: 10.1055/s-0030-1255654]
30 **Wesdorp IC**, Bartelsman JF, Huibregtse K, den Hartog Jager FC, Tytgat GN. Treatment of instrumental oesophageal perforation. *Gut* 1984; **25**: 398-404 [PMID: 6706219 DOI: 10.1136/gut.25.4.398]
31 **Yoshikane H**, Hidano H, Sakakibara A, Ayakawa T, Mori S, Kawashima H, Goto H, Niwa Y. Endoscopic repair by clipping of iatrogenic colonic perforation. *Gastrointest Endosc* 1997; **46**: 464-466 [PMID: 9402126 DOI: 10.1016/S0016-5107(97)70045-2]
32 **Minami S**, Gotoda T, Ono H, Oda I, Hamanaka H. Complete endoscopic closure of gastric perforation induced by endoscopic resection of early gastric cancer using endoclips can prevent surgery (with video). *Gastrointest Endosc* 2006; **63**: 596-601 [PMID: 16564858 DOI: 10.1016/j.gie.2005.07.029]
33 **Neuhaus H**, Costamagna G, Devière J, Fockens P, Ponchon T, Rösch T. Endoscopic submucosal dissection (ESD) of early neoplastic gastric lesions using a new double-channel endoscope (the "R-scope"). *Endoscopy* 2006; **38**: 1016-1023 [PMID: 17058167 DOI: 10.1055/s-2006-944830]
34 **Taku K**, Sano Y, Fu KI, Saito Y, Matsuda T, Uraoka T, Yoshino T, Yamaguchi Y, Fujita M, Hattori S, Ishikawa T, Saito D, Fujii T, Kaneko E, Yoshida S. Iatrogenic perforation associated with therapeutic colonoscopy: a multicenter study in Japan. *J Gastroenterol Hepatol* 2007; **22**: 1409-1414 [PMID: 17593224 DOI: 10.1111/j.1440-1746.2007.05022.x]

P- Reviewers Oda I, Fujishiro M **S- Editor** Huang XZ
L- Editor A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i4.536

World J Gastroenterol 2013 January 28; 19(4): 536-541
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Cdx2 expression and its promoter methylation during metaplasia-dysplasia-carcinoma sequence in Barrett's esophagus

Kenji Makita, Riko Kitazawa, Shuho Semba, Koto Fujiishi, Miku Nakagawa, Ryuma Haraguchi, Sohei Kitazawa

Kenji Makita, Riko Kitazawa, Koto Fujiishi, Miku Nakagawa, Ryuma Haraguchi, Sohei Kitazawa, Division of Molecular Pathology, Ehime University Graduate School of Medicine, Toon 791-0295, Japan
Shuho Semba, Division of Pathology, Kobe University Graduate School of Medicine, Kobe 650-0017, Japan
Author contributions: Makita K, Nakagawa M and Fujiishi K performed the majority of experiments and wrote the manuscript; Semba S and Haraguchi R provided vital reagents and analytical tools; Kitazawa S and Kitazawa R designed the study and were also involved in editing the manuscript.
Supported by Grant-in Aid from Ministry of Education, Sports and Culture (GP Program for Basic Science), Japan
Correspondence to: Dr. Sohei Kitazawa, Division of Molecular Pathology, Ehime University Graduate School of Medicine, Shitsukawa, Toon City, Ehime 791-0295, Japan. kitazawa@m.ehime-u.ac.jp
Telephone: +81-89-9605265 Fax: +81-89-9605267
Received: September 1, 2012 Revised: December 4, 2012
Accepted: December 22, 2012
Published online: January 28, 2013

Abstract

AIM: To examine how the expression of caudal type homebox transcription factor 2 (Cdx2) is regulated in the development of malignancy in Barrett's esophagus.

METHODS: Cdx2, mucin (MUC) series (MUC2, MUC5AC and MUC6), p53 and E-cadherin expression in Barrett's esophagus and adenocarcinoma specimens were examined by immunostaining. Isolated clusters of cells from (1) MUC2 and Cdx2-positive intestinal metaplastic mucosa; (2) MUC5AC and MUC6-positive, and MUC2 and Cdx2-negative high-grade dysplasia (HD), or intramucosal adenocarcinoma (IMC); and (3) MUC5AC, MUC6 and Cdx2-positive poorly-differentiated invasive adenocarcinoma (PDA) were analyzed by methylation-specific polymerase chain reaction using sets of primers for detecting methylation status of the *Cdx2* gene.

RESULTS: Most of the non-neoplastic Barrett's esophageal mucosa showing intestinal-type metaplasia with or without low-grade dysplasia was positive for E-cadherin, MUC series and Cdx2, but negative for p53. A portion of the low-grade to HD was positive for E-cadherin, MUC5AC, MUC6 and p53, but negative for MUC2 and Cdx2. The definite IMC area was strongly positive for MUC5AC, MUC6 and p53, but negative for MUC2 and Cdx2. Methylation of the Cdx2 promoter was not observed in intestinal metaplasia, while hypermethylation of part of its promoter was observed in hot dipped and IMC. Hypermethylation of a large fraction of the Cdx2 promoter was observed in PDA.

CONCLUSION: Cdx2 expression is restored irrespective of the methylation status of its promoter. Apparent positive immunohistochemical results can be a molecular mark for gene silencing memory.

© 2013 Baishideng. All rights reserved.

Key words: Barrett¡'s esophagus; Caudal type homebox transcription factor 2; Intestinal metaplasia; Promoter hypermethylation

Makita K, Kitazawa R, Semba S, Fujiishi K, Nakagawa M, Haraguchi R, Kitazawa S. Cdx2 expression and its promoter methylation during metaplasia-dysplasia-carcinoma sequence in Barrett's esophagus. *World J Gastroenterol* 2013; 19(4): 536-541 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i4/536.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i4.536

INTRODUCTION

Barrett's esophagus, first described in 1950 and refined in 1957, is a condition whereby the distal esophageal squamous epithelium is replaced by metaplastic columnar epithelium[1]. Three types of morphologically distinct

metaplastic columnar epithelia are recognized in Barrett's esophagus: gastric-fundic, gastric-cardiac (junctional type), and intestinal (specialized type) metaplasia[2]. Reflecting a finding that patients with intestinal-type epithelium are at increased risk of developing adenocarcinoma, the American College of Gastroenterology has recently proposed a restricted definition of Barrett's esophagus: "a change in the esophageal epithelium of any length that can be recognized at endoscopy and is confirmed to have intestinal metaplasia at biopsy"[3]. Although a recent cohort study has demonstrated that the frequency of cancer development in Barrett's esophagus is not related to the presence of intestinal metaplasia, metaplastic columnar epithelium, *per se*, is generally accepted as a precancerous process predisposed to develop discrete neoplastic lesions such as the gastric or foveolar type, the adenomatous or intestinal type, hybrid type dysplasia, and intramucosal [high-grade dysplasia (HD) or intramucosal adenocarcinoma (IMC)] and invasive cancers[4]. Cancers derived from Barrett's esophagus are histopathologically classified into two major categories: gastric and intestinal[5]. Since most Barrett-related IMC cases are either gastric or intestinal with distinct phenotypic stability during progression, two separate (gastric and intestinal) pathways of carcinogenesis have been proposed[5]. Importantly, during the progression of the intestinal pathway, a gradual decrease in transcription factor caudal type homebox transcription factor 2 (Cdx2, a caudal-related homeobox gene essential for skeletal and intestinal development has been noted, suggesting its tumor suppressor role in Barrett's esophagus[5].

We encountered a case of invasive esophageal adenocarcinoma developing into intestinal-type dysplasia and IMC, and examined Cdx2 expression and its promoter methylation status in close histopatho- logical relation to the progression stages with the use of microdissection and methylation-specific polymerase chain reaction (MSP).

MATERIALS AND METHODS

Patient

An 81-year-old Japanese man was admitted to our hospital complaining of heartburn especially after eating sweet fare. The patient had undergone stomach surgery (distal partial gastrectomy) due to gastric ulcer nearly forty years earlier. Because of gastric regurgitation, he had undergone endoscopic examination of the upper digestive tract, which revealed severe reflux esophagitis with widespread Barrett's esophagus. A biopsy was taken from irregularly elevated lesions inside the Barrett's esophagus, and a histological examination confirmed esophageal adenocarcinoma in the lesions. An esophagectomy was carried out, and the right hemicolon was rebuilt. The patient has been free of recurrence for two years since the operation.

Immunohistochemistry

The specimens of Barrett's esophagus were subjected to immunohistochemistry using diaminobenzidine as the chromogen. Deparaffinized sections of formalin-fixed tissue were stained with mucin (MUC) series (Novocastra Laboratories Ltd., Newcastle upon Tyne, United Kingdom), p53 (Lab Vision, Kalamazoo, United States) and E-cadherin (Dako, Denmark) antibody diluted at 1:100 after heat-induced antigen retrieval and with Cdx2 (Dako, Denmark) antibody diluted at 1:50. Anti-rabbit immunoglobulin G (IgG) was used as the secondary antibody for p53 and anti-mouse IgG was used for MUC series, Cdx2 and E-cadherin.

Agarose-bead mediated template preparation

Paraffin-embedded samples were deparaffinized in xylene and subjected to microdissection under light microscopic observation (Leica Microsystems, LMD7000) with the aid of both E-cadherin immunostaining and Cdx2 immunostaining. The microdissected samples were liquefied in low-melting agarose (3.2%) at 1:1, and agarose beads were made by chilling on ice. Beads were treated with proteinase K, followed by bisulfite conversion, as previously described[6].

Polymerase chain reaction amplification and sequencing

Bead fragments were analyzed by MSP using sets of primers for accessing the methylation status of the *Cdx2* gene. The promoter region of the human *Cdx2* genomic sequence (GenBank accession no. AL591024) was searched for CpG islands with an online search engine (www.ebi.ac.uk/emboss/cpgplot). One of the CpG islands (AL591024 nt 28391-28683) was further analyzed for methylation status by MSP. In the first-step polymerase chain reaction (PCR) amplification, a 183-bp amplicon containing 71-bp CpG sites, was amplified with two primers, (forward) 5'-GCCAAGGGGCCTAGGGCTGGA-3', and (reverse) 5'-GTTCACCTCCTAATACAAGCCTTTG-3' (Table 1), under the following conditions: 98°C 2 min, 30 cycles (98 ℃ 10 s, 50 ℃ 15 s, 68 ℃ 39 s). The primers used for second-step PCR were, (forward) 5'-GGAGCTGCCCCGACAGGAGCG-3', and (reverse) 5'-CGCGCCCAGCTCGGn TTTCAGCAA-3' (Table 1), under the following conditions: 98 ℃ 2 min, 25 cycles (98 ℃ 10 s, 60 ℃ 15 s, 68 ℃ 30 s). The PCR mixture contained Mighty AMP® DNA polymerase (Takara, Tokyo, Japan) and bead fragments in a final volume of 25 μL. The PCR products were electrophoresed in a 3% agarose gel, stained with ethidium bromide and visualized under ultraviolet light.

Ethics

Written informed consent was obtained from this patient, and this study was reviewed and approved by the local ethics committee at Ehime University.

RESULTS

Pathological findings

Grossly, a superficial spreading IMC surrounded by low-

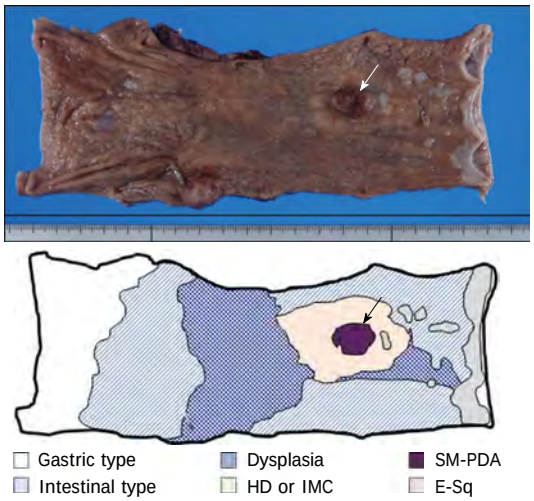


Figure 1 Macroscopic findings of excised esophagus. Surgical specimen shows the presence of superficial spreading carcinoma, extending between 30 mm from the oral and 105 mm from the anal surgical margins. The superficial spreading region is mostly composed of high-grade dysplasia (HD), or intramucosal adenocarcinoma (IMC), surrounded by dysplastic change (E-sq low-grade dysplasia). Inside this superficial spreading region, one observable elevated nodule (arrow) is composed of solid and submucosal invasive poorly-differentiated invasive adenocarcinoma (SM-PDA) with lymphatic invasion. The background non-neoplastic esophageal mucosa is extensively replaced by glandular mucosa with and without intestinal metaplasia.

Table 1 Primer sequences used in polymerase chain-based assays, product size and annealing temperature

	Primer sequence	Size (bp)	Temp (℃)
First PCR	(F)5′-GTTAAGGGGTTTAGGGTTGGA	183	60
Nested PCR	(R)5′-CAAAAACTTATATTAAAAAATAAAC		
Methylated	(F) 5′-GGAGTTGTTTCGATAGGAGCGC	71	60
	(R) 5′-TTACTAAAACCGAACTAAACGCG		
Unmethylated	(F) 5′-GGAGTTGTTTTGATAGGAGTGT	71	60
	(R) 5′-TTACTAAAACCAAACTAAACACA		

Temp: Temperature; PCR: Polymerase chain reaction; R: Reverse; F: Forward.

Table 2 Summary of immunohistochemical findings

	G-type	I-type (IM)	HD or IMC	PDA
MUC2	-	+	-	-
MUC5AC	+	-	++	+
MUC6	+	-	++	+
p53	-	-	++	++
E-cadherin	++	++	++	+
Cdx2	-	+	-	+

G-type: Gastric metaplasia; IM: Intestinal metaplasia; HD or IMC: High-grade dysplasia or intramucosal adenocarcinoma; PDA: Poorly-differentiated invasive adenocarcinoma; MUC: Mucin.

grade dysplasia and intestinal-type metaplasia extended between 30 mm from the oral and 105 mm from the anal surgical margins (Figure 1). One elevated nodule was noted inside this superficial spreading region (Figure 1). Microscopically, the background non-neoplastic esophageal mucosa was replaced, very extensively, by gastric foveolar type mucosa with (Figure 1) and without intestinal metaplasia (Figure 1). The superficial spreading IMC region was mostly composed of definite well-differentiated tubular adenocarcinoma or HD, surrounded by dysplastic change (low-grade dysplasia). The oral elevated nodular ridge was a solid, poorly-differentiated, invasive adenocarcinoma with lymphatic invasion, but no venous invasion or metastasis within the esophageal mucosa was observed. Immunohistochemically, the original esophageal squamous epithelium was positive for E-cadherin, but negative for all the MUC series (MUC2, MUC5AC and MUC6), Cdx2 and p53. Most of the non-neoplastic Barrett's esophageal mucosa showing intestinal-type metaplasia with or without low-grade dysplasia was positive for E-cadherin, MUC2, MUC5AC, MUC6 and Cdx2, but negative for p53. A portion of the low-grade to high-grade dysplasia was positive for E-cadherin, MUC5AC, MUC6 and p53, but negative for MUC2 and Cdx2. The definite IMC area was strongly positive for MUC5AC, MUC6 and p53, but negative for MUC2 and Cdx2. Figure 2 shows the transitional area between the intestinal metaplasia with low-grade dysplasia and the definite IMC area. A portion of the poorly-differentiated adenocarcinoma was positive for MUC5AC, MUC6, Cdx2 and p53, but negative for MUC2 (Figure 3). Table 2 shows a summary of the immunohistochemical findings.

Microdissection and MSP of the Cdx2 promoter

MSP revealed no methylation in Cdx2-positive Barrett's mucosa with intestinal metaplasia (Figure 4, intestinal type). Microdissected samples from the Cdx2-negative IMC area showed that a fraction of the cells was hypermethylated (Figure 4, IMC). Although Cdx2 expression was found by immunohistochemical analysis, samples from the poorly-differentiated invasive (PDA) area showed a hypermethylation pattern (Figure 4, submucosal invasive PDA).

DISCUSSION

Cdx2 is an intestine-specific transcription factor expressed in cells constituting the mucosal epithelium from the duodenum to the rectum[7]. While Cdx2 is negative for the normal foveolar mucosa of the stomach and the squamous epithelium in the esophagus stemming from the foregut, its heterotopic expression in Barrett's esophagus is observed especially in cases with intestinal-type metaplasia[8]. Among most of the terminal differentiation-specific transcription factors, Cdx2 is known to play a tumor suppressor role in cancer progression in the distal colon, a role, which in adults, is functionally and geographically distinct from the homeotic role of Cdx2 during gut development[9]. In our present case, and in confirming its tumor suppressor role, Cdx2 expression diminished during the progression from intestinal-type metaplasia to distinct IMC. Mirroring Cdx2 expression at the protein level by immunohistochemistry, the hypermethylation of the *Cdx2* gene promoter was revealed (Figure 4, IMC). Since primers used for MSP are set to amplify the *Cdx2* gene promoter with hypermethylation,

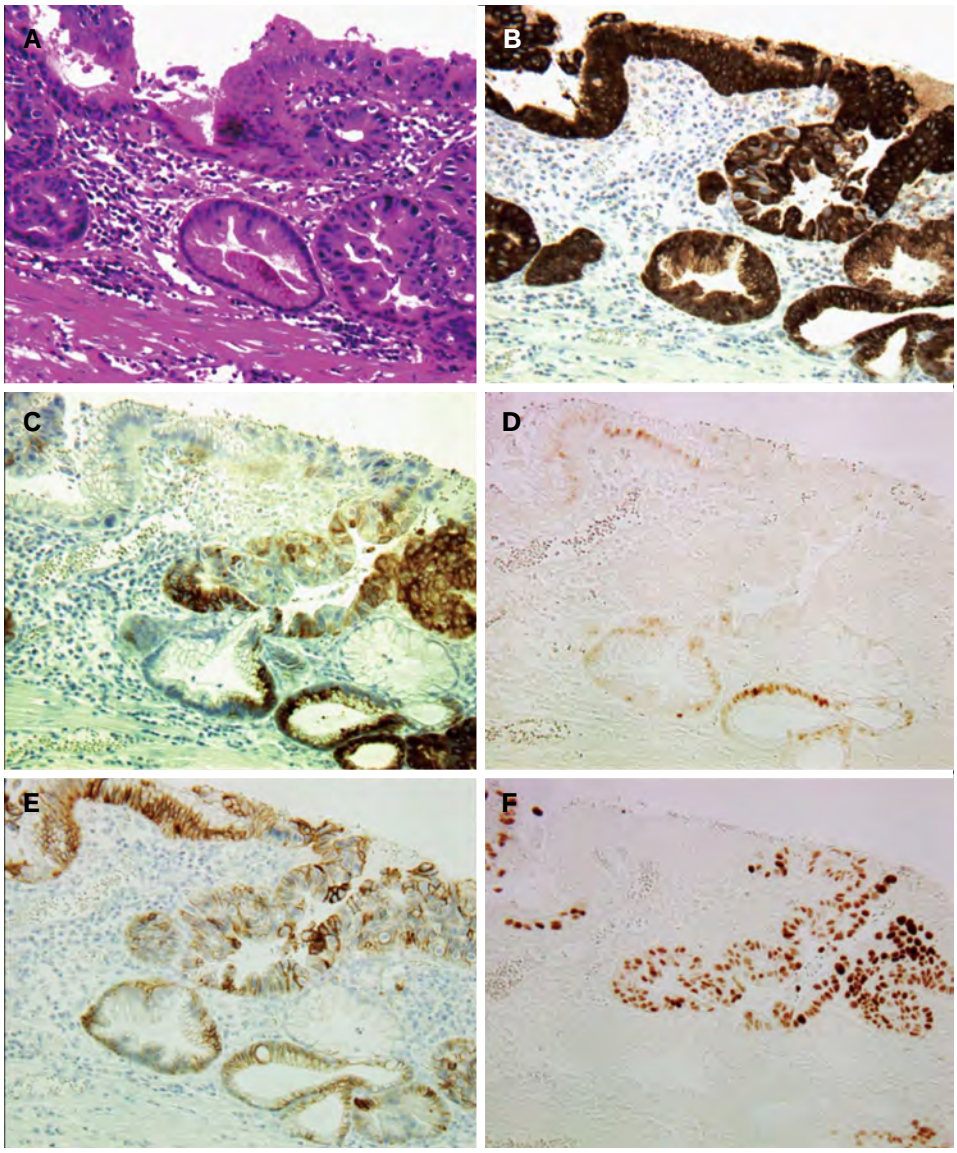


Figure 2 Histological findings of transitional area between intestinal metaplasia and high-grade dysplasia or intramucosal adenocarcinoma (× 200). A: Hematoxylin and eosin staining of transitional area. Intestinal metaplasia (IM) stretches from the upper left to the lower right corner; B: Mucin (MUC) 5AC immunostaining. Strong MUC5AC expression is observed in both IM and high-grade dysplasia (HD), or intramucosal adenocarcinoma (IMC) areas; C: MUC6 immunostaining. MUC6 expression is observed mostly in parts of the HD or IMC areas; D: Caudal type homebox transcription factor 2 (Cdx2) immunostaining. Cdx2 expression is observed only in the nuclei of the cells in the IM area; E: E-cad immunostaining. E-cad expression is observed on the membranes of cells in both IM and HD or IMC areas; F: p53 immunostaining. Strong p53 expression is observed in the nuclei of the cells in the HD or IMC area.

i.e., when all CpGs are methylated, a large fraction of the cells may acquire partial or scatter-type CpG methylation and, therefore, the *Cdx2* gene promoter may have been underestimated in our MSP. In support of our current study, Khor *et al*[5] also demonstrated the gradual down-regulation of Cdx2 expression during progression in adenomatous dysplasia, at least in the intestinal pathway of the Barrett esophageal cancers. These data suggest that Cdx2 also plays a tumor-suppressor role in the metaplasia-dysplasia-carcinoma sequence in Barrett's esophagus. In our present case, irrespective of the hypermethylation status of the *Cdx2* gene promoter, Cdx2 expression was restored in PDA as analyzed by immunohistochemistry (Figure 3F). To achieve final gene-silencing, chromatin condensation followed by modifications of histone proteins are essential[10], we therefore hypothesize that epigenetic alterations other than demethylation may lead to *Cdx2* gene reactivation during the progression phase. Indeed, our previous study showed that hypermethylation of the *E-cadherin* gene promoter and MeCP2, a methyl-CpG binding domain protein, synergistically silenced gene expression in colorectal cancers[6]. Therefore, it is evident that hypermethylation of the gene promoter, *per se*, is essential for establishing gene silencing, but not sufficient for blocking gene expression. Since in our present case, Cdx2 reactivation did not correlate with differentiated intestinal phenotype, but was observed in invasive or aggressive phenotypes, the tumor suppressive effect of Cdx2 on these invasive cancer cells might be lost. These somewhat complicated epigenetical events may partly explain the dispersion of Cdx2 expression. Therefore, when characterizing cancer cells by immunophe-

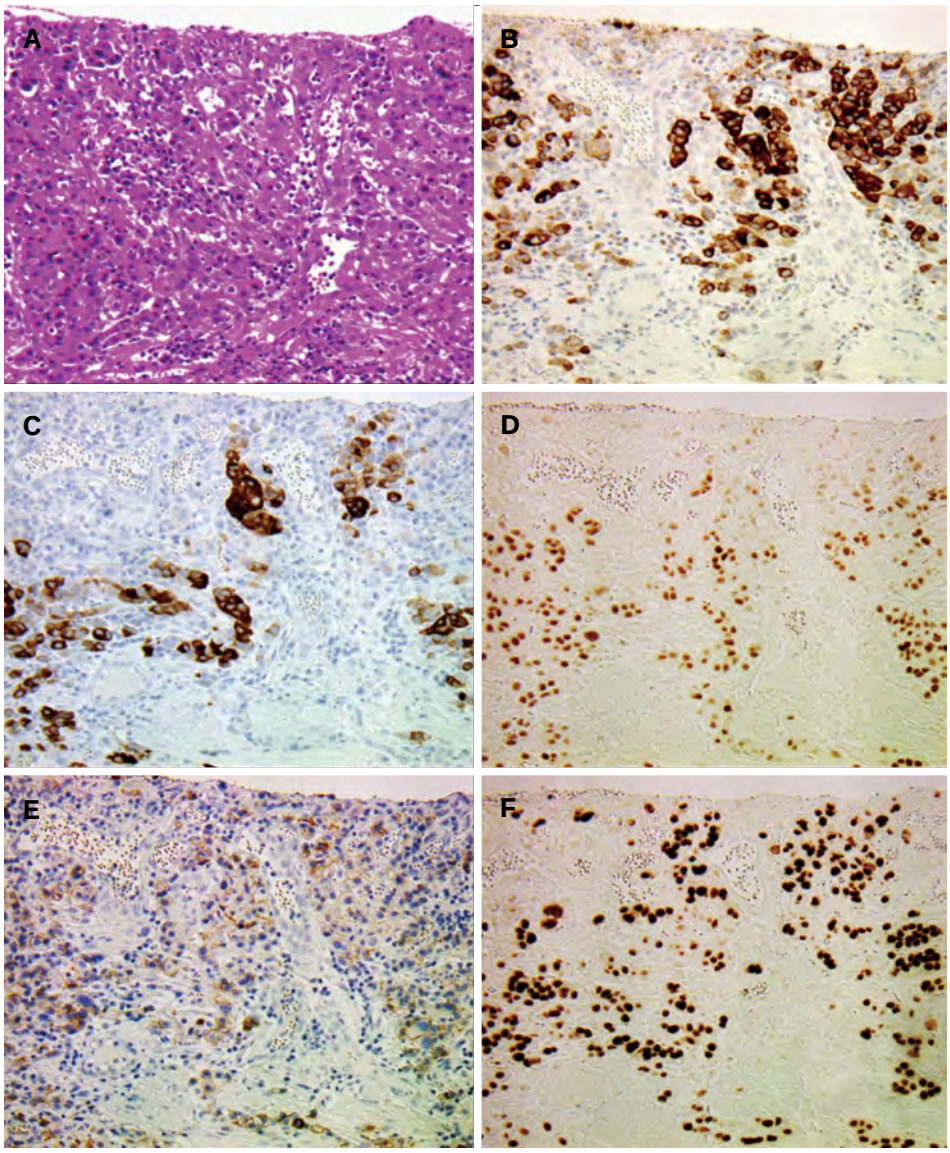


Figure 3 Histological findings of diffuse poorly-differentiated invasive adenocarcinoma (× 200). A: Hematoxylin and eosin staining of poorly-differentiated invasive adenocarcinoma (PDA). Cancer cells are diffusely scattered with prominent stromal reaction; B: Mucin (MUC) 5AC; C: MUC6; D: Caudal type homebox transcription factor 2 expression is positive in the PDA area; E: E-cad expression is markedly reduced in the PDA area; F: Strong p53 expression is observed in the nuclei of the cells in the PDA area.

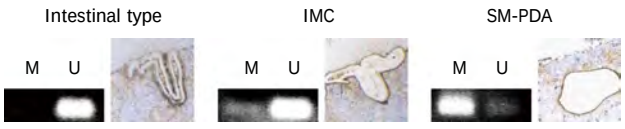


Figure 4 Detection of methylated cytosine by methylation-specific polymerase chain reaction analysis of caudal type homebox transcription factor 2 CpG-island region. Tissue samples were stained with E-cadherin and caudal type homebox transcription factor 2 (Cdx2) to assist cell identification. Cells, either isolated from intestinal metaplasia, intramucosal adenocarcinoma (IMC) or submucosal invasive poorly-differentiated adenocarcinoma (SM-PDA) by laser-assisted microdissection, were subjected to bisulfite conversion and subsequent methylation-specific polymerase chain reaction (MSP). MSP products using primers that specifically amplify only unmethylated DNA are indicated by visible polymerase chain reaction products in line unmethylated pattern (U), while visible polymerase chain reaction products in line methylated pattern (M) indicate those amplified by primers specific for methylated DNA. In intestinal-type metaplasia sections, MSP shows a U pattern, while MSP shows U patterns with partial M patterns in high-grade dysplasia or IMC sections. MSP shows a mostly M pattern in PDA where strong Cdx2 expression is observed (Figure 3D).

notyping, any apparent positive immunohistochemical results should be interpreted carefully with the help of the hypermethylation status as a molecular mark for gene silencing memory[10,11].

ACKNOWLEDGMENTS

We thank Ms. Yuki Takaoka for technical assistance.

COMMENTS

Background

Barrett's esophagus, a pathological condition in which the esophageal squamous epithelium is replaced by metaplastic columnar mucosa, is known to predispose to the development of dysplasia and subsequent cancers.

Research frontiers

Caudal type homebox transcription factor 2 (Cdx2) has recently been shown to play a tumor-suppressor role in the 'metaplasia-dysplasia-carcinoma sequence'

in Barrett's esophagus.

Innovations and breakthroughs

Recent reports have evaluated the phenotypic stability and role of Cdx2 in the neoplastic progression of different types of dysplasias. This suggests that non-intestinalized columnar metaplasia may be an unstable intermediate state at risk for neoplastic progression.

Applications

When characterizing cancer cells by immunophenotyping, any apparent positive immunohistochemical results should be interpreted carefully with the help of the hypermethylation status as a molecular mark for gene silencing memory.

Peer review

The authors examined the expression of Cdx2 and its methylation in Barrett metaplasia and esophageal adenocarcinoma. It revealed that irrespective of the hypermethylation status of the *Cdx2* gene promoter, Cdx2 expression was restored in poorly-differentiated invasive adenocarcinoma. The results are interesting and when characterizing cancer cells by immunophenotyping, any apparent positive immunohistochemical result should be interpreted carefully.

REFERENCES

1 **Bani-Hani KE**, Bani-Hani BK. Columnar-lined esophagus: time to drop the eponym of "Barrett": Historical review. *J Gastroenterol Hepatol* 2008; **23**: 707-715 [PMID: 18410605 DOI: 10.1111/j.1440-1746.2008.05386.x]

2 **Phillips RW**, Frierson HF, Moskaluk CA. Cdx2 as a marker of epithelial intestinal differentiation in the esophagus. *Am J Surg Pathol* 2003; **27**: 1442-1447 [PMID: 14576477 DOI: 10.1097/00000478-200311000-00006]

3 **Montgomery E**. Refining diagnostic criteria for high-grade dysplasia in Barrett esophagus. *Am J Clin Pathol* 2009; **132**: 7-9 [PMID: 19864227 DOI: 10.1309/AJCPPCJC71IFRVVG]

4 **Kelty CJ**, Gough MD, Van Wyk Q, Stephenson TJ, Ackroyd R. Barrett's oesophagus: intestinal metaplasia is not essential for cancer risk. *Scand J Gastroenterol* 2007; **42**: 1271-1274 [PMID: 17852872 DOI: 10.1080/00365520701420735]

5 **Khor TS**, Alfaro EE, Ooi EM, Li Y, Srivastava A, Fujita H, Park Y, Kumarasinghe MP, Lauwers GY. Divergent expression of MUC5AC, MUC6, MUC2, CD10, and CDX-2 in dysplasia and intramucosal adenocarcinomas with intestinal and foveolar morphology: is this evidence of distinct gastric and intestinal pathways to carcinogenesis in Barrett Esophagus? *Am J Surg Pathol* 2012; **36**: 331-342 [PMID: 22261707 DOI: 10.1097/PAS.0b013e31823d08d6]

6 **Darwanto A**, Kitazawa R, Maeda S, Kitazawa S. MeCP2 and promoter methylation cooperatively regulate E-cadherin gene expression in colorectal carcinoma. *Cancer Sci* 2003; **94**: 442-447 [PMID: 12824891 DOI: 10.1111/j.1349-7006.2003.tb01462.x]

7 **Werling RW**, Yaziji H, Bacchi CE, Gown AM. CDX2, a highly sensitive and specific marker of adenocarcinomas of intestinal origin: an immunohistochemical survey of 476 primary and metastatic carcinomas. *Am J Surg Pathol* 2003; **27**: 303-310 [PMID: 12604886 DOI: 10.1097/00000478-200303000-00003]

8 **Jaffee IM**, Rahmani M, Singhal MG, Younes M. Expression of the intestinal transcription factor Heaton in carcinoid tumors is a marker of midgut origin. *Arch Pathol Lab Med* 2006; **130**: 1522-1526 [PMID: 17090195 DOI: 10.1043/1543-2165]

9 **Bonhomme C**, Duluc I, Martin E, Chawengsaksophak K, Chenard MP, Kedinger M, Beck F, Freund JN, Domon-Dell C. The Cdx2 homeobox gene has a tumour suppressor function in the distal colon in addition to a homeotic role during gut development. *Gut* 2003; **52**: 1465-1471 [PMID: 12970140 DOI: 10.1136/gut.52.10.1465]

10 **Raynal NJ**, Si J, Taby RF, Gharibyan V, Ahmed S, Jelinek J, Estécio MR, Issa JP. DNA methylation does not stably lock gene expression but instead serves as a molecular mark for gene silencing memory. *Cancer Res* 2012; **72**: 1170-1181 [PMID: 22219169 DOI: 10.1158/0008-5472.CAN-11-3248]

11 **Liu T**, Zhang X, So CK, Wang S, Wang P, Yan L, Myers R, Chen Z, Patterson AP, Yang CS, Chen X. Regulation of Cdx2 expression by promoter methylation, and effects of Cdx2 transfection on morphology and gene expression of human esophageal epithelial cells. *Carcinogenesis* 2007; **28**: 488-496 [PMID: 16990345 DOI: 10.1093/carcin/bgl176]

P- Reviewers Pei ZH, Smith MDC **S- Editor** Gou SX
L- Editor Webster JR **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i4.542

World J Gastroenterol 2013 January 28; 19(4): 542-549
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Relationship between diversion colitis and quality of life in rectal cancer

Dong Nyoung Son, Dong Jin Choi, Si Uk Woo, Jin Kim, Bo Ra Keom, Chul Hwan Kim, Se Jin Baek, Seon Hahn Kim

Dong Nyoung Son, Jin Kim, Se Jin Baek, Seon Hahn Kim, Colorectal Division, Department of Surgery, Korea University Anam Hospital, Korea University College of Medicine, Seoul 136-705, South Korea
Dong Jin Choi, Department of Surgery, Sun General Hospital, Daejeon 301-747, South Korea
Si Uk Woo, Department of Surgery, Gangseo Song-do Hospital, Seoul 139-711, South Korea
Bo Ra Keom, Department of Gastroenterology, Korea University Anam Hospital, Korea University College of Medicine, Seoul 136-705, South Korea
Chul Hwan Kim, Department of Pathology, Korea University Anam Hospital, Korea University College of Medicine, Seoul 136-705, South Korea
Author contributions: Son DN, Choi DJ, Kim J and Kim SH designed the research; Son DN, Choi DJ, Woo SU and Kim J collected the data; Son DN, Baek SJ, Geom BR, Kim CH and Kim SH analyzed and interpreted the data; Son DN wrote the manuscript; Kim SH critically reviewed the manuscript.
Supported by The 2007 SK Chemical Grant of the Korean Society of Gastroenterology
Correspondence to: Seon Hahn Kim, MD, Colorectal Division, Department of Surgery, Korea University Anam Hospital, Korea University College of Medicine, 126-1, 5 Ga, Anam-Dong, Seongbuk-Gu, Seoul 136-705,
South Korea. drkimsh@korea.ac.kr
Telephone: +82-2-9206644 Fax: +82-2-9281631
Received: September 3, 2012 Revised: November 7, 2012
Accepted: December 15, 2012
Published online: January 28, 2013

Abstract

AIM: To investigated the incidence of diversion colitis (DC) and impact of DC symptoms on quality of life (QoL) after ileostomy reversal in rectal cancer.

METHODS: We performed a prospective study with 30 patients who underwent low anterior resection and the creation of a temporary ileostomy for the rectal cancer between January 2008 and July 2009 at the Department of Surgery, Korea University Anam Hospital. The participants totally underwent two rounds of the examinations. At first examination, endoscopies, tissue biopsies, and questionnaire survey about the symptom were performed 3-4 mo after the ileostomy creations. At second examination, endoscopies, tissue biopsies, and questionnaire survey about the symptom and QoL were performed 5-6 mo after the ileostomy reversals. Clinicopathological data were based on the histopathological reports and clinical records of the patients.

RESULTS: At the first examination, all of the patients presented with inflammation, which was mild in 15 (50%) patients, moderate in 11 (36.7%) and severe in 4 (13.3%) by endoscopy and mild in 14 (46.7%) and moderate in 16 (53.3%) by histology. At the second examination, only 11 (36.7%) and 17 (56.7%) patients had mild inflammation by endoscopy and histology, respectively. There was no significant difference in DC grade between the endoscopic and the histological findings at first or second examination. The symptoms detected on the first and second questionnaires were mucous discharge in 12 (40%) and 5 (17%) patients, bloody discharge in 5 (17%) and 3 (10%) patients, abdominal pain in 4 (13%) and 2 (7%) patients and tenesmus in 9 (30%) and 5 (17%) patients, respectively. We found no correlation between the endoscopic or histological findings and the symptoms such as mucous discharge, bleeding, abdominal pain and tenesmus in both time points. Diarrhea was detected in 9 patients at the second examination; this number correlated with the severity of DC (0%, 0%, 66.7%, 33.3% *vs* 0%, 71.4%, 23.8%, 4.8%, $P = 0.001$) and the symptom-related QoL ($r = -0.791$, $P < 0.001$).

CONCLUSION: The severity of DC is related to diarrhea after an ileostomy reversal and may adversely affect QoL.

© 2013 Baishideng. All rights reserved.

Key words: Diversion colitis; Quality of life; Diarrhea; Ileostomy; Rectal cancer

Son DN, Choi DJ, Woo SU, Kim J, Keom BR, Kim CH, Baek SJ, Kim SH. Relationship between diversion colitis and quality of life in rectal cancer. *World J Gastroenterol* 2013; 19(4): 542-549 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i4/542.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i4.542

INTRODUCTION

The surgical interruption of fecal flow may induce inflammation in the non-functional region of the distal colon[1]. This condition, diversion colitis (DC), is particularly common after an ileostomy or a colostomy. Theoretically, the inflammation typically resolves when the fecal passage resumes[2]. In Western countries, the estimated incidence of DC ranges from 70% to 100%[1,3], but the incidence in Asian countries is unknown. Glotzer *et al*[4] first described the clinical symptom related to defunctioned colon. DC symptoms include abdominal pain, bleeding, mucous discharge, and tenesmus; however, many patients do not present with definitive symptoms[5]. Retrospective and prospective studies report abnormal endoscopic and histological findings in most DC patients[3,6-8]. For patients having rectal surgery for cancer, the functional outcome and quality of life (QoL) are related to the length of the remnant rectum, the radiation therapy parameters and the extent of anastomotic leakage[9-11]. After ileostomy reversal, DC may also influence QoL, but the significance of this influence has not been reported. We hypothesized that the endoscopic and histological incidence of DC before and after ileostomy reversal in rectal cancer patients will correspond with patient's symptoms and impact QoL. Thus, the aims of this study are to determine the incidence of DC and its impact on QoL after ileostomy reversal.

MATERIALS AND METHODS

Forty-eight consecutive patients had surgery for rectal cancer and the creation of a temporary ileostomy at our institution between January 2008 and July 2009. Rectal cancer was defined as tumor 16 cm or less from the anal verge measured with a rigid rectosigmoidoscope (lower third, < 6 cm; middle third, 6-12 cm; upper third, 12-16 cm). The eligible patients were aged 18 years or older with, an American Society of Anesthesiologists class 1 to 3 and diagnosed with rectal cancer within 16 cm from the anal verge. The initial exclusion criteria included inflammatory bowel disease, such as Crohn's disease or ulcerative colitis, preoperative or postoperative radiation therapy and anastomotic leakage after rectal cancer surgery. The indications for chemoradiation prior to surgery were as follows: T4 lesion involvement below the peritoneal reflection, bulky tumor mass or lateral pelvic lymph node metastasis. The surgical indications for rectal cancer included clinical T1-3 lesions based on a pelvic computed tomography and an magnetic resonance imaging, irrespective of any locally lymph node metastasis the within mesorectal fascia. For the enrolled patients, the stoma closure was performed 3-4 mo after the first surgery to allow for 8 patients with stage Ⅲ or Ⅳ disease to complete intravenous chemotherapy. Each patient was hospitalized on 1 d before the ileostomy reversal. At this time, they underwent the first round of endoscopy, tissue biopsy and completed a questionnaire. A single endoscopist conducted the colonoscopies without an enema to ensure the collection of abnormal tissues. Random biopsies were also performed on the descending or transverse colon, even in case of normal colonoscopic findings. A single pathologist examined the tissues. Both the endoscopist and the pathologist were blinded to the patient's symptoms. The first DC symptom questionnaire was also performed during these 3 to 4 mo. Five to six months after the ileostomy reversal, the same endoscopist and pathologist conducted endoscopic and histological examinations to evaluate for complete resolution of DC. In the second questionnaire, we investigated symptom changes and related QoL changes. The endoscopic findings were scored according to edema, mucosal hemorrhage and contact bleeding. These features were scored individually from 0 to 3, 0 to 3, and 0 to 1, respectively, for a total score of 0 to 7. The total score was defined as mild (1 to 2), moderate (3 to 5), or severe (6 to 7). The histological findings were scored according to acute inflammation, chronic inflammation, eosinophilic infiltration, crypt architecture distortion, follicular lymphoid hyperplasia and crypt abscess. These features were scored from 0 to 1; 0 to 2; 0 to 2; 0 to 1; 0 to 1; and 0 to 1, respectively, for a total score of 0 to 8. The total score was further defined as mild (1 to 3), moderate (4 to 6), or severe (7 to 8). Both of the questionnaires investigated DC symptoms such as mucous discharge, bleeding, abdominal pain and tenesmus. However, we excluded fecal frequency, incontinence and the symptoms that are associated with intestinal obstruction (abdominal distension and pain, and emesis). We also excluded the bleeding from hemorrhoids and anal fissures. The QoL section of the questionnaire assessed symptomatic changes from before to after ileostomy reversal as follows: "much worse", "slightly worse", "similar", "slightly better", or "much better". The institutional review board of our institution approved this study and all of the subjects provided informed consent.

Statistical analysis

The data were represented as the median (minimum-maximum), as appropriate for quantitative variables and frequency (percentage) for qualitative variables. The χ^2 test and Fisher's exact test were used to compare between the endoscopic and histological findings about incidence rates of DC, and to test difference or relation with symptoms according to severity of DC and QoL. *P* values less than 0.05 were considered statistically significant. The calculations were performed using SPSS version 12.0 (SPSS Inc, Chicago, IL, United States).

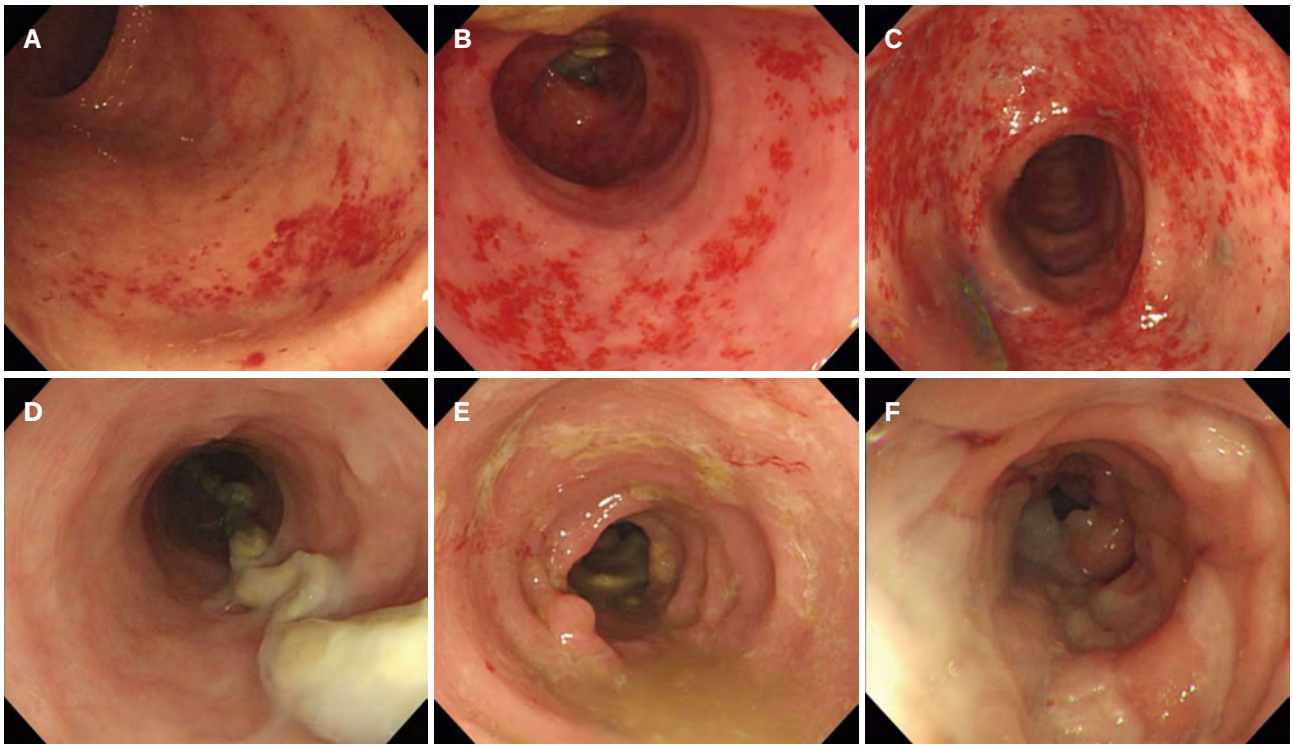


Figure 1 Example of major endoscopic findings in diversion colitis according to relative severity. A-C: Mucosal hemorrhage (A: Score 1; B: Score 2; C: Score3); D-F: Edema (D: Score 1; E: Score 2; F: Score 3).

Table 1 Characteristics for 30 patients with rectal cancer *n* (%)

Parameter	Patients
Age (yr)	59 (31-73)[1]
Gender	
Male	21 (70.0)
Female	9 (30.0)
ASA	
Ⅰ	11 (36.7)
Ⅱ	15 (50)
Ⅲ	4 (13.3)
Ⅳ	0
Tumor location	
Upper rectum	6 (20.0)
Mid rectum	5 (16.7)
Lower rectum	19 (63.3)
Intravenous chemotherapy	
No	22 (73.3)
Yes	8 (26.7)
pTNM staging	
0	4 (13.3)
Ⅰ	6 (20.0)
Ⅱ	11 (36.7)
Ⅲ	7 (23.3)
Ⅳ	2 (6.7)

[1]Values are median (range). ASA: American Society of Anesthesiologists; pTNM: Pathological tumor node metastasis.

RESULTS

Between January 2008 and July 2009, 48 patients were enrolled in the study. However, preceding the study, 18 patients were excluded from the analysis because they underwent a bowel enema procedure (7 patients) that could alter mucosal findings or symptoms, had excessive bowel content that hindered a clear colonoscopic view (9 patients), or had an anastomotic stricture observed during colonoscopy that prevented the visualization of the proximal portion of the colon (2 patients). The final prospective analysis included 30 patients.

The patients' characteristics are listed in Table 1. The median age was 59 years (range: 31-73 years) and the male: female ratio was 7:3. The tumor was located in the upper rectum for 6 patients (20%), the mid-rectum for 5 patients (16.7%) and the lower rectum for 19 patients (63.3%). Only 8 patients received intravenous chemotherapy. Using the endoscopic and histological criteria, all of the patients (100%) developed DC after their ileostomy creation (Table 2). There was no significant difference between the endoscopic and the histological findings in terms of the DC grade prior to ileostomy reversal ($P = 0.084$) or 5-6 mo after reversal ($P = 0.195$), and the endoscopic and histological features decreased after the ileostomy reversal. The incidence of symptomatic DC was 63.3% after ileostomy creation, and 46.6% 5-6 mo after ileostomy reversal. Figure 1 shows the endoscopic findings by the mucosal hemorrhage and edema severity. Figure 2 shows histological findings by the eosinophilic infiltration and chronic inflammation severity. Of the endoscopic findings, edema and mucosal hemorrhage were most common, and contact bleeding was rare. The most common histological finding was chronic inflammation (100%), followed by eosinophilic infiltration (Table 3). The endoscopic and histological abnormalities disappeared or decreased after the ileostomy reversal (Table 3).

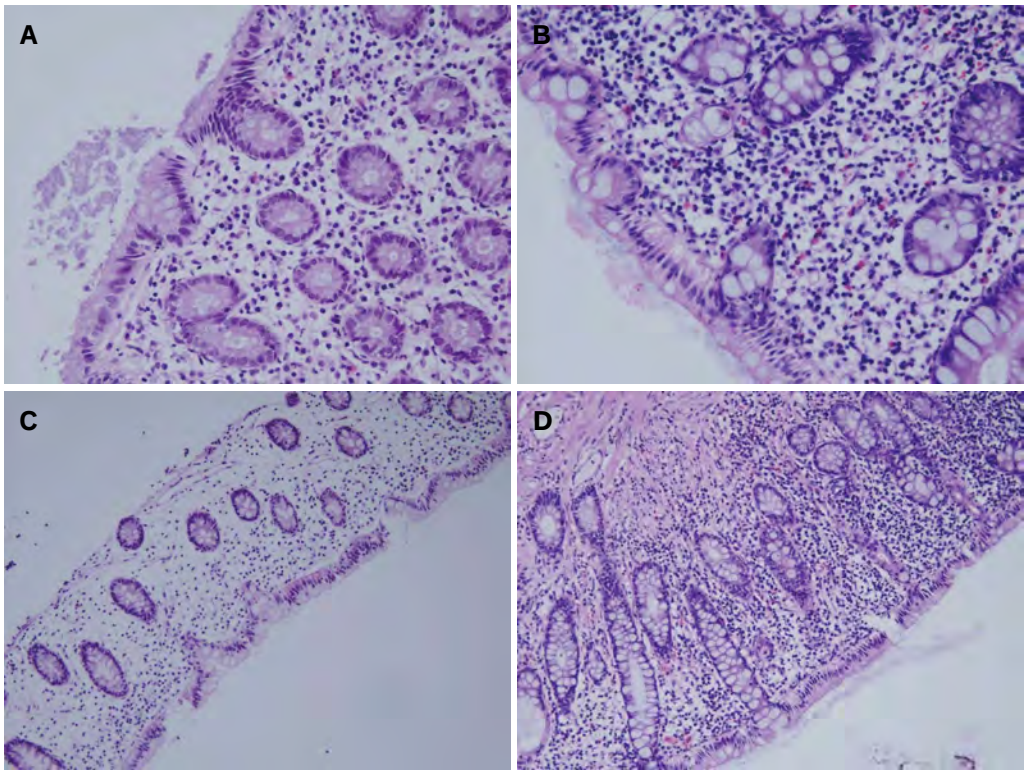


Figure 2 Example of major histological findings in diversion colitis according to relative severity. A, B: Eosinophilic infiltration including numerous cytoplasmic granules and bilobed nucleus (A: Score 1; B: Score 2; hematoxylin and eosin stain × 400); C, D: Chronic inflammation with mononuclear cell such as macrophage,lymaphocytes and plasma cells (C: Score 1; D: Score 2; hematoxylin and eosin stain × 200).

Table 2 Incidence of diversion colitis *n* (%)

Grade of DC	1 d prior to ileostomy reversal			5-6 mo after ileostomy reversal		
	Endoscopic (*n* = 30)	Histological (*n* = 30)	*P* value	Endoscopic (*n* = 30)	Histological (*n* = 30)	*P* value
None	0	0	0.084	19 (63.3)	13 (43.3)	0.195
Mild	15 (50.0)	14 (46.7)		11 (36.7)	17 (56.7)	
Moderate	11 (36.7)	16 (53.3)		0	0	
Severe	4 (13.3)	0		0	0	

DC: Diversion colitis.

Table 3 Endoscopic and histological findings *n* (%)

	1 d prior to ileostomy reversal (*n* = 30)	5-6 mo after ileostomy reversal (*n* = 30)
Endoscopic finding		
Edema	30 (100)	9 (30.0)
Mucosal hemorrhage	22 (73.3)	3 (10.0)
Contact bleeding	6 (20.0)	0
Histological finding		
Acute inflammation	7 (23.3)	0
Chronic inflammation	30 (100)	17 (56.7)
Eosinophilic infiltration	29 (96.7)	9 (30.0)
Crypt architecture distorsion	6 (20.0)	0
Follicular lymphoid hyperplasia	12 (40.0)	0
Crypt abscess	7 (23.3)	0

Before the ileostomy reversal, the most frequent initial symptom, according to the questionnaires, was mucous discharge, followed by tenesmus, bleeding and abdominal pain (Table 4). There were no reports of anal defecation caused by an ileostomy with incomplete diversion prior to the ileostomy reversal. The answers to the questionnaire conducted 5-6 mo after the ileostomy reversal showed that the symptoms had generally decreased since the first survey (Table 4). However, nine patients developed diarrhea as a new symptom (Table 4). We found no correlation between the endoscopic or histological findings and the symptoms before ileostomy reversal.

For example, there were mucous discharge ($P = 0.073$; $P = 0.284$), bleeding ($P = 0.338$; $P = 1.000$), abdominal pain ($P = 0.529$; $P = 0.602$), and tenesmus ($P = 0.075$; $P = 0.440$) (Table 4). Of the symptoms reported after the ileostomy reversal, only diarrhea correlated with the endoscopic and histological findings before the ileostomy reversal ($P = 0.001$; Table 4). In the second questionnaires, 4 patients indicated that they felt 'much worse', 8

Table 4 Correlation between symptoms before and after ileostomy reversal and grade of diversion colitis[1] *n* (%)

Symptom	Endoscopic finding (*n* = 30)				Histological finding (*n* = 30)			
	Mild	Moderate	Severe	*P* value	Mild	Moderate	Severe	*P* value
Symptoms before ileostomy reversal								
Mucous discharge				0.073				0.284
Yes	3 (25.0)	7 (58.3)	2 (16.7)		4 (33.3)	8 (66.7)	0	
No	12 (66.7)	4 (22.2)	2 (11.1)		10 (55.6)	8 (44.4)	0	
Bleeding				0.338				1.000
Yes	1 (20.0)	3 (60.0)	1 (20.0)		2 (40.0)	3 (60.0)	0	
No	14 (56.0)	8 (32.0)	3 (12.0)		12 (48.0)	13 (52.0)	0	
Abdominal pain				0.529				0.602
Yes	1 (25.0)	2 (50.0)	1 (25.0)		1 (25.0)	3 (75.0)	0	
No	14 (53.9)	9 (34.6)	3 (11.5)		13 (50.0)	13 (50.0)	0	
Tenesmus				0.075				0.440
Yes	2 (22.2)	6 (66.7)	1 (11.1)		3 (33.3)	6 (66.7)	0	
No	13 (61.9)	5 (23.8)	3 (14.3)		11 (52.4)	10 (47.6)	0	
Symptoms after ileostomy reversal								
Mucous discharge				0.679				0.336
Yes	3 (60.0)	1 (20.0)	1 (20.0)		1 (20.0)	4 (80.0)	0	
No	12 (48.0)	10 (40.0)	3 (12.0)		13 (52.0)	12 (48.0)	0	
Bleeding				0.550				0.228
Yes	1 (33.3)	1 (33.3)	1 (33.3)		0 (0)	3 (100)	0	
No	14 (51.9)	10 (37.0)	3 (11.1)		14 (51.9)	13 (48.1)	0	
Abdominal pain				0.229				0.485
Yes	1 (50.0)	0	1 (50.0)		0	2 (100)	0	
No	14 (53.8)	11 (39.3)	3 (10.7)		14 (50.0)	14 (50.0)	0	
Tenesmus				0.844				0.336
Yes	2 (40.0)	2 (40.0)	1 (20.0)		1 (20.0)	4 (80.0)	0	
No	13 (52.0)	9 (36.0)	3 (12.0)		13 (52.0)	12 (48.0)	0	
Diarrhea				0.001				0.001
Yes	0	6 (66.7)	3 (33.3)		0	9 (100)	0	
No	15 (71.4)	5 (23.8)	1 (4.8)		14 (66.7)	7 (33.3)	0	

[1]Grade of diversion colitis means result that we examined 1 d prior to ileostomy reversal.

Table 5 Correlation between symptoms and quality of life 5 to 6 mo after ileostomy reversal *n* (%)

Symptom	Much worse	Slightly worse	Similar	Slightly better	Much better	*P* value
Mucous discharge						0.137
Yes	2 (40.0)	0	2 (40.0)	1 (20.0)	0	
No	2 (8.0)	8 (32.0)	13 (52.0)	2 (8.0)	0	
Bleeding						0.797
Yes	0	1 (33.3)	2 (66.7)	0	0	
No	4 (14.8)	7 (25.9)	13 (48.1)	3 (11.1)	0	
Abdominal pain						0.816
Yes	0	1 (50.0)	1 (50.0)	0	0	
No	4 (14.3)	7 (25.0)	14 (50.0)	3 (10.7)	0	
Tenesmus						0.516
Yes	1 (20.0)	2 (40.0)	1 (20.0)	1 (20.0)	0	
No	3 (12.0)	6 (24.0)	14 (56.0)	2 (8.0)	0	
Diarrhea						< 0.001[1]
Yes	4 (44.4)	5 (55.6)	0	0	0	
No	0	3 (14.3)	15 (71.4)	3 (14.3)	0	

[1]Spearman's correlation coefficient = -0.791.

patients felt 'slightly worse', 15 patients felt "similar" and 3 patients felt "slightly better" (Table 5). Finally, diarrhea showed a significant effect in the QoL relevance analysis of symptoms (r = -0.791, P < 0.001; Table 5).

DISCUSSION

Both the endoscopic and histological findings showed that 100% of the patients developed DC following the ileostomy creation. This figure exceeds the rates reported in Western populations (91% and 76%)[5,8]. The symptoms previously associated with DC include mucous discharge, rectal bleeding, abdominal pain and tenesmus[5]. We can check for diarrhea in patients with normal bowel continuity of bowel, but in patients with an ileostomy, we cannot. We expected that diarrhea would occur in DC after the ileostomy reversal as it commonly occurs in other forms of colitis (ulcerative colitis, Crohn's colitis and ischemic colitis). We tested this hypothesis using a questionnaire concerning symptoms, including diarrhea, with a study group that excluded the patients with inflammatory bowel disease and ischemic colitis.

DC symptom rates vary from 6% to 48%, although the overall occurrence is low[5,12,13]. Nineteen patients (63.3%) in our study had at least one symptom after ileostomy creation, and the incidence of symptoms after the ileostomy reversal was 46.6%. Thus, the incidence appears to have decreased slightly; however, these rates represent a dynamic situation. The current analysis showed that mucous discharge, bleeding, abdominal pain and tenesmus resolved, in 11 of 12 patients, 4 of 5 patients, all of 4 patients and 6 of 9 patients, respectively, after the

ileostomy reversal. Most of the symptoms disappeared; however, the symptoms experienced after the ileostomy reversal were either were continued or new but likely not caused by DC. For example, after the ileostomy reversal, 6 patients reported tenesmus; it was a new symptom in 3 patients and continued in 3 patients. Tenesmus can occur as the result of the rectal resection itself. Some authors have suggested that tenesmus resulted from altered motility and the reservoir function of the neorectum[14]. Considering the lack of a relationship between the DC symptoms (except diarrhea), and DC severity in this study, we assume that it is difficult to judge DC severity by these symptoms, particularly in patients with a temporary ileostomy. The endoscopic and histological findings, indicate that the inflammatory changes in DC resolve quickly after intestinal continuity is reestablished[5]. Some authors report normal sigmoidoscopy within 2 mo after restoring colonic continuity[4]. The patients in our study still had mild DC 5-6 mo after the ileostomy reversal. Therefore, the time to complete resolution of DC may vary and may not be predictable. To confirm this hypothesis, further evaluation is needed.

We must consider more active treatment selectively in symptomatic patients who have severe endoscopic/histological findings. There are two options for treating of symptomatic DC. DC symptoms may persist in patients with a permanent stoma. One patient with hemiplegia, for whom stoma reversal was not feasible, underwent rectal resection for severe DC[15]. Surgical treatment may have been appropriate here, but other studies show that medical treatment may also be effective. Early studies suggested that a deficiency of short chain fatty acids (SCFAs) in the excluded colon would contributes to DC[1]. Bacteria produce SCFAs as byproducts of carbohydrate fermentation in the colonic lumen, and SCFAs provide the primary energy source for colonic mucosal cells[15]. Some authors even argue that SCFAs enema may improve the symptoms and endoscopic findings[1]. In human neutrophils, SCFAs reduce the production of reactive oxygen species, which are the agents of oxidative tissue damage[16]. Therefore, SCFAs may be applicable to symptomatic DC patients who have temporary ileostomies.

The current study showed that there were no severe DC symptoms in the patients who had temporary ileostomies. This finding explains why the patients easily endured the ileostomy or, even though they were developed, and were able to wait for timing of ileostomy reversal after clinician's explanation. Most clinicians can predict which symptoms, may not merit treatment clinically.

Patients may not demand active treatment for other symptoms, but they will for diarrhea. The diarrhea appeared soon after the ileostomy reversal, and continued for a minimum of 3 wk and a maximum of 8 wk in this study. Most clinicians previously thought of diarrhea as low anterior resection syndrome (LARS) and others mistook it for a symptom of general enterocolitis. Most of the patients complained of anal skin irritation, lethargy and nervousness. Some went so far as to continue with a stoma rather than have their ileostomy reversed. In the present study, the patients who had diarrheal symptoms and no signs of obstruction could obtain some degree of control with anti-diarrheal agents such as loperamide.

Several factors may affect functional outcome and in QoL of rectal cancer. Hoerske *et al*[11] reported that patients diagnosed with lower rectal cancer have lower QoL score than those with upper and middle rectal cancer because of the smaller reservoir volume. Low anterior resection (LAR) for rectal cancer can leads to severe bowel dysfunction with fecal frequency, urgency and incontinence[17-19]. Other evidence suggests that patients who receive preoperative radiotherapy have a higher rate of urgency, diarrhea and fecal incontinence[20,21]. Therefore, we excluded the patients who received preoperative radiotherapy to limit the confusion with DC symptoms. Generally, radiation therapy can be used as local treatment for rectal cancer. Simunovic *et al*[22] reported that the local recurrence rate of rectal cancer was 6% in a preoperative radiation group *vs* 3% in a non-radiation group. This rate may indicate that radiation therapy can be omitted if surgery is performed on the proper surgical plane with exact preoperative evaluation of the mesorectal fascia. In this study, most of the patients with locally advanced cancer already had lymph node metastases within the mesorectal fascia in the preoperative evaluation. In the United States, however, postoperative chemoradiation therapy has been the standard treatment because of improved local control, without any effect on the overall survival rate[23]. In this study, the patients an advanced cancer stage, underwent chemotherapy only. Hypoxia in the post-surgical bed requires more radiation dose than preoperative state. Postoperative radiation can increase the possibility of acute and chronic gastrointestinal toxicities, such as radiation enterocolitis (including proctitis) and adhesions affecting the small bowel[24]. These events can negatively affect a patient's QoL. Postoperative complications such as anastomotic leakage after LAR can also result in poor long-term functional outcomes[9].

Although some important factors in functional outcome or QoL in this context are known, the role of DC after restoration of fecal movement is unknown. We conducted this study for this reason. The nine patients who developed diarrhea after the ileostomy reversal subsequently reported a moderate to large decrease in QoL. Most of the other symptoms did not correlate with the QoL. Diarrhea was also the only symptom that showed a significant correlation with the severity of DC; hence, we suggest that severe DC can be predicted by the occurrence of diarrhea alone after an ileostomy reversal. In the clinical environment, we propose that clinicians continue treatment with SCFA enemas instead of ileostomy reversal in patients with severe DC.

There are a few limitations in this study. First, the small sample size limited the statistical significance of our findings. Second, we did not use a validated questionnaire,

such as the Fecal Incontinence Quality of Life score analyzing multidimensional aspects that can indirectly reflect QoL. However, considering that the effectiveness of a questionnaire as a data collection tool depends on the nature of the study and the age of patients, we believe that modification of the questionnaire is needed. Third, 25 patients developed LARS with fecal frequency, urgency or incontinence after rectal cancer surgery. During the survey, most patients mistook fecal frequency or urgency for diarrheal symptom. Diarrhea is generally defined as the passage of abnormally liquid stool at an increased frequency, exceeding 200 g/d[25]. Although we tried to survey using an accurate definition, it was difficult to measure the total amount of stool per day. Future studies with a larger sample size, such as a multi-variate analysis of risk factors will be required to ascertain the relationship between LARS and diarrheal symptom. Finally, the pathogenesis of DC has been little reported until now. Some authors suggested that nitrate-reducing bacteria was found to be increased in the colon that is not receiving fecal flow[26]. We are investigating this issue at our hospital based on the hypothesis that differences in the colonic bacterial spectra may play a role in this variation.

In conclusion, in this study of South Korean patients diagnosed with rectal cancer, we observed a higher incidence of DC after temporary ileostomy than that reported for patients in Western countries. This is also the first study to indicate that DC after a temporary ileostomy may adversely affect patient QoL. The severity of DC after the closure of a diverting stoma appeared to influence patient QoL, primarily by causing diarrhea.

COMMENTS

Background

Up to now, the incidence of diversion colitis (DC) in western countries has been published in English literature, but not yet in eastern. Theorectically, DC can happen to patients with ileostomy after low anterior resection (LAR) in rectal cancer, in which patient's quality of life (QoL) after rectal resection is not only related to the remnant rectal length, but can be hypothesized to related to DC.

Research frontiers

Studies regarding the DC have mainly been limited to analyze clinicopathologic finding after surgical situation of the benign disease. Therefore, in patients with temporary ileostomy after LAR in rectal cancer, it would be great interest to examine the DC incidence in Asia and to evaluate if the severity of DC is related to patient's QoL after the ileostomy reversal.

Innovations and breakthroughs

The results of this study showed that DC developed in all patients with ileostomy after LAR and the symptoms in the period of diverting stoma were not correlated with severity of DC. But, diarrhea after the ileostomy reversal was related to the severity of DC.

Applications

There is a need to take notice of DC effect on the patient's QoL in rectal cancer. Severity of DC before an ileostomy reversal in rectal cancer can predict the patient's QoL after an ileostomy reversal and lead the patients into proper education and more intensive management. Also, on the base of this result, clinicians need to proceed the further study to clarify why the severity of DC is variable between the individual with patient's symptoms and impact QoL.

Terminology

DC is defined as the surgical interruption of fecal flow induce inflammation in the non-functional region of the distal colon and this is characterized that the inflammation typically can resolve when the fecal passage resumes.

Peer review

This paper is very well written in spite of a small sample size and has a possible message that the severity of DC seems to have an influence on patient's QoL, particularly on diarrhea, after reversal of a diverting stoma.

REFERENCES

1 **Harig JM**, Soergel KH, Komorowski RA, Wood CM. Treatment of diversion colitis with short-chain-fatty acid irrigation. *N Engl J Med* 1989; **320**: 23-28 [PMID: 2909876]
2 **Roe AM**, Warren BF, Brodribb AJ, Brown C. Diversion colitis and involution of the defunctioned anorectum. *Gut* 1993; **34**: 382-385 [PMID: 8472988]
3 **Ferguson CM**, Siegel RJ. A prospective evaluation of diversion colitis. *Am Surg* 1991; **57**: 46-49 [PMID: 1796797]
4 **Glotzer DJ**, Glick ME, Goldman H. Proctitis and colitis following diversion of the fecal stream. *Gastroenterology* 1981; **80**: 438-441 [PMID: 7450438]
5 **Whelan RL**, Abramson D, Kim DS, Hashmi HF. Diversion colitis. A prospective study. *Surg Endosc* 1994; **8**: 19-24 [PMID: 8153859]
6 **Haque S**, Eisen RN, West AB. The morphologic features of diversion colitis: studies of a pediatric population with no other disease of the intestinal mucosa. *Hum Pathol* 1993; **24**: 211-219 [PMID: 8432517]
7 **Grant NJ**, Van Kruiningen HJ, Haque S, West AB. Mucosal inflammation in pediatric diversion colitis: a quantitative analysis. *J Pediatr Gastroenterol Nutr* 1997; **25**: 273-280 [PMID: 9285377]
8 **Orsay CP**, Kim DO, Pearl RK, Abcarian H. Diversion colitis in patients scheduled for colostomy closure. *Dis Colon Rectum* 1993; **36**: 366-367 [PMID: 8458263]
9 **Hallböök O**, Sjödahl R. Anastomotic leakage and functional outcome after anterior resection of the rectum. *Br J Surg* 1996; **83**: 60-62 [PMID: 8653367]
10 **Dahlberg M**, Glimelius B, Graf W, Påhlman L. Preoperative irradiation affects functional results after surgery for rectal cancer: results from a randomized study. *Dis Colon Rectum* 1998; **41**: 543-549; discussion 549-551 [PMID: 9593234]
11 **Hoerske C**, Weber K, Goehl J, Hohenberger W, Merkel S. Long-term outcomes and quality of life after rectal carcinoma surgery. *Br J Surg* 2010; **97**: 1295-1303 [PMID: 20602501]
12 **Haas PA**, Fox TA, Szilagy EJ. Endoscopic examination of the colon and rectum distal to a colostomy. *Am J Gastroenterol* 1990; **85**: 850-854 [PMID: 2371986]
13 **Ma CK**, Gottlieb C, Haas PA. Diversion colitis: a clinicopathologic study of 21 cases. *Hum Pathol* 1990; **21**: 429-436 [PMID: 2318485]
14 **van Duijvendijk P**, Slors F, Taat CW, Heisterkamp SH, Obertop H, Boeckxstaens GE. A prospective evaluation of anorectal function after total mesorectal excision in patients with a rectal carcinoma. *Surgery* 2003; **133**: 56-65 [PMID: 12563238]
15 **Murray FE**, O'Brien MJ, Birkett DH, Kennedy SM, LaMont JT. Diversion colitis. Pathologic findings in a resected sigmoid colon and rectum. *Gastroenterology* 1987; **93**: 1404-1408 [PMID: 3678755]
16 **Liu Q**, Shimoyama T, Suzuki K, Umeda T, Nakaji S, Sugawara K. Effect of sodium butyrate on reactive oxygen species generation by human neutrophils. *Scand J Gastroenterol* 2001; **36**: 744-750 [PMID: 11444474]
17 **Camilleri-Brennan J**, Steele RJ. Quality of life after treatment for rectal cancer. *Br J Surg* 1998; **85**: 1036-1043 [PMID: 9717993]
18 **Camilleri-Brennan J**, Ruta DA, Steele RJ. Patient generated index: new instrument for measuring quality of life in patients with rectal cancer. *World J Surg* 2002; **26**: 1354-1359 [PMID: 12297930]
19 **Emmertsen KJ**, Laurberg S. Low anterior resection syndrome score: development and validation of a symptom-

based scoring system for bowel dysfunction after low anterior resection for rectal cancer. *Ann Surg* 2012; **255**: 922-928 [PMID: 22504191]

20 **Bruheim K**, Guren MG, Skovlund E, Hjermstad MJ, Dahl O, Frykholm G, Carlsen E, Tveit KM. Late side effects and quality of life after radiotherapy for rectal cancer. *Int J Radiat Oncol Biol Phys* 2010; **76**: 1005-1011 [PMID: 19540058]

21 **Pollack J**, Holm T, Cedermark B, Altman D, Holmström B, Glimelius B, Mellgren A. Late adverse effects of short-course preoperative radiotherapy in rectal cancer. *Br J Surg* 2006; **93**: 1519-1525 [PMID: 17054311 DOI: 10.1002/bjs.5525]

22 **Simunovic M**, Sexton R, Rempel E, Moran BJ, Heald RJ. Optimal preoperative assessment and surgery for rectal cancer may greatly limit the need for radiotherapy. *Br J Surg* 2003; **90**: 999-1003 [PMID: 12905555 DOI: 10.1002/bjs.4210]

23 **NIH consensus conference.** Adjuvant therapy for patients with colon and rectal cancer. *JAMA* 1990; **264**: 1444-1450 [PMID: 2202842 DOI: 10.1001/jama.1990.03450110090034]

24 **Minsky BD**, Cohen AM, Enker WE, Kelsen DP, Kemeny N, Frankel J. Efficacy of postoperative 5-FU, high-dose leucovorin, and sequential radiation therapy for clinically resectable rectal cancer. *Cancer Invest* 1995; **13**: 1-7 [PMID: 7834464]

25 **Thomas PD**, Forbes A, Green J, Howdle P, Long R, Playford R, Sheridan M, Stevens R, Valori R, Walters J, Addison GM, Hill P, Brydon G. Guidelines for the investigation of chronic diarrhoea, 2nd edition. *Gut* 2003; **52** Suppl 5: v1-15 [PMID: 12801941]

26 **Neut C**, Guillemot F, Colombel JF. Nitrate-reducing bacteria in diversion colitis: a clue to inflammation? *Dig Dis Sci* 1997; **42**: 2577-2580 [PMID: 9440640]

P- Reviewer Benjamin P **S- Editor** Gou SX
L- Editor A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i4.550

World J Gastroenterol 2013 January 28; 19(4): 550-555
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Colon transit time according to physical activity and characteristics in South Korean adults

Kang Ok Cho, Yun Ju Jo, Bong Kil Song, Jung Woo Oh, Yeon Soo Kim

Kang Ok Cho, Institute of Korea Exercise and Development, Seoul 151-877, South Korea
Yun Ju Jo, Department of Internal Medicine, Eulji Universitiy School of Medicine, Seoul 139-711, South Korea
Bong Kil Song, Jung Woo Oh, Yeon Soo Kim, Health and Exercise Science Laboratory, Institute of Sports Science, Seoul National University, Seoul 151-742, South Korea
Author contributions: Song BK and Oh JW performed the majority of experiments; Jo YJ provided vital reagents and analytical tools and was also involved in editing the manuscript; Kim YS provided the collection of all the human material in addition to providing financial support for this work; Cho KO designed the study and wrote the manuscript.
Correspondence to: Dr. Yeon Soo Kim, Health and Exercise Science Laboratory, Institute of Sports Science, Seoul National University, Seoul 151-742, South Korea. kys0101@snu.ac.kr
Telephone: +82-2-8891926 Fax: +82-2-8847616
Received: August 16, 2012 Revised: October 22, 2012
Accepted: November 24, 2012
Published online: January 28, 2013

Abstract

AIM: To investigate factors contributing to the colon transit time (CTT), physical activity and characteristics were examined.

METHODS: Forty-seven Korean adults (males, *n* = 23; females, *n* = 24) took a capsule containing 20 radio-opaque markers to measure the CTT. The subjects used an accelerometer to measure the physical activity and underwent a bioelectrical impedance analysis to determine the physical characteristics. Macro-nutrient was also surveyed.

RESULTS: The mean total CTTs (TCTT) in the males and females were 8.8 and 24.7 h (*P* = 0.002), respectively. In the male subjects, the right CTT (3.5 ± 4.9 h *vs* 10.0 ± 11.6 h, *P* = 0.023) and recto-sigmoid CTT (4.4 ± 4.7 *vs* 13.6 ± 12.5 h, *P* = 0.004) were significantly shorter and the total energy expenditure (637.6 ± 44.3 kcal *vs* 464.3 ± 64.9 kcal, *P* = 0.003), total activity count (247 017 ± 75 022 count *vs* 178 014 ± 75 998 count, *P* = 0.003), energy expenditure of light intensity (148.5 ± 6.9 kcal *vs* 120.0 ± 16.8 kcal, *P* = 0.006), energy expenditure of moderate intensity (472.0 ± 36.2 kcal *vs* 281.4 ± 22.2 kcal, *P* < 0.001), fat intake (65.5 ± 23.3 g *vs* 51.2 ± 17.4 g, *P* = 0.010), and water consumption (1714.3 ± 329.4 g *vs* 1164.7 ± 263.6 g, *P* = 0.009) were significantly higher than in the female subjects. Regarding correlations, when adjusted for gender, fiber (*r* = -0.545, *P* < 0.001) and water intake (*r* = -0.257, *P* < 0.05) correlated significantly with the TCTT in all subjects. In addition, the body mass index (*r* = -0.424, *P* < 0.05) and fiber intake (*r* = -0.417, *P* < 0.05) in the males as well as the fiber intake (*r* = -0.655, *P* < 0.001) in the females showed significant correlations with the TCTT.

CONCLUSION: The subjects showed significant gender differences in the TCTT, right CTT, and recto-sigmoid CTT. Furthermore, the intake of the fiber and water contributed to the CTT.

© 2013 Baishideng. All rights reserved.

Key words: Colon transit time; Physical activity; Characteristics; Macro-nutrient; South Korean

Cho KO, Jo YJ, Song BK, Oh JW, Kim YS. Colon transit time according to physical activity and characteristics in South Korean adults. *World J Gastroenterol* 2013; 19(4): 550-555 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i4/550.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i4.550

INTRODUCTION

Colorectal cancer is the third most prevalent human cancer worldwide, with 1 million estimated new case annually, of which, about 50% lead to death[1]. The recog-

nized risk factors include westernized eating habits, obesity, insufficient physical activity and genetic factors[2,3]. However, the effects of gender and individual behavior on the colon transit time (CTT) remain controversial. In some studies, some factors affecting the CTT include age, gender, body mass index (BMI), dietary fiber, water intake, and living habits[4-6]. On the other hand, in a literature review of factors affecting the CTT, age[7], gender[2,3,7], BMI[8], and dietary fiber[9] were not found to be statistically significant.

The impact of exercise and physical activity (PA) on the gastrointestinal and colonic tracts is an area of emerging interest, with recent research focusing on the potential benefits of PA[3,4]. PA is known to be associated with colonic function and constipation[9,10]. In addition, walking, running, and strength training are known to reduce the CTT[10,11]. Also, a period of physical inactivity in subjects who had been engaged in regular PA for several years significantly increased the CTT[12,13]. At present, leisure time activities and transport activities constitute the majority of one's daily physical over all activity[14-16]. Increasing PA is an important health goal for everyone, but doing this requires an accurate method of measuring daily PA. To solve this problem, accelerometers are now being utilized in studies of PA. Accelerometers measure the acceleration of movement and can quantify the intensity, frequency and duration of movement. Lightweight, small and very durable, accelerometers are practical for all ages and activities and can be used indoors or outdoors. Several studies to date have supported the use of accelerometers to assess energy expenditure during locomotion and have confirmed a validity range of 0.77-0.89[11,12,16,17]. For this reason, to measure PA, we analyzed total energy expenditure and energy expenditure of low-, moderate-, and vigorous intensity physical activities using an accelerometer.

Measuring the CTT by the radio-opaque marker method is simple, widely available and important for the diagnosis of slow transit constipation[18-20]. Although there are several reports which sought to measure the CTT using Kolomark™, a radio-opaque marker, normative data on South Koreans are still lacking. Thus, to understand colonic motor function in Koran adults, the CTT was analyzed using Kolomark™ in the present study. To investigate the factors which influence the CTT in South Korean adults, we examined the effects of gender, physical characteristics, physical activity, and macro-nutrient and water intake on the CTT.

MATERIALS AND METHODS

Participants

The study subjects were 47 adults, 23 of whom were male and 24 female. The mean age was 36.0 years with a range of 25-59 years, and all voluntarily gave written informed consent. Of all potential subjects, those with any possible restriction on their normal PA or diet, those with cardiovascular or orthopedic disease which may affect the CTT, menstruating woman those who cannot ingest drugs due to functional stomach diseases, and those on any prescription of anti-constipation drugs or for diabetes mellitus or hypertension were excluded from the current analysis.

Measurement of physical characteristics

A bioelectrical impedance analysis (Inbody, Biospace, Seoul, South Korea) was used to measure the height, weight, BMI, lean body mass (LBM), and percentage of body fat (BF) at the beginning of the study in a fasted state.

Measurement of physical activity

PA was measured over a 1-wk period using an accelerometer (Actical, Mini Mitter, Chicago, IL, United States). To measure PA accurately, the study subjects were advised to perform their daily PA as they normally would. The accelerometer was attached to the iliac crest using a belt. Prior to measurement, the age, sex, height and weight of each participant were entered into the device. The total energy expenditure, activity energy expenditure, and time of activity depending on the PA intensity and frequency were individually measured according to the time. The results were automatically stored. Using the recorded data, the energy expenditure was calculated based on Muffin's formula to calculate the basal metabolic rate[11,17].

Analysis of macro-nutrient intake

To determine the average daily energy, carbohydrate, protein, fat, fiber and water intake, all participants recorded their daily dietary information using the dietary recall method over a 1 wk period during the accelerometer phase. Foods that could not fit into a standard measure were described by the dimensions of their shape, with a grid measured in centimeters to allow these measurements to be done accurately. The dietary records were analyzed using the nutrient analysis program.

Measurement of the CTT

To measure the colonic motor function, the CTT was measured using a multiple marker technique with a radio-opaque marker (Kolomark™, MI Tech, Pyeongtaek, South Korea). The subjects took one capsule containing 20 radio-opaque markers at the same time every day for three consecutive days. On the fourth day, following the first administration, a supine abdominal radiograph was performed. The mean CTT (hours) was calculated by counting the number of radio-opaque markers that were left in the total colon and in each segment of the colon and then multiplying this number by 1.2[18-20].

Ethics

This work was carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association. This study was approved ethically by the Institutional Review Board of Seoul National University (IRB No. 2011/1006). All patients provided informed written consent.

Table 1 Physical characteristic of the participants

Variable	Male (n = 23)	Female (n = 24)	Total (n = 47)	P value
Age (yr)	37.3 ± 5.8	35.9 ± 5.6	36.0 ± 5.9	0.108
Height (cm)	172.1 ± 5.1	159.6 ± 6.3	165.2 ± 8.5	< 0.001
Weight (kg)	75.6 ± 12.5	55.2 ± 6.8	64.4 ± 14.1	< 0.001
BMI (kg/m^2)	24.5 ± 3.5	21.7 ± 2.5	23.4 ± 3.5	< 0.001
LBM (kg)	57.1 ± 15.3	41.1 ± 5.0	48.3 ± 13.5	< 0.001
BF (%)	21.1 ± 8.7	25.5 ± 3.5	23.5 ± 6.7	0.009

Data are presented as mean ± SD. BMI: Body mass index; LBM: Lean body mass; BF: Body fat.

Table 2 Physical activity amount and intensity of the subjects

Variable	Male	Female	Total	P value
TEE (kcal)	637.6 ± 44.3	464.3 ± 64.9	542.4 ± 54.9	0.003
TAC (count)	247 017 ± 75 022	178 014 ± 75 998	212 515.5 ± 75 501	0.003
EEL (kcal)	148.5 ± 6.9	120.0 ± 16.8	133.1 ± 16.8	0.006
EEM (kcal)	472.0 ± 36.2	281.4 ± 22.2	367.4 ± 22.2	< 0.001
EEV (kcal)	24.5 ± 6.7	11.1 ± 47.8	17.8 ± 13.8	0.214

Data are presented as mean ± SD. TEE: Total energy expenditure; TAC: Total activity count; EEL: Energy expenditure of light intense activity; EEM: Energy expenditure of moderate intense activity; EEV: Energy expenditure of vigorous intense activity.

Table 3 Daily macro-nutrients intake of the subjects

Variable	Male	Female	Total	P value
Totalenergy (kcal)	2193.1 ± 259.2	2091.5 ± 229.2	2147.2 ± 233.6	0.292
Protein (g)	85.0 ± 18.6	82.5 ± 20.9	83.6 ± 19.6	0.493
Fat (g)	65.5 ± 23.3	51.2 ± 17.4	59.0 ± 21.9	0.010
Carbohydrate (g)	338.8 ± 71.7	298.5 ± 42.4	305.8 ± 61.2	0.054
Fiber(g)	8.1 ± 4.5	9.9 ± 4.7	8.9 ± 4.6	0.133
Water (g)	1714.3 ± 329.4	1164.7 ± 263.6	1227.0 ± 286.8	0.009

Data are presented as mean ± SD.

Table 4 Segmental colon transit time of the subjects

Variable	Male	Female	Total	P value
LCTT (h)	0.9 ± 2.2	1.1 ± 3.0	1.0 ± 2.6	0.592
RCTT (h)	3.5 ± 4.9	10.0 ± 11.6	6.8 ± 9.6	0.023
RSCTT (h)	4.4 ± 4.7	13.6 ± 12.5	9.0 ± 8.6	0.004
TCTT (h)	8.8 ± 9.4	24.7 ± 23.6	16.8 ± 16.2	0.002

Data are presented as mean ± SD. RCTT: Right colon transit time; LCTT: Left colon transit time; RSCTT: Recto-sigmoid colon transit time; TCTT: Total colon transit time.

Statistical analysis

Data analysis was conducted using the SPSS software package (version 18.0, SPSS Inc., Chicago, IL, United States), and descriptive statistics were presented using mean ± SD. The differences in the variables between the males and females were verified by an independent samples t-test. To assess the correlation between the total CTT and all of the experiment variables, Partial's correlation coefficient (r) was determined. An adjustment was made for gender. The level of significance (P) was 0.05 for all statistical analyses.

RESULTS

Physical characteristics

The physical characteristics of all subjects are presented in Table 1. The mean age was 37.3 and 35.9 years in the male and female subjects, respectively. The variables associated with the physical characteristics of the height, body weight, BMI and LBM were significantly higher in the males than in the females (P < 0.001). On the other hand, the BF was significantly lower in the male subjects than in the female subjects (P = 0.009).

Physical activity amount and intensity

The amounts and intensities of the PA of the male and female subjects are shown in Table 2. The total energy expenditure (173.3 kcal, P = 0.003), total activity count (69 003 count, P = 0.003), energy expenditure of light intensity (EEL) (28.5 kcal, P = 0.006), and energy expenditure of moderate intensity (EEM) (190.6 kcal, P < 0.001) were significantly higher in the male subjects than in the female subjects.

Macro-nutrient intake

Daily macro-nutrient and water intake by the subjects are presented in Table 3. The intake levels of fat (14.3 g, P = 0.010) and water (549.6 g, P = 0.009) were significantly higher in the males than in the females.

Segmental CTT and total CTT

The mean total CTT (TCTT) was 8.8 h and 24.7 h in the male and female subjects, respectively. This gender difference was statistically significant (P = 0.002). Although there was no significant difference in the left CTT, the right CTT (P = 0.023) and recto-sigmoid CTT (P = 0.004) showed significant gender difference (Table 4).

Correlation between the TCTT and parameters

Correlations between the TCTT and related parameters for the male and female subjects are shown in Table 5. In the male subjects, the BMI (r = -424, P < 0.05) and fiber intake levels (r = -417, P < 0.05) showed significant correlations with the TCTT. On the other hand, only the fiber intake (r = -655, P < 0.001) showed a significant correlation with the TCTT in the female subjects. Moreover, when adjusted for gender, the fiber (r = -545, P < 0.001) and water (r = -257, P < 0.05) intake levels showed significant correlations with the TCTT in all subjects.

DISCUSSION

All physical characteristics were higher in the male subjects, except for the BF, but even those values were within the normal range. These values indicate that the South Korean adults who participated in this study were relatively healthy.

Table 5 Correlations between the total colon transit time and parameters

Variable	Male	Female	Total[1]
Physicalcharacteristics			
Age	0.060	-0.112	-0.067
Height	0.071	-0.157	-0.108
Weight	-0.344	-0.014	-0.098
BMI	-0.424	0.127	-0.031
LBM	-0.053	-0.107	-0.050
BF	-0.243	0.149	0.051
Physicalactivity			
TEE	-0.244	-0.022	-0.031
TAC	-0.191	-0.164	-0.180
EEL	-0.067	-0.211	-0.182
EEM	-0.197	-0.092	-0.101
EEV	-0.256	0.092	0.078
Macro-nutrientintake			
Energy	0.196	0.158	0.290
Protein	-0.102	-0.247	-0.123
Fat	0.024	-0.155	-0.008
Carbohydrate	0.346	-0.221	0.250
Fiber	-0.417	-0.655	-0.545
Water	-0.266	-0.326	-0.257

[1]Adjusted for gender. BMI: Body mass index; LBM: Lean body mass; BF: Body fat; TEE: Total energy expenditure; TAC: Total activity count; EEL: Energy expenditure of light intense activity; EEM: Energy expenditure of moderate intense activity; EEV: Energy expenditure of vigorous intense activity.

Despite longstanding disputes, it is uncertain as to whether or not there is any difference in the CTT between males and females. In our study, the comparison of the TCTTs according to gender showed that the mean TCTTs of the males and females were 8.8 and 24.7 h, respectively. Drossman *et al*[21] and Heaton *et al*[22] reported that the prevalence of constipation is higher in women in than in man, and that it becomes more prominent in woman of childbearing age, suggesting a role of the female sex hormone. Jung *et al*[6] reported that the mean TCTT of healthy South Korean subjects was 22.3 h in male subjects and 30.1 h in female subjects and that was no significant difference in the TCTT between male and female subjects. In our study, however, a significant difference was noted in the TCTT according to gender. Moreover, the RCTT and RSCTT in females showed were significantly longer than they were in males. A radio-opaque marker study can provide us with information about the segmental CTT. Our study showed that gender and the LCTT were unrelated. Jung *et al*[6] showed that the RSCTT was significant longer in the female subjects, whereas TCTT, RCTT, and LCTT differences in terms of gender were not significant. Rao *et al*[23] in a study that used ambulatory 24 h colonic manometry revealed that women showed less pressure activity than men and that this difference was particularly significant in the transverse descending colon. Therefore, a gender difference may contribute to the prevalence of constipation in women. However, more studies of variables and sample sizes regarding this mechanism are required.

Because a sedentary lifestyle is a risk factor for chronic disease, promoting PA is very important[24,25]. Some studies reported that factors that can influence the CTT are regular PA and regular physical exercise, both of which are considered to be useful in the management of chronic constipation[26,27]. This is based partly on the assumption that exercise shortens the CTT through the gastrointestinal tract. However, studies testing the influence of exercise on the CTT in healthy young subjects show conflicting results. Some studies showed a decrease in the CTT after physical training[11-13], whereas other studies did not show a reduction partly owing to large intra-individual differences[28-30]. In our study, although South Korean male adults engaged in more PA with or without an adjustment for gender, the CTT did not correlate with the PA levels.

Regarding the intake of dietary fiber and water, several studies have shown that the CTT was shortened and the stool frequency increased when the amounts of dietary fiber and water increased[31-34]. In our study, the mean CTT also showed a significant correlation with the amount of dietary fiber intake in all subjects. This result is similar to those of previous studies, which found that the CTT is significantly correlated with dietary fiber intake. We observed an inverse association between the TCTT and fiber intake in males, females, and in all subjects with or without an adjustment for gender. This result indicates that fiber intake is a strong contributing factor which affects the CTT in South Korean adults. For water intake, although all subjects showed significant correlation between this and the TCTT, the discrepancy in water consumption may be due to the limitation of the measurement methods based on the subjects' food diaries. The Can-pro program by the Korean Nutrition Society does not analyze the moisture content of food. Thus, the surveyed amounts of consumed water were used for the water intake amounts. As a result, the amounts of water contained in the subject's food intake level were excluded from total water consumption in our study. However, although our ability to analyze water consumption was limited, water consumption was shown to help the CTT in all subjects in the present study. The current study was also limited by the small sample size, which prevents any generalization of the study results. Therefore, further studies should be conducted with more subjects.

Other factors that can influence the CTT include physical characteristics such as weight, and height and age[4,5]. In our study, the average BMI of the subjects was 24.5 kg/m^2 for the men and 21.7 kg/m^2 for the women. Considering that the normal range of BMI reported by the World Health Organization was 18.5-25.0 kg/m^2 for adults, these values indicate that the South Korean adults who participated in this study were relatively healthy. The finding of a correlation between the TCTT and BMI in the male subjects, but not in the female subjects, showed that the BMI increased the CTT, as in previous studies. The precise mechanism between the BMI and CTT could not be explained, although possible reasons include differences in eating habits and living patterns.

In our study, the BMI was a factor contributing to the CTT in only males. Martelli *et al*[4] showed that the CTT of healthy older subjects (age 55-74 years) was longer on average than that of healthy young subjects (age 21-27 years). However, in our study, age did not correlate with the CTT for those aged 25-59 years.

Based on these results, significant gender differences were noted in the TCTT, RCTT and RSCTT. The BMI and fiber intake in males and fiber intake in females were shown to assist the CTT. Moreover, the intake of the fiber and water intake was shown to help the CTT in all subjects.

COMMENTS

Background

The recognized risk factors of colorectal cancer include westernized eating habits, obesity, insufficient physical activity and genetic factors. However, the effects of gender and individual behavior on the colon transit time (CTT) remain controversial.

Research frontiers

The impact of exercise and physical activity (PA) on the gastrointestinal and colonic tracts is an area of emerging interest, with recent research focusing on the potential benefits of PA.

Innovations and breakthroughs

The authors measured physical activity by accelerometer, colon transit time by radio-opaque marker method and food intake by the nutrient analysis program that are all very good and relevant. Colon transit time in South Korean general population showed gender difference and modulation of CTT by the intake of fiber and water.

Applications

CTT has a gender difference which are involved total calories, energy expenditure, physical activity, and water consumption. Regardless the gender, the intake of fiber and water modulated to reduce colon transit time. It can help the general advise for life habit with patients of constipation. A further study that including larger sample size and physical activity intervention may assist to elucidate the precise relationship between physical activity and colon movement.

Peer review

The authors have studied CTT according to physical activity and characteristics in South Korean, finding that significant gender differences in the total CTT, right CTT, and recto-sigmoid CTT adults. The manuscript is a good study and should be acceptable.

REFERENCES

1 **Parkin DM**, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108 [PMID: 15761078 DOI: 10.3322/canjclin.55.2.74]

2 **White E**, Jacobs EJ, Daling JR. Physical activity in relation to colon cancer in middle-aged men and women. *Am J Epidemiol* 1996; **144**: 42-50 [PMID: 8659484 DOI: 10.1093/oxfordjournals.aje.a008853]

3 **Johnsen NF**, Christensen J, Thomsen BL, Olsen A, Loft S, Overvad K, Tjønneland A. Physical activity and risk of colon cancer in a cohort of Danish middle-aged men and women. *Eur J Epidemiol* 2006; **21**: 877-884 [PMID: 17160429 DOI: 10.1007/s10654-006-9076-z]

4 **Martelli H**, Devroede G, Arhan P, Duguay C, Dornic C, Faverdin C. Some parameters of large bowel motility in normal man. *Gastroenterology* 1978; **75**: 612-618 [PMID: 710830]

5 **Meir R**, Beglinger C, Dederding JP, Meyer-Wyss B, Fumagalli M, Rowedder A, Turberg J, Brignoli R. [Age- and sex-specific standard values of colonic transit time in healthy subjects]. *Schweiz Med Wochenschr* 1992; **122**: 940-943 [PMID: 1615304]

6 **Jung HK**, Kim DY, Moon IH. Effects of gender and menstrual cycle on colonic transit time in healthy subjects. *Korean J Intern Med* 2003; **18**: 181-186 [PMID: 14619388]

7 **Hinds JP**, Stoney B, Wald A. Does gender or the menstrual cycle affect colonic transit? *Am J Gastroenterol* 1989; **84**: 123-126 [PMID: 2916519]

8 **Wald A**, Van Thiel DH, Hoechstetter L, Gavaler JS, Egler KM, Verm R, Scott L, Lester R. Gastrointestinal transit: the effect of the menstrual cycle. *Gastroenterology* 1981; **80**: 1497-1500 [PMID: 7227774]

9 **Stephenson LE**, Bebb DG, Reimer RA, Culos-Reed SN. Physical activity and diet behaviour in colorectal cancer patients receiving chemotherapy: associations with quality of life. *BMC Gastroenterol* 2009; **9**: 60 [PMID: 19635164 DOI: 10.1186/1471-230X-9-60]

10 **Colbert LH**, Hartman TJ, Malila N, Limburg PJ, Pietinen P, Virtamo J, Taylor PR, Albanes D. Physical activity in relation to cancer of the colon and rectum in a cohort of male smokers. *Cancer Epidemiol Biomarkers Prev* 2001; **10**: 265-268 [PMID: 11303597]

11 **Song BK**, Cho KO, Jo Y, Oh JW, Kim YS. Colon transit time according to physical activity level in adults. *J Neurogastroenterol Motil* 2012; **18**: 64-69 [PMID: 22323989 DOI: 10.5056/jnm.2012.18.1.64]

12 **Bartram HP**, Wynder EL. Physical activity and colon cancer risk? Physiological considerations. *Am J Gastroenterol* 1989; **84**: 109-112 [PMID: 2644818]

13 **Robertson G**, Meshkinpour H, Vandenberg K, James N, Cohen A, Wilson A. Effects of exercise on total and segmental colon transit. *J Clin Gastroenterol* 1993; **16**: 300-303 [PMID: 8331262 DOI: 10.1097/00004836-199306000-00006]

14 **Zhang Y**, Cantor KP, Dosemeci M, Lynch CF, Zhu Y, Zheng T. Occupational and leisure-time physical activity and risk of colon cancer by subsite. *J Occup Environ Med* 2006; **48**: 236-243 [PMID: 16531827 DOI: 10.1097/01.jom.0000199521.72764.26]

15 **Chow WH**, Dosemeci M, Zheng W, Vetter R, McLaughlin JK, Gao YT, Blot WJ. Physical activity and occupational risk of colon cancer in Shanghai, China. *Int J Epidemiol* 1993; **22**: 23-29 [PMID: 8449643]

16 **Irwin ML**. Physical activity interventions for cancer survivors. *Br J Sports Med* 2009; **43**: 32-38 [PMID: 18948351 DOI: 10.1136/bjsm.2008.053843]

17 **Hendelman D**, Miller K, Baggett C, Debold E, Freedson P. Validity of accelerometry for the assessment of moderate intensity physical activity in the field. *Med Sci Sports Exerc* 2000; **32**: S442-S449 [PMID: 10993413 DOI: 10.1097/00005768-200009001-00002]

18 **Chaussade S**, Roche H, Khyari A, Couturier D, Guerre J. [Measurement of colonic transit time: description and validation of a new method]. *Gastroenterol Clin Biol* 1986; **10**: 385-389 [PMID: 3732742]

19 **Metcalf AM**, Phillips SF, Zinsmeister AR, MacCarty RL, Beart RW, Wolff BG. Simplified assessment of segmental colonic transit. *Gastroenterology* 1987; **92**: 40-47 [PMID: 3023168]

20 **Degen LP**, Phillips SF. Variability of gastrointestinal transit in healthy women and men. *Gut* 1996; **39**: 299-305 [PMID: 8977347 DOI: 10.1136/gut.39.2.299]

21 **Drossman DA**, Li Z, Andruzzi E, Temple RD, Talley NJ, Thompson WG, Whitehead WE, Janssens J, Funch-Jensen P, Corazziari E. U.S. householder survey of functional gastrointestinal disorders. Prevalence, sociodemography, and health impact. *Dig Dis Sci* 1993; **38**: 1569-1580 [PMID: 8359066 DOI: 10.1007/BF01303162]

22 **Heaton KW**, Radvan J, Cripps H, Mountford RA, Braddon FE, Hughes AO. Defecation frequency and timing, and stool form in the general population: a prospective study. *Gut* 1992; **33**: 818-824 [PMID: 1624166 DOI: 10.1136/gut.33.6.818]

23 **Rao SS**, Sadeghi P, Beaty J, Kavlock R, Ackerson K. Ambulatory 24-h colonic manometry in healthy humans. *Am J Physiol Gastrointest Liver Physiol* 2001; **280**: G629-G639 [PMID: 11254489]

24 **Mathieu ME**, Brochu M, Béliveau L. DiabetAction: changes in physical activity practice, fitness, and metabolic syndrome in type 2 diabetic and at-risk individuals. *Clin J Sport Med* 2008; **18**: 70-75 [PMID: 18185042 DOI: 10.1097/JSM.0b013e31815c1d68]

25 **Graff J**, Brinch K, Madsen JL. Gastrointestinal mean transit times in young and middle-aged healthy subjects. *Clin Physiol* 2001; **21**: 253-259 [PMID: 11318834 DOI: 10.1046/j.1365-2281.2001.00308.x]

26 **Papathanasopoulos A**, Camilleri M. Dietary fiber supplements: effects in obesity and metabolic syndrome and relationship to gastrointestinal functions. *Gastroenterology* 2010; **138**: 65-72.e1-65-72.e2 [PMID: 19931537 DOI: 10.1053/j.gastro.2009.11.045]

27 **Meshkinpour H**, Selod S, Movahedi H, Nami N, James N, Wilson A. Effects of regular exercise in management of chronic idiopathic constipation. *Dig Dis Sci* 1998; **43**: 2379-2383 [PMID: 9824122]

28 **Cordain L**, Latin RW, Behnke JJ. The effects of an aerobic running program on bowel transit time. *J Sports Med Phys Fitness* 1986; **26:** 101-104 [PMID: 3713155]

29 **Oettlé GJ**. Effect of moderate exercise on bowel habit. *Gut* 1991; **32**: 941-944 [PMID: 1885077 DOI: 10.1136/gut.32.8.941]

30 **Coenen C**, Wegener M, Wedmann B, Schmidt G, Hoffmann S. Does physical exercise influence bowel transit time in healthy young men? *Am J Gastroenterol* 1992; **87**: 292-295 [PMID: 1539562]

31 **Howard RA**, Freedman DM, Park Y, Hollenbeck A, Schatzkin A, Leitzmann MF. Physical activity, sedentary behavior, and the risk of colon and rectal cancer in the NIH-AARP Diet and Health Study. *Cancer Causes Control* 2008; **19**: 939-953 [PMID: 18437512 DOI: 10.1007/s10552-008-9159-0]

32 **Cheskin LJ**, Kamal N, Crowell MD, Schuster MM, Whitehead WE. Mechanisms of constipation in older persons and effects of fiber compared with placebo. *J Am Geriatr Soc* 1995; **43**: 666-669 [PMID: 7775727]

33 **Davies GJ**, Crowder M, Reid B, Dickerson JW. Bowel function measurements of individuals with different eating patterns. *Gut* 1986; **27**: 164-169 [PMID: 3005140 DOI: 10.1136/gut.27.2.164]

34 **Roberfroid M**. Dietary fiber, inulin, and oligofructose: a review comparing their physiological effects. *Crit Rev Food Sci Nutr* 1993; **33**: 103-148 [PMID: 8257475 DOI: 10.1080/10408399309527616]

P- Reviewer Gordon AG **S- Editor** Zhai HH
L- Editor A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i4.556

World J Gastroenterol 2013 January 28; 19(4): 556-560
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

New endoscopic classification of achalasia for selection of candidates for peroral endoscopic myotomy

Hui-Kai Li, En-Qiang Linghu

Hui-Kai Li, En-Qiang Linghu, Department of Gastroenterology, PLA General Hospital, Beijing 100853, China
Author contributions: Linghu EQ designed the research, and both authors performed the research and data analysis, and prepared the manuscript.
Correspondence to: En-Qiang Linghu, MD, Department of Gastroenterology, PLA General Hospital, Beijing 100853, China. linghuenqiang@vip.sina.com
Telephone: +86-10-66936998 Fax: +86-10-66937485
Received: August 23, 2012 Revised: October 13, 2012
Accepted: December 27, 2012
Published online: January 28, 2013

Abstract

AIM: To propose a new endoscopic classification of achalasia for selecting patients appropriate for undergoing peroral endoscopic myotomy (POEM).

METHODS: We screened out the data of patients with achalasia examined from October 2000 to September 2011 at our Digestive Endoscopic Center with endoscopic pictures clear enough to reveal the morphology of middle and lower esophagus. After analyzing the correlation between the endoscopic morphology of the esophageal lumen and POEM, we proposed a new endoscopic classification (Ling classification) of achalasia according to three kinds of endoscopically viewed structures: multi-ring structure, crescent-like structure and diverticulum structure. There were three types based on the criteria of Ling classification: type Ⅰ, smooth without multi-ring, crescent-like structure or diverticulum structure; type Ⅱ, with multi-ring or crescent-like structure but without diverticulum structure; and type Ⅲ, with diverticulum structure. Type Ⅱ was classified into three subtypes: Ling Ⅱa, Ling Ⅱb and Ling Ⅱc; and type Ⅲ also had three subtypes: Ling Ⅲl, Ling Ⅲr and Ling Ⅲlr. Two endoscopists made a final decision upon mutual agreement through discussion if their separately recorded characteristics were different.

RESULTS: Among the 976 screened patients with achalasia, 636 patients with qualified endoscopic pictures were selected for the analysis, including 405 males and 231 females. The average age was 42.7 years, ranging from 6 to 93 years. Type Ⅰ was the most commonly observed type of achalasia, accounting for 64.5% (410/636), and type Ⅲ was the least commonly observed type of achalasia, accounting for 2.8% (18/636). And type Ⅱ accounted for 32.7% (208/636) and subtype of Ling Ⅱa, Ling Ⅱb and Ling Ⅱc accounted for 14.6% (93/636), 9.9% (63/636) and 8.2% (52/636), respectively. And subtype of Ling Ⅲl, Ling Ⅲr and Ling Ⅲlr accounted for 0.8% (5/636), 0.3% (2/636) and 1.7% (11/636), respectively.

CONCLUSION: A new endoscopic classification of achalasia is proposed that might help in determining the proper candidates for POEM.

© 2013 Baishideng. All rights reserved.

Key words: Endoscopy; Classification; Achalasia; Selection; Peroral endoscopic myotomy

Li HK, Linghu EQ. New endoscopic classification of achalasia for selection of candidates for peroral endoscopic myotomy. *World J Gastroenterol* 2013; 19(4): 556-560 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i4/556.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i4.556

INTRODUCTION

Treatments for achalasia include pharmacological therapy, endoscopic injection of botulinum toxin, endoscopic dilation, and laparoscopic or open surgery[1-5], among which laparoscopic surgery with fundoplication has been widely accepted as the primary therapy because of its low complication rate, durable symptom relief, and low incidence of postoperative gastroesophageal reflux[6-10]. However, a new alternative therapy named peroral endoscopic myot-

omy (POEM) has recently been reported to be effective clinically for esophageal achalasia without serious complications at least in a short term[11-13]. We have performed POEM since 2010, and found that not all the patients with achalasia were eligible to undergo POEM. If the esophageal lumen was too tortuous or if a diverticulum was formed in the middle or the lower part of the esophagus, to establish an appropriate submucosal tunnel would, to a great extent, cause a penetrative damage to the mucosa or the muscularis propria, which obviously destroyed the completeness of the submucosal tunnel, leading to a serious outcome such as mediastinitis if not successfully treated in time. Mucosal penetration at the gastric cardia has been firstly reported to occur respectively in 2 out of 5 and 2 out of 17 patients in two studies, although it did not cause serious consequences[12-14]. The occurrence of mucosal penetration at the cardia was also confirmed by several other studies[15-19]. Therefore, it is very important and necessary to make a precise judgment whether or not a patient with achalasia is a proper candidate for POEM. Inoune *et al*[12] suggested that a patient was not fit for a POEM therapy if a double-lumen sign was detected in the esophageal computed tomography (CT). However, no other studies have, to our best knowledge, reported the correlation between the morphology of the esophageal lumen and the indications of POEM. To better judge if a patient was suitable for POEM, we analyzed the correlation between the endoscopic morphology of the esophageal lumen and POEM, and proposed a new endoscopic classification of achalasia (Ling classification), hoping to assist in determining the indications of POEM.

MATERIALS AND METHODS

We reviewed the data of endoscopic examinations of patients diagnosed with achalasia from October 2000 to September 2011 at our Digestive Endoscopic Center and screened out the data of patients with endoscopic pictures clear enough to reveal the morphology of the middle and the lower parts of the esophagus. Two endoscopists (Li HK and Linghu EQ) independently recorded the characteristics of the morphology of the middle and the lower parts of the esophagus, which included three endoscopically detected intraluminal structures: multi-ring structure, crescent-like structure and diverticulum structure. There were three types according to the Ling classification[20]: type Ⅰ, smooth without multi-ring, crescent-like structure or diverticulum structure; type Ⅱ, with multi-ring or crescent-like structure but without diverticulum structure; and type Ⅲ, with diverticulum structure (Figure 1). Type Ⅱ was further divided into three subtypes: Ling Ⅱ$_a$, Ling Ⅱ$_b$ and Ling Ⅱ$_c$, and type Ⅲ also had three subtypes: Ling Ⅲ$_l$, Ling Ⅲ$_r$ and Ling Ⅲ$_{lr}$. The criteria for classifying subtypes Ⅱ and III were as follows: Ling Ⅱ$_a$, with multi-ring structure; Ling Ⅱ$_b$, with crescent-like structure and the midpoint of its inner edge not larger than 1/3 of the esophageal lumen; Ling Ⅱ$_c$, with crescent-like structure and the midpoint of its inner edge over 1/3 of the esophageal lumen; Ling Ⅲ$_l$, diverticulum structure in the left wall of esophagus; Ling Ⅲ$_r$, diverticulum structure in the right wall of esophagus; and Ling Ⅲ$_{lr}$, diverticulum structure in both the left and right walls of esophagus. If the two endoscopists recorded different characteristics, they would discuss together and made a final decision upon mutual agreement.

RESULTS

Data of 976 patients with achalasia were collected and analyzed, and 636 of them were screened out with clear endoscopic pictures revealing the morphology of middle and lower esophagus, while 340 were excluded for unqualified pictures. Among the 636 patients, 405 were male and 231 were female. The average age was 42.7 years, ranging from 6 to 93 years.

We named our proposed classification Ling classification since Ling is short for Linghu, which is the family name of the corresponding author. As shown in Table 1, 64.5% (410/636) of the patients were classified as type Ⅰ, which was the most common type of achalasia based on the Ling classification; 32.7% (208/636) of the patients were classified as type Ⅱ; and only 2.8% (18/636) as type Ⅲ. The typical pictures of each type or subtype are shown in Figure 1. The proportion of each type or subtype is described in Table 1 according to the Ling classification.

DISCUSSION

As early as in 1980, Ortega *et al*[21] for the first time performed cutting esophageal muscles using the needle knife through the peroral endoscope, but few doctors followed them due to the possible mediastinal contamination. Until 2007, Pasricha *et al*[22] reported the possibility of POEM through a submucosal tunnel in a porcine model. In 2010, Inoue *et al*[12] started to use POEM to treat patients with achalasia after several refinements, including applications of endoscopic submucosal dissection technique, the triangle-tip knife, positive pressure ventilation and CO_2 insufflations through the endoscope during POEM, and they reported a satisfactory short-term effects. Swanström *et al*[13] also reported their experience of POEM in patients with achalasia and esophageal motility disorders. Both of the above-mentioned clinical trials demonstrated that small mucosal penetration at the gastric cardia occurred respectively in 2 out of 5 and 2 out of 17 patients, but were treated differently. Inoue observed the penetrations without special treatment, while Swanström used traditional hemostatic clips to seal the penetrations. Even though no serious clinical outcomes were observed, we should keep alert about the incompleteness of the submucosal tunnel caused by mucosal penetration at the cardia, and we used fibrin sealant to treat this mucosal penetration[15]. If the penetrations were *in situ*, fluids from the stomach or the esophagus might flow into the tunnel, which might affect the healing of the incised inner circular muscles and further influence the effects of POEM.

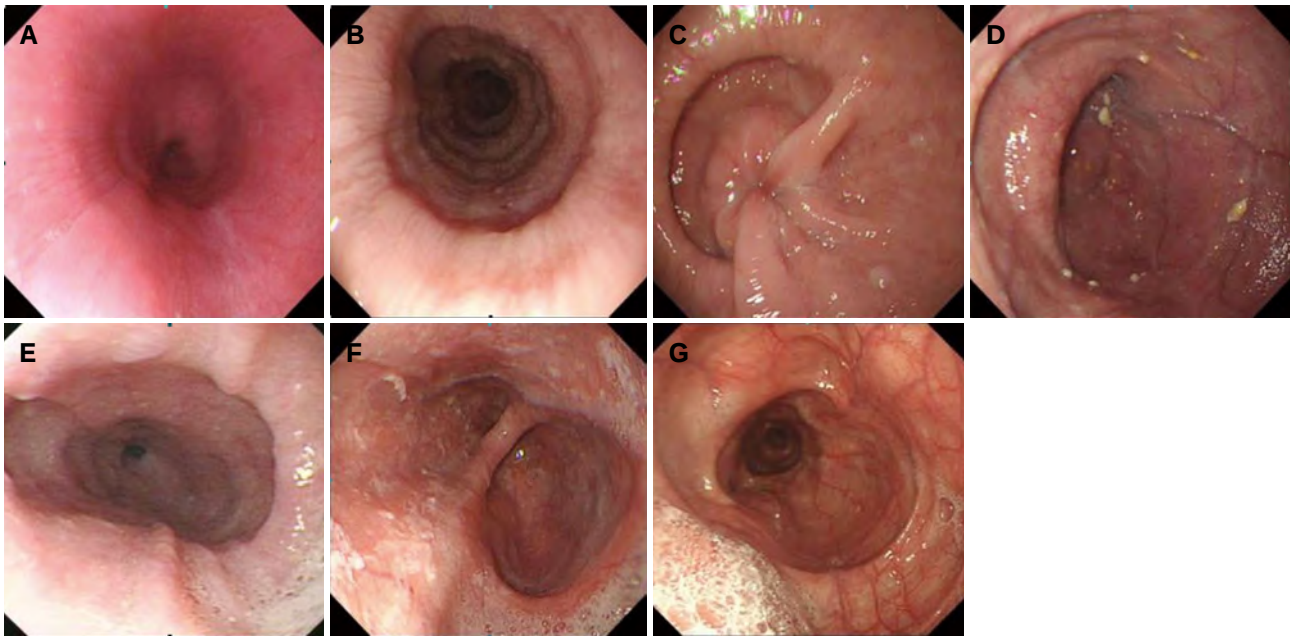


Figure 1 Typical pictures of each type or subtype in Ling classification. A: Type Ⅰ; B: Type Ⅱa; C: Type Ⅱb; D: Type Ⅱc; E: Type Ⅲl; F: Type Ⅲr; G: Type Ⅲlr.

Table 1 Proportion of each type or subtype according to Ling classification *n* (%)

Type	Proportion
Ling Ⅰ	410 (64.5)
Ling Ⅱ	208 (32.7)
Ling Ⅱa	93 (14.6)
Ling Ⅱb	63 (9.9)
Ling Ⅱc	52 (8.2)
Ling Ⅲ	18 (2.8)
Ling Ⅲl	5 (0.8)
Ling Ⅲr	2 (0.3)
Ling Ⅲlr	11 (1.7)

We think that the reason why mucosal penetrations are more likely to occur in the cardia is that the working space in the submucosal tunnel near the cardia is narrower than in other parts, which makes a precise electrocautery without damaging the cardiac mucosa very difficult. Similarly, if POEM was performed in patients with a very tortuous esophagus, penetrations of mucosa or muscularis propria could happen where the working space is confined by the tortuousness of the esophagus. So, from the point of minimizing complications like penetrations of mucosa or muscularis propria, it is very important to make a precise judgment whether a patient with achalasia is or not a proper candidate for POEM. Inoune mentioned that a patient was not fit for a POEM therapy if a double-lumen sign was detected in the CT scan[12], however, no other studies on POEM have reported the correlation between the morphology of esophageal lumen and the indications of POEM[13-14,16-18]. We, therefore, proposed the endoscopic Ling classification of achalasia, hoping to assist in determining the indications of POEM after analyzing the correlation between the endoscopic morphology of the esophageal lumen and POEM.

After reviewing the endoscopic pictures of nearly 1000 patients, we screened out 636 patients with pictures clear enough to reveal the middle and lower esophagus and analyzed the characteristics of the morphology of esophagus. Because the submucosal tunnel established during POEM generally starts from the middle esophagus and ends at about 3 cm distal to the cardia[12,13], we focused on the morphology of the middle and lower esophagus. Based on the Ling classification, the esophagus of type Ⅰ is very smooth and without multi-ring structure, crescent-like structure or diverticulum structure, so type Ⅰ patients are the most safe type to undergo POEM. The esophagus of type Ling Ⅱa with multi-ring structure was not so smooth as type Ⅰ, but not much difficulties were encountered during establishment of the submucosal tunnel in our practice of POEM. The crescent-like structure present in type Ling Ⅱb or Ling Ⅱc actually reflexes the tortuousness of the esophageal lumen, and the degree of tortuousness is more severe in Ling Ⅱc than in Ling Ⅱb, and therefore to establish a submucosal tunnel across the tortuous point of the esophagus is more difficult and more likely to cause a penetration in Ling Ⅱc than in Ling Ⅱb. Diverticulum structure occurred in type Ⅲ, making it the most challenging type to establish a submucosal tunnel across the diverticulum.

Location of the crescent-like structure or the diverticulum structure can greatly affect the success of a POEM therapy. Since we and other operators[12,13] preferred the establishment of submucosal tunnel in the right wall of the esophagus leading to the lesser curvature of stomach, the submucosal tunnel would be very difficult to be established if a crescent-like structure or diverticulum structure was located in the right wall of the esophagus. Otherwise, if a crescent-like structure or diverticulum

structure was located in the left wall of the esophagus, the submucosal tunnel would be established with much less difficulty.

Based on our experiences, we suggest that type Ling I and Ling II$_a$ patients are safe for POEM, and that Ling II$_b$ patients can also be considered for POEM, but more cautions should be used to avoid damaging the mucosa or the muscularis propria when the submucosal tunnel is being established across the crescent-like structure. For Ling II$_c$ and Ling III patients, POEM can be performed when the crescent-like structure is on the left side of the esophageal lumen, but it is not recommended when the crescent-like structure is on the right side. These suggestions may be modified in the future with improvement of technology. For example, when a new and safe technique to establish a tunnel across a crescent-like structure is available, a POEM therapy would be recommended for all the Ling II patients free of concerns of penetrations irrespective of the location of the crescent-like structures.

One classification of achalasia categorized by high-resolution manometry has been reported to be promising in predicting the efficacy of treatment of achalasia, such as balloon dilation[23-25]. But up to now, there has been no report about its usefulness for selecting candidates for POEM. And the relationship between the proposed endoscopic classification and manometric classification remains an interesting point worth further studying.

Since not all patients were eligible for undergoing POEM, we analyzed the characteristics of the esophageal lumen and proposed the Ling classification, hoping that Ling classification might help in determining the proper candidates for POEM, thus minimizing the complications such as penetration of mucosa or muscularis propria because other therapies such as endoscopic injection of botulinum toxin, endoscopic dilation or surgery would be recommended instead of POEM in patients with a severely tortuous esophagus. However, further prospective studies are needed to determine the usefulness of Ling classification for selecting candidates for POEM.

COMMENTS

Background

Peroral endoscopic myotomy (POEM) has been primarily confirmed to be effective for patients with achalasia in the short term by several studies since it was first clinically reported in 2010, but not all the patients with achalasia were eligible for a POEM therapy. If the esophageal lumen was too tortuous or a diverticulum was formed in the middle or the lower part of the esophagus, it would be quite challenging to establish a submucosal tunnel.

Research frontiers

Although no serious clinical outcomes of complications like mucosal perforation in the cardia has been reported, it is still of vital importance to select proper candidates for POEM so as to minimize those complications.

Innovations and breakthroughs

The authors proposed a new endoscopic classification of achalasia after analyzing the endoscopic pictures which clearly revealed the morphology of middle and lower esophagus and this new Ling classification of achalasia might assist in determining the indications of POEM.

Applications

The authors suggest the followings for choosing the candidates for POEM: Ling I and Ling II$_a$ patients are safe for POEM, and Ling II$_b$ patients can also be considered for POEM but more cautions should be used to avoid damaging the mucosa or the muscularis propria when the submucosal tunnel is being established across the crescent-like structure. For Ling II$_c$ and Ling III patients, POEM can be performed when the crescent-like structure is on the left side of the esophageal lumen, but it is not recommended when the crescent-like structure is on the right side.

Terminology

POEM is a newly developed endoscopic therapy for achalasia by myotomy of the inner circular muscle of the lower esophagus, thus reducing the lower esophageal sphincter pressure and relieving symptoms of the patients.

Peer review

This study proposed a new endoscopic classification of achalasia based on the morphology of the middle and lower part of the esophagus, and role of the new classification in determining proper candidates for POEM was explored, and the authors suggested indications based on this new classification,which is very interesting. However, further prospective studies are needed to determine the validity of this Ling classification for selecting candidates for POEM.

REFERENCES

1 **Roll GR**, Rabl C, Ciovica R, Peeva S, Campos GM. A controversy that has been tough to swallow: is the treatment of achalasia now digested? *J Gastrointest Surg* 2010; **14** Suppl 1: S33-S45 [PMID: 19760373 DOI: 10.1007/s11605-009-1013-5]

2 **Cheatham JG**, Wong RK. Current approach to the treatment of achalasia. *Curr Gastroenterol Rep* 2011; **13**: 219-225 [PMID: 21424734 DOI: 10.1007/s11894-011-0190-z]

3 **Gockel I**, Sgourakis G, Drescher DG, Lang H. Impact of minimally invasive surgery in the spectrum of current achalasia treatment options. *Scand J Surg* 2011; **100**: 72-77 [PMID: 21737381]

4 **Leyden JE**, Moss AC, MacMathuna P. Endoscopic pneumatic dilation versus botulinum toxin injection in the management of primary achalasia. *Cochrane Database Syst Rev* 2006; (4): CD005046 [PMID: 17054234]

5 **Boeckxstaens GE**, Annese V, des Varannes SB, Chaussade S, Costantini M, Cuttitta A, Elizalde JI, Fumagalli U, Gaudric M, Rohof WO, Smout AJ, Tack J, Zwinderman AH, Zaninotto G, Busch OR. Pneumatic dilation versus laparoscopic Heller's myotomy for idiopathic achalasia. *N Engl J Med* 2011; **364**: 1807-1816 [PMID: 21561346 DOI: 10.1056/NEJMoa1010502]

6 **Mikaeli J**, Islami F, Malekzadeh R. Achalasia: a review of Western and Iranian experiences. *World J Gastroenterol* 2009; **15**: 5000-5009 [PMID: 19859991 DOI: 10.3748/wjg.15.5000]

7 **Patti MG**, Herbella FA. Achalasia and other esophageal motility disorders. *J Gastrointest Surg* 2011; **15**: 703-707 [PMID: 21394546 DOI: 10.1007/s11605-011-1478-x]

8 **Wang L**, Li YM, Li L. Meta-analysis of randomized and controlled treatment trials for achalasia. *Dig Dis Sci* 2009; **54**: 2303-2311 [PMID: 19107596 DOI: 10.1007/s10620-008-0637-8]

9 **Moawad FJ**, Wong RKh. Modern management of achalasia. *Curr Opin Gastroenterol* 2010; **26**: 384-388 [PMID: 20502326 DOI: 10.1097/MOG.0b013e32833aaf4a]

10 **Gutschow CA**, Töx U, Leers J, Schäfer H, Prenzel KL, Hölscher AH. Botox, dilation, or myotomy? Clinical outcome of interventional and surgical therapies for achalasia. *Langenbecks Arch Surg* 2010; **395**: 1093-1099 [PMID: 20845045 DOI: 10.1007/s00423-010-0711-5]

11 **Inoue H**, Minami H, Kaga M, Sato Y, Kudo SE. Endoscopic mucosal resection and endoscopic submucosal dissection for esophageal dysplasia and carcinoma. *Gastrointest Endosc Clin N Am* 2010; **20**: 25-34, v-vi [PMID: 19951792 DOI: 10.1016/j.giec.2009.08.005]

12 **Inoue H**, Minami H, Kobayashi Y, Sato Y, Kaga M, Suzuki M, Satodate H, Odaka N, Itoh H, Kudo S. Peroral endoscopic myotomy (POEM) for esophageal achalasia. *Endoscopy* 2010;

42: 265-271 [PMID: 20354937 DOI: 10.1055/s-0029-1244080]
13 **Swanström LL**, Rieder E, Dunst CM. A stepwise approach and early clinical experience in peroral endoscopic myotomy for the treatment of achalasia and esophageal motility disorders. *J Am Coll Surg* 2011; **213**: 751-756 [PMID: 21996484 DOI: 10.1016/j.jamcollsurg.2011.09.001]
14 **Stavropoulos SN**, Harris MD, Hida S, Brathwaite C, Demetriou C, Grendell J. Endoscopic submucosal myotomy for the treatment of achalasia (with video). *Gastrointest Endosc* 2010; **72**: 1309-1311 [PMID: 21111876 DOI: 10.1016/j.gie.2010.04.016]
15 **Li H**, Linghu E, Wang X. Fibrin sealant for closure of mucosal penetration at the cardia during peroral endoscopic myotomy (POEM). *Endoscopy* 2012; **44** Suppl 2 UCTN: E215-E216 [PMID: 22622752 DOI: 10.1055/s-0032-1309358]
16 **Costamagna G**, Marchese M, Familiari P, Tringali A, Inoue H, Perri V. Peroral endoscopic myotomy (POEM) for oesophageal achalasia: preliminary results in humans. *Dig Liver Dis* 2012; **44**: 827-832 [PMID: 22609465]
17 **von Renteln D**, Inoue H, Minami H, Werner YB, Pace A, Kersten JF, Much CC, Schachschal G, Mann O, Keller J, Fuchs KH, Rösch T. Peroral endoscopic myotomy for the treatment of achalasia: a prospective single center study. *Am J Gastroenterol* 2012; **107**: 411-417 [PMID: 22068665 DOI: 10.1038/ajg.2011.388]
18 **Zhou PH**, Cai MY, Yao LQ, Zhong YS, Ren Z, Xu MD, Chen WF, Qin XY. [Peroral endoscopic myotomy for esophageal achalasia: report of 42 cases]. *Zhonghua Weichang Waike Zazhi* 2011; **14**: 705-708 [PMID: 21948538]
19 **Ren Z**, Zhong Y, Zhou P, Xu M, Cai M, Li L, Shi Q, Yao L. Perioperative management and treatment for complications during and after peroral endoscopic myotomy (POEM) for esophageal achalasia (EA) (data from 119 cases). *Surg Endosc* 2012; **26**: 3267-3272 [PMID: 22609984 DOI: 10.1007/s00464-012-2336-y]
20 **Linghu EQ**, Li HK. A new endoscopic classification of achalasia. *Zhonghua Qiangjing Waike Zazhi* 2011; **4:** 8-10 [DOI: 10.3877/cma.j.issn.1674-6899.2011.05.004]
21 **Ortega JA**, Madureri V, Perez L. Endoscopic myotomy in the treatment of achalasia. *Gastrointest Endosc* 1980; **26**: 8-10 [PMID: 7358270 DOI: 10.1016/S0016-5107(80)73249-2]
22 **Pasricha PJ**, Hawari R, Ahmed I, Chen J, Cotton PB, Hawes RH, Kalloo AN, Kantsevoy SV, Gostout CJ. Submucosal endoscopic esophageal myotomy: a novel experimental approach for the treatment of achalasia. *Endoscopy* 2007; **39**: 761-764 [PMID: 17703382 DOI: 10.1055/s-2007-966764]
23 **Pandolfino JE**, Kwiatek MA, Nealis T, Bulsiewicz W, Post J, Kahrilas PJ. Achalasia: a new clinically relevant classification by high-resolution manometry. *Gastroenterology* 2008; **135**: 1526-1533 [PMID: 18722376 DOI: 10.1053/j.gastro.2008.07.022]
24 **Pratap N**, Kalapala R, Darisetty S, Joshi N, Ramchandani M, Banerjee R, Lakhtakia S, Gupta R, Tandan M, Rao GV, Reddy DN. Achalasia cardia subtyping by high-resolution manometry predicts the therapeutic outcome of pneumatic balloon dilatation. *J Neurogastroenterol Motil* 2011; **17**: 48-53 [PMID: 21369491 DOI: 10.5056/jnm.2011.17.1.48]
25 **Roman S**, Zerbib F, Quenehervé L, Clermidy H, Varannes SB, Mion F. The Chicago classification for achalasia in a French multicentric cohort. *Dig Liver Dis* 2012; **44**: 976-980 [PMID: 22938702]

P- Reviewers Patti MG, Traube M **S- Editor** Zhai HH
L- Editor A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i4.561

World J Gastroenterol 2013 January 28; 19(4): 561-568
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Pilot study on efficacy of reduced cathartic bowel preparation with polyethylene glycol and bisacodyl

Zhi-Yuan Chen, He-Song Shen, Ming-Yue Luo, Chai-Jie Duan, Wen-Li Cai, Hong-Bing Lu, Guo-Peng Zhang, Yang Liu, Jerome Z Liang

Zhi-Yuan Chen, He-Song Shen, Ming-Yue Luo, Department of Radiology, the Sixth Affiliated Hospital of Sun Yat-Sen University, Guangzhou 510655, Guangdong Province, China
Zhi-Yuan Chen, Department of Radiology, Guangdong Provincial Hospital of Traditional Chinese Medicine, Guangzhou 510006, Guangdong Province, China
Chai-Jie Duan, Research Center of Biomedical Engineering, Graduate School at Shenzhen, Tsinghua University, Shenzhen 518055, Guangdong Province, China
Wen-Li Cai, Department of Radiology, Massachusetts General Hospital and Harvard Medical School, Boston, MA 02114, United States
Hong-Bing Lu, Guo-Peng Zhang, Yang Liu, Department of Biomedical Engineering, The Fourth Military Medical University, Xi'an 710032, Shaanxi Province, China
Jerome Z Liang, Departments of Radiology and Computer Science, State University of New York at Stony Brook, New York, NY 11794, United States
Author contributions: Chen ZY and Shen HS performed the experiments, analyzed the data and wrote the manuscript; Luo MY designed the study and provided financial support for this work; Duan CJ, Lu HB, Zhang GP and Liu Y were involved in the study conception and design; Cai WL and Liang JZ critically revised the manuscript; all the authors have read and approved the final version.
Supported by Grant from Guangdong Provincial Science and Technology Program, No. 2011B031800182
Correspondence to: Dr. Ming-Yue Luo, Department of Radiology, the Sixth Affiliated Hospital of Sun Yat-Sen University, Guangzhou 510655, Guangdong Province, China. myluo720@163.com
Telephone: +86-20-38254100 Fax: +86-20-38254100
Received: October 30, 2012 Revised: December 15, 2012
Accepted: January 5, 2013
Published online: January 28, 2013

Abstract

AIM: To evaluate the efficacy of reduced cathartic bowel preparation with 2 L polyethylene glycol (PEG)-4000 electrolyte solution and 10 mg bisacodyl enteric-coated tablets for computed tomographic colonography (CTC).

METHODS: Sixty subjects who gave informed consent were randomly assigned to study group A, study group B or the control group. On the day prior to CTC, subjects in study group A were given 20 mL 40% wt/vol barium sulfate suspension before 3 mealtimes, 60 mL 60% diatrizoate meglumine diluted in 250 mL water after supper, and 10 mg bisacodyl enteric-coated tablets 1 h before oral administration of 2 L PEG-4000 electrolyte solution. Subjects in study group B were treated identically to those in study group A, with the exception of bisacodyl which was given 1 h after oral PEG-4000. Subjects in the control group were managed using the same strategy as the subjects in study group A, but without administration of bisacodyl. Residual stool and fluid scores, the attenuation value of residual fluid, and discomfort during bowel preparation in the three groups were analyzed statistically.

RESULTS: The mean scores for residual stool and fluid in study group A were lower than those in study group B, but the differences were not statistically significant. Subjects in study group A showed greater stool and fluid cleansing ability than the subjects in study group B. The mean scores for residual stool and fluid in study groups A and B were lower than those in the control group, and were significantly different. There was no significant difference in the mean attenuation value of residual fluid between study group A, study group B and the control group. The total discomfort index during bowel preparation was 46, 45 and 45 in the three groups, respectively, with no significant difference.

CONCLUSION: Administration of 10 mg bisacodyl enteric-coated tablets prior to or after oral administration of 2 L PEG-4000 electrolyte solution enhances stool and fluid cleansing ability, and has no impact on the attenuation value of residual fluid or the discomfort index. The former is an excellent alternative for CTC colorectum cleansing

© 2013 Baishideng. All rights reserved.

Key words: Colorectal cancer; Screening; Computed tomographic colonography; Polyethylene glycol; Bisacodyl

Chen ZY, Shen HS, Luo MY, Duan CJ, Cai WL, Lu HB, Zhang GP, Liu Y, Liang JZ. Pilot study on efficacy of reduced cathartic bowel preparation with polyethylene glycol and bisacodyl. *World J Gastroenterol* 2013; 19(4): 561-568 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i4/561.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i4.561

INTRODUCTION

Computed tomographic colonography (CTC) has been shown to be an effective tool for colorectal cancer screening, due to its non-invasiveness and high sensitivity for polyp and neoplasia detection[1-6]. This sensitivity is comparable to that obtained using optical colonoscopy[7,8]. However, this examination still requires bowel cleansing similar to that used prior to optical colonoscopy[9]. The need for thorough colorectal cleansing using cathartics remains a major barrier limiting subject acceptance[10]. Reduced cathartic bowel preparation will increase subject compliance to CTC screening[11-13]. To date, there is no general consensus on reduced cathartic bowel preparation, which combines easy preparation and good acceptance[14-18].

Polyethylene glycol (PEG) electrolyte solution is an isosmotic laxative which does not cause electrolyte imbalance[19]. To date, a little research has been conducted on reduced cathartic bowel preparation with PEG-3350 and 10 mg bisacodyl for CTC; however, there are no studies on reduced cathartic bowel preparation combining PEG-4000 with 10 mg bisacodyl[9,12]. Hence, the purpose of this pilot study was to prospectively evaluate the efficacy of reduced cathartic bowel preparation with 2 L PEG-4000 electrolyte solution and 10 mg bisacodyl enteric-coated tablets for CTC.

MATERIALS AND METHODS

Study subjects

Our randomized, prospective and investigator-blinded study group was composed of 60 subjects (34 men and 26 women; age range 22-76 years, mean age 43.6 years). Indications for participation were as follows: asymptomatic subjects with increased colorectal cancer risk due to family or personal history; subjects with recent onset of alarm symptoms, i.e., positive fecal occult blood test, blood in feces, abdominal pain, alternating bowel, refractory iron-deficient anemia, constipation or diarrhea. Subjects with age younger than 18 years, inflammatory bowel disease, end-stage renal disease, and women of child-bearing age were excluded.

After a subject was enrolled in the study, he or she was assigned either to study group A, study group B or the control group on the basis of random numbers. The study protocol was approved by the institutional review board, and informed consent was obtained from each subject.

Bowel preparation

Subjects were instructed to avoid solid food on the day prior to the colorectal examinations. Subjects in study group A were given 20 mL 40% wt/vol barium sulfate suspension (Qingdao Dongfeng Chemical Co. Ltd., Shandong, China) before 3 mealtimes (breakfast, 7:00; lunch, 12:00; supper, 19:00) to achieve fecal tagging; for fluid tagging, 60 mL 60% diatrizoate meglumine (Hunan Hansen Pharmacy Co. Ltd, Hunan, China) diluted in 250 mL water was taken orally after supper; and then two 5 mg bisacodyl enteric-coated tablets (Shaanxi Chuanlong Pharmacy Co. Ltd, Shaanxi, China) were administered at 19:00; and finally, at 20:00, 2 L PEG-4000 electrolyte solution (Jiangxi Hengkang Pharmacy Co. Ltd, Jiangxi, China; each liter solution consisted of 15 mEq PEG-4000, 125 mEq Na^+, 10 mEq K^+, 20 mEq HCO_3^-, 80 mEq SO_4^{2-}, and 35 mEq Cl^-) was administered orally with the first dose of 750 mL, and the remainder was divided into five 250 mL aliquots separated by 15 min. Bowel preparation of the subjects in study group B was identical to that in study group A, with the exception of bisacodyl administration at 21:00. Bowel preparation in subjects in the control group was the same as that in study group A, without administration of bisacodyl.

CTC protocol

Anisodamine hydrochloride (10 mg) was injected intramuscularly 10 min before helical computed tomography (CT) scanning to allow optimal colonic distention, minimize peristalsis and alleviate spasms. Subjects were placed in the right lateral decubitus position on the CT table and a rectal catheter with a retention cuff was inserted. After inflation of the retention cuff, the cuff was gently pulled back until its proximal end rested on the anal sphincter. To distend the colorectum as fully as possible, subjects were then moved into the supine position and room air was gently insufflated into the colorectum to maximal subject tolerance using an automated delivery (JS-628, Guangzhou Jinjian Co. Ltd, Guangdong, China). The delivery was stopped if the rectal pressure was consistently over 3.5 kPa.

Scanning was performed with a 128-slice CT scanner (Somatom Definition AS 128, Siemens AG, Erlangen, Germany) or a 4-slice CT scanner (Aquilion 4, Toshiba Co., Japan) using the following parameters: collimation, 0.625; thickness, 1 mm; pitch, 1.2; electric current, 60 mA; voltage, 120 kV; matrix, 512 × 512; field of view, 3500-4000 mm. Subjects were instructed to hold their breath during data acquisition. A standard CT scout image of the abdomen and pelvis was acquired to assess the degree of colorectal distention, and more room air was insufflated if required. With the scout image, each examination was tailored to encompass the entire colorectum. Scanning was then performed in the prone position, using the same parameters as those in the supine position.

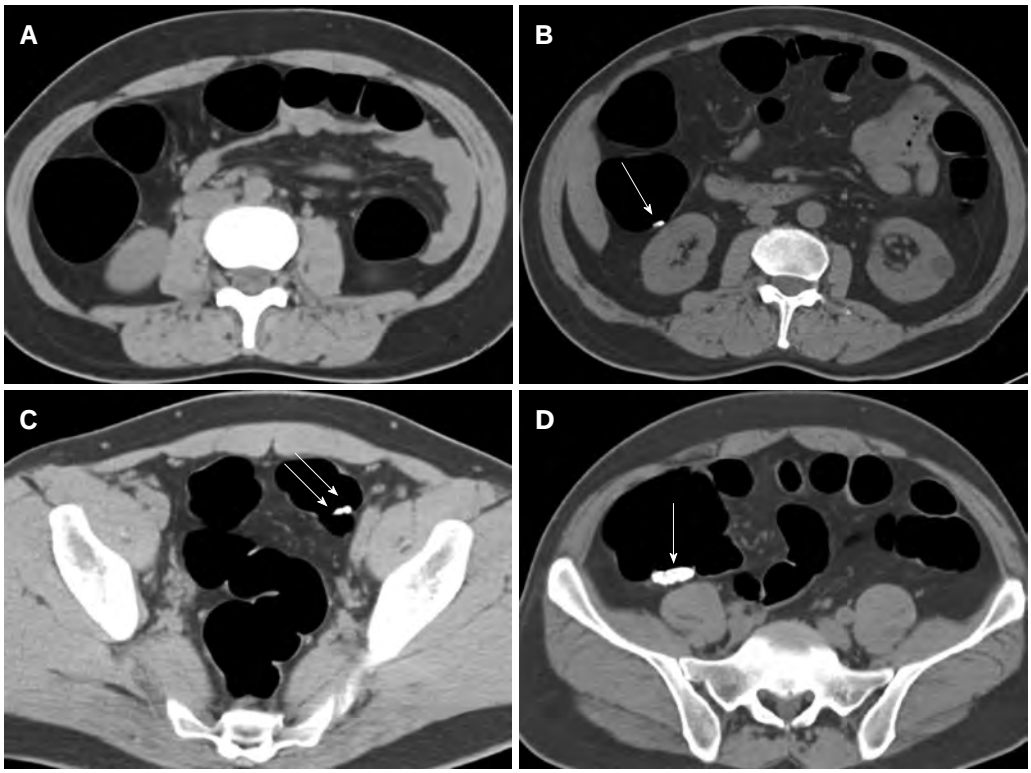


Figure 1 Four-point scoring system of residual stool. A: Stool score of 1: no residual stool; B: Stool score of 2: one stool particle (arrow) smaller than 5 mm in diameter; C: Stool score of 3: two stool particles (arrows) smaller than 5 mm in diameter; D: Stool score of 4: stool particle (arrow) larger than 5 mm in diameter.

Discomfort during bowel preparation

Subjects were invited to complete a questionnaire to describe their discomfort during bowel preparation. Possible discomfort included thirst, hunger, bloating, abdominal pain, sleep disturbance, malaise, nausea, vomiting, cramping, anal discomfort, dizziness and others.

Image evaluation

Axial two-dimensional images in the supine position were evaluated. Axial two-dimensional images in the prone position and multiplanar reformation images were also evaluated when necessary. All images were evaluated by two independent experienced readers, who were blind to histories, clinical symptoms and signs, and reduced cathartic regimens in the subjects, in random order on a picture archiving and communication system workstation with regard to residual stool, residual fluid and attenuation value of the residual fluid. If their evaluations differed, the two readers reached a consensus after reviewing and discussing the controversial images with another senior radiologist. The scores from the two readers were averaged to give an overall score for each segment in each subject. The entire colorectum was divided into six anatomic segments: cecum, ascending colon, transverse colon, descending colon, sigmoid colon and rectum.

Residual stool evaluation

A previously established four-point scoring system was used to evaluate residual stool in each of the six colorectum segments with lower scores corresponding to decreased residual stool[18]. A colorectum segment with no stool was given a score of 1; a segment with a single residual stool particle smaller than 5 mm in diameter, a score of 2; a segment with two or three particles of stool all smaller than 5 mm in diameter, a score of 3; and a segment with stool particles larger than 5 mm or more numerous than three, a score of 4 (Figure 1). A score of 1 or 2, indicated good stool cleansing; and a score of 3 or 4, indicated poor stool cleansing.

Residual fluid evaluation

A similar four-point scale was used to assess residual fluid with lower scores corresponding to a decreased percentage of the distended colorectum segment occupied by residual fluid[18]. The score reflected the percentage of the colorectum lumen filled with fluid. A segment with no fluid was given a score of 1; a segment with less than 25% of the lumen filled, a score of 2; a segment with 25%-50% of the lumen filled, a score of 3; and a segment with more than 50% of the lumen filled, a score of 4 (Figure 2). A score of 1 or 2, indicated good fluid cleansing; and a score of 3 or 4, indicated poor fluid cleansing.

Measurement of the attenuation value of residual fluid

The attenuation value of residual fluid was obtained by placing a single region of interest which was approximately 2 cm in diameter in the largest fluid collection. The mean of these measurements was calculated to establish the fluid attenuation value for each fluid collection.

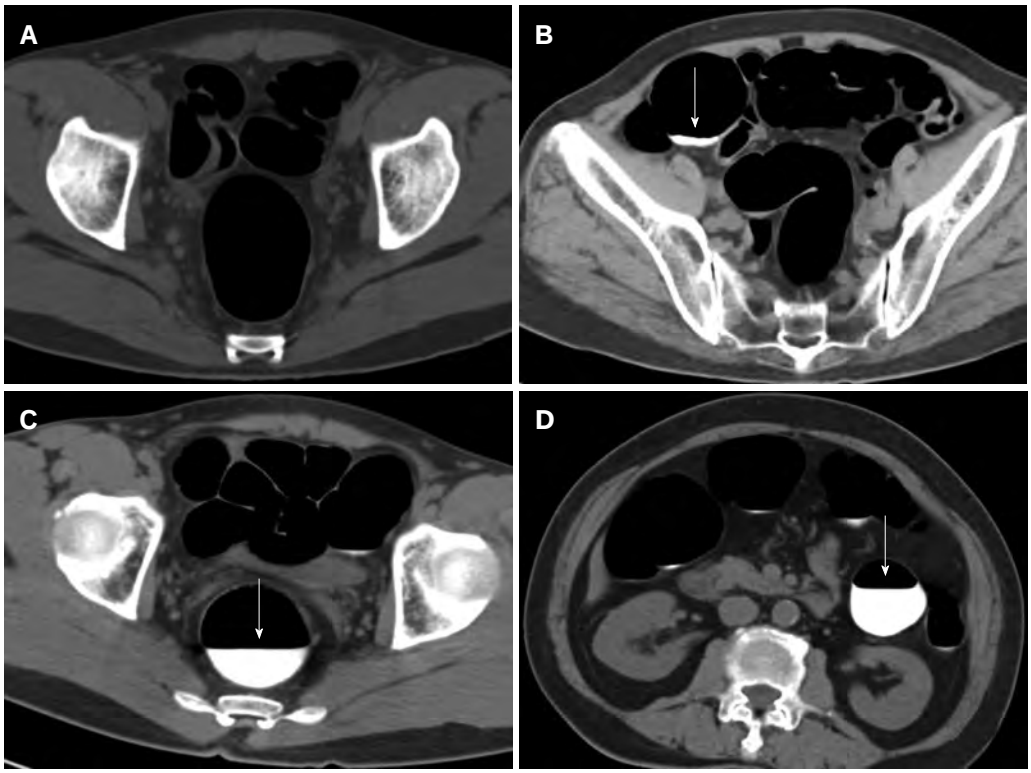


Figure 2 Four-point scoring system of residual fluid. A: Fluid score of 1: no residual fluid; B: Fluid score of 2: less than 25% of the lumen filled with fluid (arrow); C: Fluid score of 3: 25%-50% of the lumen filled with fluid (arrow); D: Fluid score of 4: more than 50% of the lumen filled with fluid (arrow).

Statistical analysis

Continuous data were expressed as means with standard deviations unless otherwise specified. Unpaired Student's *t* tests with the Welch correction were used to compare residual stool and fluid scores, attenuation value of residual fluid, and discomfort during bowel preparation among the three groups using SPSS version 13.0 for Windows. A P value < 0.05 was considered statistically significant.

RESULTS

Residual stool score

In study group A, a residual stool score of 1 was observed in 66 colorectum segments, 2 in 21 colorectum segments, 3 in 5 colorectum segments, 4 in 28 colorectum segments, with a mean score of 1.96 ± 0.11. In study group B, a residual stool score of 1 was observed in 68 colorectum segments, 2 in 15 colorectum segments, 3 in 5 colorectum segments, 4 in 32 colorectum segments, with a mean score of 2.01 ± 0.12. In the control group, a residual stool score of 1 was observed in 37 colorectum segments, 2 in 15 colorectum segments, 3 in 23 colorectum segments, 4 in 45 colorectum segments, with a mean score of 2.63 ± 0.12. There were no significant differences when study group A was compared with study group B ($P > 0.05$); however, when study group A was compared with the control group ($P < 0.001$), and study group B was compared with the control group ($P < 0.002$) significant differences were observed (Table 1).

Good and poor stool cleansing was 72.5% (87/120) and 27.5% (33/120), respectively, in study group A; 69.2% (83/120) and 30.8% (37/120), respectively, in study group B; and 43.3% (52/120) and 56.7% (68/120), respectively, in the control group.

Residual fluid score

In study group A, a residual fluid score of 1 was observed in 67 colorectum segments, 2 in 46 colorectum segments, 3 in 7 colorectum segments, 4 in 0 colorectum segments, with a mean score of 1.50 ± 0.06. In study group B, a residual fluid score of 1 was observed in 66 colorectum segments, 2 in 45 colorectum segments, 3 in 8 colorectum segments, 4 in 1 colorectum segment, with a mean score of 1.53 ± 0.06. In the control group, a residual fluid score of 1 was observed in 58 colorectum segments, 2 in 37 colorectum segments, 3 in 18 colorectum segments, 4 in 7 colorectum segments, with a mean score of 1.78 ± 0.08. There were no significant differences when study group A was compared with study group B ($P > 0.05$); however, when study group A was compared with the control group ($P < 0.05$), and study group B was compared with the control group ($P < 0.05$) significant differences were observed (Table 1).

Good and poor fluid cleansing was 94.2% (113/120) and 5.8% (7/120), respectively, in study group A; 92.5% (111/120) and 7.5% (9/120), respectively, in study group B; and 79.2% (95/120) and 20.8% (25/120), respectively, in the control group.

Attenuation value of residual fluid

In study group A, the attenuation value of residual fluid

Table 1 Residual stool and fluid score in the three groups *n* (%)

Group	Residual stool stool and fluid score				mean ± SD
	1	2	3	4	
Residual stool stool					
Study group A	66 (50.0)	21 (17.5)	5 (4.2)	28 (23.3)	1.96 ± 0.11
Study group B	68 (56.7)	15 (12.5)	5 (4.2)	32 (26.6)	2.01 ± 0.12
Control group	37 (30.8)	15 (12.5)	23 (19.2)	45 (37.5)	2.63 ± 0.12
Residual fluid score					
Study group A	67 (55.9)	46 (38.3)	7 (5.8)	0 (0.0)	1.50 ± 0.06
Study group B	66 (55.0)	45 (37.5)	8 (6.7)	1 (0.8)	1.53 ± 0.06
Control group	58 (48.4)	37 (30.8)	18 (15.0)	7 (5.8)	1.78 ± 0.08

in the cecum was 718 HU, ascending colon was 840 HU, transverse colon was 704 HU, descending colon was 761 HU, sigmoid colon was 694 HU and rectum was 655 HU, with a mean attenuation value of residual fluid of 729 HU. In study group B, the attenuation value of residual fluid in the cecum was 713 HU, ascending colon was 795 HU, transverse colon was 692 HU, descending colon was 665 HU, sigmoid colon was 532 HU, and rectum was 521 HU, with a mean attenuation value of residual fluid of 653 HU. In the control group, the attenuation value of residual fluid in the cecum was 647 HU, ascending colon was 662 HU, transverse colon was 593 HU, descending colon was 643 HU, sigmoid colon was 534 HU, and rectum was 503 HU, with a mean attenuation value of residual fluid of 597 HU. The *P* values in study group A, study group B and the control group were all > 0.05 and not statistically significant (Table 2).

Discomfort during bowel preparation

The three most common complaints related to bowel preparation were hunger (n = 43), bloating (n = 37), and thirst (n = 23). Other discomfort experienced during bowel preparation included nausea (n = 10), abdominal pain (n = 9), dizziness (n = 7), sleep disturbance (n = 6), and vomiting (n = 1). There were no serious adverse events during bowel preparation in the three groups. Total discomfort index during bowel preparation in study group A, study group B, and the control group were 46, 45 and 45, respectively, and the differences were not significant (P > 0.05, Table 3).

DISCUSSION

Despite being a preventable neoplasm, colorectal cancer is the fourth leading cause of cancer death in China[20]. Early detection and removal of the precursor lesion significantly reduces the incidence and mortality associated with this neoplasm[21,22]. CTC has been demonstrated to be a feasible and promising new technique in colorectal cancer screening[23-25]. However, with the current methods of CTC, it is necessary to undertake adequate colorectal cleansing to achieve acceptable CTC sensitivity and specificity, as excess stool creates pseudopolyps and obscures true soft-tissue polyp visualization[26,27]. Full bowel preparation with cathartics is the major barrier to screening, due to the discomfort and inconvenience of

Table 2 Attenuation value of residual fluid in the three groups

Group	Cecum	Asc c	Tra c	Des c	Sig c	Rectum	Mean
Study group A	718	840	704	761	694	655	729
Study group B	713	795	692	665	532	521	653
Control group	647	662	593	643	534	503	597

Asc c: Ascending colon; Tra c: Transverse colon; Des c: Descending colon; Sig c: Sigmoid colon.

the associated diarrhea, and negatively affects examination compliance[28]. Reduced cathartic bowel preparation will increase subject compliance, and is under extensive study at present[29-32]. There is no general consensus as to which reduced cathartic bowel preparation to use[33,34]. However, the prospect of replacing conventional preparation with reduced cathartics has driven researchers to identify a new method which combines diagnostic reliability, ease of preparation and subject acceptance[35].

PEG electrolyte solution is an isosmotic laxative which does not cause electrolyte imbalance, and results in improved tagging of residual stool. However, the use of PEG preparations often results in excess residual fluid within the colorectum[19]. As a stimulant laxative, bisacodyl decreases residual fluid. Hence, bowel preparation combining PEG electrolyte solution with bisacodyl enteric-coated tablet may have an ameliorative effect on colorectal cleansing. The standard volume of PEG-3350 is 4 L; each liter of PEG-3350 solution consists of 100 g PEG-3350, 7.5 g sodium sulfate, 2.7 g sodium chloride, 1.0 g potassium chloride, 5.9 g sodium ascorbate, and 4.7 g ascorbic acid. The common dosage of bisacodyl is 20 mg or 10 mg. In our study, a reduced volume of 2 L PEG-4000 electrolyte solution and 10 mg bisacodyl enteric-coated tablets were administered orally; each liter of PEG-4000 electrolyte solution consisted of 15 mEq PEG-4000, 125 mEq Na^+, 10 mEq K^+, 20 mEq HCO_3^-, 80 mEq SO_4^{2-}, and 35 mEq Cl^-. The molecular weight of PEG-4000 is greater than that of PEG-3350 and has higher viscosity and greater ability to form solids than PEG-3350, and their chemical performance is different.

In our series, the residual stool score in study group A (1.96 ± 0.11) was lower than that in study group B (2.01 ± 0.12) and the difference was not significant, which suggests that the cleansing stool ability was not affected by administration of 10 mg bisacodyl enteric-coated tablets prior to or after oral administration of 2 L PEG-4000 electrolyte solution. The residual stool score in study group A (1.96 ± 0.11) was lower than that in the control group (2.63 ± 0.12) and the difference was significant, which suggests that the stool cleansing ability was enhanced by administration of 10 mg bisacodyl enteric-coated tablets prior to oral administration of 2 L PEG-4000 electrolyte solution. The residual stool score in study group B (2.01 ± 0.12) was lower than that in the control group (2.63 ± 0.12) and the difference was significant, which suggests that oral administration of 10 mg bisacodyl enteric-coated tablets after oral administration of 2 L PEG-4000 electrolyte solution increases

Table 3 Discomfort during bowel preparation

Group	Hunger	Bloating	Thirst	Nausea	Abd p	Diz	Sle d	Vom	Total
Study group A	15	12	7	3	3	3	2	1	46
Study group B	14	13	8	3	3	2	2	0	45
Control group	14	12	8	4	3	2	2	0	45

Abd p: Abdominal pain; Diz: Dizziness; Sle d: Sleep disturbance; Vom: Vomiting.

stool cleansing ability. Therefore, oral administration of 10 mg bisacodyl enteric-coated tablets prior to or after oral administration of 2 L PEG-4000 electrolyte solution improves stool cleansing ability.

Although the residual fluid score in study group A (1.50 ± 0.06) was lower than that in study group B (1.53 ± 0.06) and the difference was not significant, which suggests that the fluid cleansing ability was not influenced by oral administration of 10 mg bisacodyl enteric-coated tablets prior to or after oral administration of 2 L PEG-4000 electrolyte solution. The residual stool score in study group A (1.50 ± 0.06) was lower than that in the control group (1.78 ± 0.08) and the difference was significant, which suggests that oral administration of 10 mg bisacodyl enteric-coated tablets prior to oral administration of 2 L PEG-4000 electrolyte solution heightened the fluid cleansing ability. The residual fluid score in study group B (1.53 ± 0.06) was lower than that in the control group (1.78 ± 0.08) and the difference was significant, which suggests that the fluid cleansing ability was improved by oral administration of 10 mg bisacodyl enteric-coated tablets after oral administration of 2 L PEG-4000 electrolyte solution. Hence, oral administration of 10 mg bisacodyl enteric-coated tablets prior to or after oral administration of 2 L PEG-4000 electrolyte solution increases the fluid cleansing ability. Borden *et al*[18] established a four-point scoring system to evaluate residual fluid in each of the six colorectum segments when comparing magnesium citrate and sodium phosphate. Using the same four-point scoring system, good and poor fluid cleansing was 52% and 48%, respectively, in the study by Hara *et al*[9] following the ingestion of 10 mg bisacodyl tablets after administration of 4 L PEG-3350. Good and poor fluid cleansing was 92.2% and 7.8%, respectively, in our study after oral administration of 10 mg bisacodyl enteric-coated tablets 1 h after oral administration of 2 L PEG-4000 electrolyte solution.

The mean attenuation value of residual fluid in study group A, study group B, and the control group was 729 HU, 653 HU and 597 HU, respectively, with no significant differences. This suggested that oral administration of 10 mg bisacodyl enteric-coated tablets prior to or after oral administration of 2 L PEG-4000 electrolyte solution did not affect the mean attenuation value of residual fluid. The attenuation value of residual fluid has been shown to have a significant effect on polyp conspicuity. Previous research has suggested that optimal viewing conditions in the two-dimensional format are met with an attenuation value of residual fluid of approximately 700 HU, due to high conspicuity of all polyps at this level. Such an increase in polyp conspicuity could lead to increased sensitivity and specificity of CTC. With a mean attenuation value of residual fluid of 729 HU, study group A was closer to this optimal level than study group B and the control group.

Total discomfort index during bowel preparation in study group A, study group B, and the control group was 46, 45 and 45, respectively; however, these differences were not significant. There were no serious adverse events during bowel preparation in the three groups. These results suggest that oral administration of 10 mg bisacodyl enteric-coated tablets prior to or after oral administration of 2 L PEG-4000 electrolyte solution does not influence subject discomfort during bowel preparation for CTC.

No significant differences were observed between study group A and B with regard to residual stool and fluid scores. However, the residual stool score in study group A (1.96 ± 0.11) was lower than that in study group B (2.01 ± 0.12), and the residual fluid score in study group A (1.50 ± 0.06) was also lower than that in study group B (1.53 ± 0.06), which indicated that oral administration of 10 mg bisacodyl enteric-coated tablets prior to oral administration of 2 L PEG-4000 electrolyte solution resulted in a trend toward greater stool and fluid cleansing ability than when given after PEG-4000 electrolyte solution. It would be preferable to administer 10 mg bisacodyl enteric-coated tablets prior to oral administration of 2 L PEG-4000 electrolyte solution for CTC bowel preparation.

There were two limitations in our pilot study. First, the number of subjects was relatively small and the study was likely underpowered to detect subtle differences in preparation adequacy. Second, we compared bowel preparation outcomes obtained with these three bowel preparation regimens, but did not assess the possible diagnostic performance of these regimens. Our study was not designed to address diagnostic performance, which will be studied at a later date.

In conclusion, oral administration of 10 mg bisacodyl enteric-coated tablets prior to or after oral administration of 2 L PEG-4000 electrolyte solution not only enhances the stool and fluid cleansing ability, but also has no impact on the attenuation value of residual fluid or discomfort during bowel preparation. Oral administration of 10 mg bisacodyl enteric-coated tablets prior to oral administration of 2 L PEG-4000 electrolyte solution is an excellent alternative for CTC colorectum cleansing.

COMMENTS

Background

Computed tomographic colonography (CTC) has been shown to be an effective tool for colorectal cancer screening. At present, this examination still requires bowel cleansing similar to that used prior to optical colonoscopy. The need for thorough colorectal cleansing using cathartics remains a major barrier limiting subject acceptance. Reduced cathartic bowel preparation will increase subject compliance in CTC screening. There is no general consensus on reduced cathartic bowel preparation.

Research frontiers

Reduced cathartic bowel preparation is under extensive study at present. The prospect of replacing conventional preparation with reduced cathartics has driven researchers to identify a new method which combines ease of preparation and subject acceptance.

Innovations and breakthroughs

Polyethylene glycol (PEG)-4000 electrolyte solution is an isosmotic laxative that does not cause electrolyte imbalance, and results in improved tagging of residual stool and excess residual fluid. Bisacodyl enteric-coated tablet, a stimulant laxative, decreases the residual fluid. Bowel preparation with combined PEG-4000 electrolyte solution and bisacodyl enteric-coated tablets has an ameliorative effect on colorectum cleansing.

Applications

Oral administration of 10 mg bisacodyl enteric-coated tablets prior to or after oral administration of 2 L PEG-4000 electrolyte solution enhances stool and fluid cleansing ability, and has no impact on the attenuation value of residual fluid or discomfort during bowel preparation. Oral administration of 10 mg bisacodyl enteric-coated tablets prior to oral administration of 2 L PEG-4000 electrolyte solution is an excellent alternative for CTC colorectum cleansing.

Terminology

CTC uses 2D computed tomographic (CT) images of the colorectum, rendered into 3D images and is used to screen for polyps and other abnormalities. The examination consists of non-invasive CT scans, obtained in a few minutes. At the workstation, the images are reconstructed into a 3D model of the colorectum, and the physician may begin clinical analysis of the images.

Peer review

It is a good reason for physician to find a convenient and effective method for patients to carry bowel preparation. But the cost and benefit and the discomfort score of the patient need to be considered.

REFERENCES

1 **Cash BD**, Riddle MS, Bhattacharya I, Barlow D, Jensen D, del Pino NM, Pickhardt PJ. CT colonography of a Medicare-aged population: outcomes observed in an analysis of more than 1400 patients. *AJR Am J Roentgenol* 2012; **199**: W27-W34 [PMID: 22733929 DOI: 10.2214/AJR.11.7729]

2 **Pooler BD**, Baumel MJ, Cash BD, Moawad FJ, Riddle MS, Patrick AM, Damiano M, Lee MH, Kim DH, Muñoz del Rio A, Pickhardt PJ. Screening CT colonography: multicenter survey of patient experience, preference, and potential impact on adherence. *AJR Am J Roentgenol* 2012; **198**: 1361-1366 [PMID: 22623549 DOI: 10.2214/AJR.11.7671]

3 **de Haan MC**, Nio CY, Thomeer M, de Vries AH, Bossuyt PM, Kuipers EJ, Dekker E, Stoker J. Comparing the diagnostic yields of technologists and radiologists in an invitational colorectal cancer screening program performed with CT colonography. *Radiology* 2012; **264**: 771-778 [PMID: 22771881 DOI: 10.1148/radiol.12112486]

4 **Laghi A**, Iafrate F, Rengo M, Hassan C. Colorectal cancer screening: the role of CT colonography. *World J Gastroenterol* 2010; **16**: 3987-3994 [PMID: 20731011 DOI: 10.3748/wjg.v16.i32.3987]

5 **Johnson CD**, Chen MH, Toledano AY, Heiken JP, Dachman A, Kuo MD, Menias CO, Siewert B, Cheema JI, Obregon RG, Fidler JL, Zimmerman P, Horton KM, Coakley K, Iyer RB, Hara AK, Halvorsen RA, Casola G, Yee J, Herman BA, Burgart LJ, Limburg PJ. Accuracy of CT colonography for detection of large adenomas and cancers. *N Engl J Med* 2008; **359**: 1207-1217 [PMID: 18799557 DOI: 10.1056/NEJMoa0800996]

6 **Lieberman DA**. Clinical practice. Screening for colorectal cancer. *N Engl J Med* 2009; **361**: 1179-1187 [PMID: 19759380 DOI: 10.1056/NEJMcp0902176]

7 **Ghanouni A**, Smith SG, Halligan S, Plumb A, Boone D, Magee MS, Wardle J, von Wagner C. Public perceptions and preferences for CT colonography or colonoscopy in colorectal cancer screening. *Patient Educ Couns* 2012; **89**: 116-121 [PMID: 22705250 DOI: 10.1016/j.pec.2012.05.007]

8 **Jensch S**, Bipat S, Peringa J, de Vries AH, Heutinck A, Dekker E, Baak LC, Montauban van Swijndregt AD, Stoker J. CT colonography with limited bowel preparation: prospective assessment of patient experience and preference in comparison to optical colonoscopy with cathartic bowel preparation. *Eur Radiol* 2010; **20**: 146-156 [PMID: 19626326 DOI: 10.1007/s00330-009-1517-0]

9 **Hara AK**, Kuo MD, Blevins M, Chen MH, Yee J, Dachman A, Menias CO, Siewert B, Cheema JI, Obregon RG, Fidler JL, Zimmerman P, Horton KM, Coakley K, Iyer RB, Halvorsen RA, Casola G, Johnson CD. National CT colonography trial (ACRIN 6664): comparison of three full-laxative bowel preparations in more than 2500 average-risk patients. *AJR Am J Roentgenol* 2011; **196**: 1076-1082 [PMID: 21512073 DOI: 10.2214/AJR.10.4334]

10 **Zueco Zueco C**, Sobrido Sampedro C, Corroto JD, Rodriguez Fernández P, Fontanillo Fontanillo M. CT colonography without cathartic preparation: positive predictive value and patient experience in clinical practice. *Eur Radiol* 2012; **22**: 1195-1204 [PMID: 22246146 DOI: 10.1007/s00330-011-2367-0]

11 **Johnson CD**, Kriegshauser JS, Lund JT, Shiff AD, Wu Q. Partial preparation computed tomographic colonography: a feasibility study. *Abdom Imaging* 2011; **36**: 707-712 [PMID: 21614573 DOI: 10.1007/s00261-011-9760-7]

12 **Keedy AW**, Yee J, Aslam R, Weinstein S, Landeras LA, Shah JN, McQuaid KR, Yeh BM. Reduced cathartic bowel preparation for CT colonography: prospective comparison of 2-L polyethylene glycol and magnesium citrate. *Radiology* 2011; **261**: 156-164 [PMID: 21873253 DOI: 10.1148/radiol.11110217]

13 **Liedenbaum MH**, Denters MJ, Zijta FM, van Ravesteijn VF, Bipat S, Vos FM, Dekker E, Stoker J. Reducing the oral contrast dose in CT colonography: evaluation of faecal tagging quality and patient acceptance. *Clin Radiol* 2011; **66**: 30-37 [PMID: 21147296 DOI: 10.1016/j.crad.2010.06.016]

14 **Pollentine A**, Mortimer A, McCoubrie P, Archer L. Evaluation of two minimal-preparation regimes for CT colonography: optimising image quality and patient acceptability. *Br J Radiol* 2012; **85**: 1085-1092 [PMID: 22422379 DOI: 10.1259/bjr/22421731]

15 **Liedenbaum MH**, Denters MJ, de Vries AH, van Ravesteijn VF, Bipat S, Vos FM, Dekker E, Stoker J. Low-fiber diet in limited bowel preparation for CT colonography: Influence on image quality and patient acceptance. *AJR Am J Roentgenol* 2010; **195**: W31-W37 [PMID: 20566777 DOI: 10.2214/AJR.09.3572]

16 **Campanella D**, Morra L, Delsanto S, Tartaglia V, Asnaghi R, Bert A, Neri E, Regge D. Comparison of three different iodine-based bowel regimens for CT colonography. *Eur Radiol* 2010; **20**: 348-358 [PMID: 19711082 DOI: 10.1007/s00330-009-1553-9]

17 **Liedenbaum MH**, de Vries AH, Gouw CI, van Rijn AF, Bipat S, Dekker E, Stoker J. CT colonography with minimal bowel preparation: evaluation of tagging quality, patient acceptance and diagnostic accuracy in two iodine-based preparation schemes. *Eur Radiol* 2010; **20**: 367-376 [PMID: 19707769 DOI: 10.1007/s00330-009-1570-8]

18 **Borden ZS**, Pickhardt PJ, Kim DH, Lubner MG, Agriantonis DJ, Hinshaw JL. Bowel preparation for CT colonography: blinded comparison of magnesium citrate and sodium phosphate for catharsis. *Radiology* 2010; **254**: 138-144 [PMID: 20032148 DOI: 10.1148/radiol.09090398]

19 **Ell C**, Fischbach W, Bronisch HJ, Dertinger S, Layer P, Rünzi M, Schneider T, Kachel G, Grüger J, Köllinger M, Nagell W, Goerg KJ, Wanitschke R, Gruss HJ. Randomized trial of low-volume PEG solution versus standard PEG + electrolytes for bowel cleansing before colonoscopy. *Am J Gastroenterol* 2008; **103**: 883-893 [PMID: 18190651 DOI: 10.1111/j.1572-0241.2007.01708.x]

20 **Wu F,** Lin GZ, Zhang JX. An overview of cancer incidence and trend in China. *Zhongguo Zhongliu* 2012; **21:** 81-85

21 **Park SH**, Yee J, Kim SH, Kim YH. Fundamental elements for successful performance of CT colonography (virtual colonoscopy). *Korean J Radiol* 2007; **8**: 264-275 [PMID: 17673837 DOI: 10.3348/kjr.2007.8.4.264]

22 **Lefere PA**, Gryspeerdt SS, Dewyspelaere J, Baekelandt M, Van Holsbeeck BG. Dietary fecal tagging as a cleansing method before CT colonography: initial results polyp detection and patient acceptance. *Radiology* 2002; **224**: 393-403 [PMID: 12147834 DOI: 10.1148/radiol.2241011222]

23 **Heresbach D**, Djabbari M, Riou F, Marcus C, Le Sidaner A, Pierredon-Foulogne MA, Ponchon T, Boudiaf M, Seyrig JA, Laumonier H, Luet D, Giraud-Cohen M, Pelletier AL, Charachon A, Ramaholimihaso F, Bouillet P, Veyrac M, Ficarelli S, Vahedi K, Keruhel J, Lamouliatte H, Ridereau-Zins C, Bouhnik Y, Tissier M, Diris B, Zagdanski AM, Josselin JM, Hamonic S, Gandon Y. Accuracy of computed tomographic colonography in a nationwide multicentre trial, and its relation to radiologist expertise. *Gut* 2011; **60**: 658-665 [PMID: 21266723 DOI: 10.1136/gut.2010.225623]

24 **Howard K**, Salkeld G, Pignone M, Hewett P, Cheung P, Olsen J, Clapton W, Roberts-Thomson IC. Preferences for CT colonography and colonoscopy as diagnostic tests for colorectal cancer: a discrete choice experiment. *Value Health* 2011; **14**: 1146-1152 [PMID: 22152186 DOI: 10.1016/j.jval.2011.07.012]

25 **Moawad FJ**, Maydonovitch CL, Cullen PA, Barlow DS, Jenson DW, Cash BD. CT colonography may improve colorectal cancer screening compliance. *AJR Am J Roentgenol* 2010; **195**: 1118-1123 [PMID: 20966316 DOI: 10.2214/AJR.10.4921]

26 **Neri E**, Turini F, Cerri F, Vagli P, Bartolozzi C. CT colonography: same-day tagging regimen with iodixanol and reduced cathartic preparation. *Abdom Imaging* 2009; **34**: 642-647 [PMID: 18726646 DOI: 10.1007/s00261-008-9453-z]

27 **Nagata K**, Okawa T, Honma A, Endo S, Kudo SE, Yoshida H. Full-laxative versus minimum-laxative fecal-tagging CT colonography using 64-detector row CT: prospective blinded comparison of diagnostic performance, tagging quality, and patient acceptance. *Acad Radiol* 2009; **16**: 780-789 [PMID: 19375954 DOI: 10.1016/j.acra.2008.12.027]

28 **Yoon SH**, Kim SH, Kim SG, Kim SJ, Lee JM, Lee JY, Han JK, Choi BI. Comparison study of different bowel preparation regimens and different fecal-tagging agents on tagging efficacy, patients' compliance, and diagnostic performance of computed tomographic colonography: preliminary study. *J Comput Assist Tomogr* 2009; **33**: 657-665 [PMID: 19820488 DOI: 10.1097/RCT.0b013e3181926570]

29 **Taylor SA**, Slater A, Burling DN, Tam E, Greenhalgh R, Gartner L, Scarth J, Pearce R, Bassett P, Halligan S. CT colonography: optimisation, diagnostic performance and patient acceptability of reduced-laxative regimens using barium-based faecal tagging. *Eur Radiol* 2008; **18**: 32-42 [PMID: 17404739 DOI: 10.1007/s00330-007-0631-0]

30 **Jensch S**, de Vries AH, Peringa J, Bipat S, Dekker E, Baak LC, Bartelsman JF, Heutinck A, Montauban van Swijndregt AD, Stoker J. CT colonography with limited bowel preparation: performance characteristics in an increased-risk population. *Radiology* 2008; **247**: 122-132 [PMID: 18292475]

31 **Jensch S**, de Vries AH, Pot D, Peringa J, Bipat S, Florie J, van Gelder RE, Stoker J. Image quality and patient acceptance of four regimens with different amounts of mild laxatives for CT colonography. *AJR Am J Roentgenol* 2008; **191**: 158-167 [PMID: 18562740 DOI: 10.2214/AJR.07.3128]

32 **Florie J**, van Gelder RE, Schutter MP, van Randen A, Venema HW, de Jager S, van der Hulst VP, Prent A, Bipat S, Bossuyt PM, Baak LC, Stoker J. Feasibility study of computed tomography colonography using limited bowel preparation at normal and low-dose levels study. *Eur Radiol* 2007; **17**: 3112-3122 [PMID: 17549490 DOI: 10.1007/s00330-007-0668-0]

33 **Nagata K**, Singh AK, Sangwaiya MJ, Näppi J, Zalis ME, Cai W, Yoshida H. Comparative evaluation of the fecal-tagging quality in CT colonography: barium vs. iodinated oral contrast agent. *Acad Radiol* 2009; **16**: 1393-1399 [PMID: 19596591 DOI: 10.1016/j.acra.2009.05.003]

34 **Kim MJ**, Park SH, Lee SS, Byeon JS, Choi EK, Kim JH, Kim YN, Kim AY, Ha HK. Efficacy of barium-based fecal tagging for CT colonography: a comparison between the use of high and low density barium suspensions in a Korean population - a preliminary study. *Korean J Radiol* 2009; **10**: 25-33 [PMID: 19182500 DOI: 10.3348/kjr.2009.10.1.25]

35 **Mahgerefteh S**, Fraifeld S, Blachar A, Sosna J. CT colonography with decreased purgation: balancing preparation, performance, and patient acceptance. *AJR Am J Roentgenol* 2009; **193**: 1531-1539 [PMID: 19933644 DOI: 10.2214/AJR.09.2342]

P- Reviewers Bhosale PR, Mascalchi M, Lian SL
S- Editor Gou SX **L- Editor** A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i4.569

World J Gastroenterol 2013 January 28; 19(4): 569-574
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Retrospective study of steroid therapy for patients with autoimmune pancreatitis in a Chinese population

Bin Liu, Jing Li, Lu-Nan Yan, Hao-Ran Sun, Tong Liu, Zhi-Xiang Zhang

Bin Liu, Tong Liu, Zhi-Xiang Zhang, Department of General Surgery, General Hospital of Tianjin Medical University, Tianjin 300052, China
Jing Li, Department of Anesthesiology, General Hospital of Tianjin Medical University, Tianjin 300052, China
Lu-Nan Yan, Department of General Surgery, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China
Hao-Ran Sun, Department of Radiology, General Hospital of Tianjin Medical University, Tianjin 300052, China
Author contributions: Liu B and Li J performed the majority of experiments; Liu T and Zhang ZX revised the manuscript; Yan LN and Sun HR coordinated and provided financial support for this work, and collected clinical materials; Liu B, Li J and Yan LN designed the study and wrote the manuscript.
Supported by National Natural Science Foundation of China, No. 81170453
Correspondence to: Bin Liu, MD, Department of General Surgery, General Hospital of Tianjin Medical University, Tianjin 300052, China. liubin78@163.com
Telephone: +86-22-60363951 Fax: +86-22-60363951
Received: September 13, 2012 Revised: December 19, 2012
Accepted: December 25, 2012
Published online: January 28, 2013

Abstract

AIM: To explore the optimal steroid therapeutic strategy for autoimmune pancreatitis (AIP).

METHODS: This study was conducted retrospectively in two large institutions in China. Patients with clinically, radiologically and biochemically diagnosed AIP were enrolled. The performed radiological investigations and biochemical tests, the regimen of the given steroid treatment, remission and relapse whether with and without steroid therapy were analyzed.

RESULTS: Twenty-eight patients with AIP received steroid treatment, while 40 patients were treated surgically by pancreatoduodenectomy, distal pancreatectomy and choledochojejunostomy, radiofrequency ablation for the enlarged pancreatic head, percutaneous transhepatic biliary drainage and endoscopic biliary drainage. The starting oral prednisolone dose was 30 mg/d in 18 (64.3%) patients and 40 mg/d in 10 (35.7%) patients administered for 3 wk. The remission rate of AIP patients with steroid treatment (96.4%) was significantly higher than in those without steroid treatment (75%). Maintenance therapy (oral prednisolone dose 5 mg/d) was performed after remission for at least 6-12 mo to complete the treatment course. Similarly, the relapse rate was significantly lower in AIP patients with steroid treatment (28.6%) than in those without steroid treatment (42.5%). Steroid re-treatment was effective in all relapsed patients with or without steroid therapy.

CONCLUSION: Steroid therapy should be considered in all patients with active inflammatory phase of AIP. However, the optimal regimen still should be trailed in larger numbers of patients with AIP.

© 2013 Baishideng. All rights reserved.

Key words: Autoimmune pancreatitis; Chinese population; Steroid therapy; Remission; Relapse

Liu B, Li J, Yan LN, Sun HR, Liu T, Zhang ZX. Retrospective study of steroid therapy for patients with autoimmune pancreatitis in a Chinese population. *World J Gastroenterol* 2013; 19(4): 569-574 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i4/569.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i4.569

INTRODUCTION

Autoimmune pancreatitis (AIP) has recently been described as a type of chronic pancreatitis characterized by an autoimmune inflammatory process with prominent lymphocyte infiltration[1]. Since the concept of autoimmune pancreatitis was firstly introduced by Yoshida *et al*[2]

in 1995, the number of AIP reports has been recently increased in the medical literature.

AIP is characterized by diffuse or focal enlargement of the pancreas, irregular narrowing of the main pancreatic duct, elevated serum immunoglobulin G4 (IgG4) and presence of autoantibodies, and/or IgG4-positive plasma cells and/or dense lymphocyte infiltration with fibrosis[3]. Before the availability of the diagnostic criteria of AIP proposed by the Japan Pancreas Society[4], AIP was frequently misdiagnosed as pancreatic cancer, which consequently imposed the patients a superfluous pancreatic resection[5].

Since the fibroinflammatory process of AIP has a favorable response to steroids, steroid therapy has been accepted as a standard treatment for AIP[6]. Treatment protocols for AIP are still evolving. Although the initial and maintenance dose of oral steroids are clearly recommend by the Japanese literature[7], there is little consensus worldwide on a steroid treatment regimen for patients with AIP[8]. Hence, to explore the optimal steroid therapeutic strategy for AIP, we conducted a retrospective study on AIP treatment in two large institutions in China.

MATERIALS AND METHODS

Subjects

This retrospective study was done to evaluate steroid therapeutic strategy for patients with AIP. The study was conducted in the General Hospital of Tianjin Medical University and West China Hospital of Sichuan University. A total of 68 patients with clinically, radiologically and biochemically diagnosed AIP were enrolled.

Diagnostic criteria

AIP was diagnosed according to the Asian diagnostic criteria for AIP[9]. Consequently, the diagnosis was based on the following clinicopathological findings: (1) the imaging criteria including diffuse/segmental/focal enlargement of the pancreas and diffuse/segmental/focal pancreatic ductal narrowing, often with the stenosis of the bile duct; (2) the serological criteria including elevated serum IgG or IgG4 levels, or detection of autoantibodies; and (3) and/or the histopathological criteria including lymphoplasmacytic infiltration and fibrosis, with abundant IgG4-positive cell infiltration. Thus, AIP was diagnosed when the imaging criteria and one of the other two criteria, the serological and histopathological, were satisfied. Optionally, AIP can also be diagnosed with fulfillment of both the imaging criteria and a good response to steroid treatment.

Assessment of the investigations and therapeutic strategy

We assessed the performed radiological investigations and biochemical tests for all AIP patients. We analyzed remission and relapse whether with or without steroid treatment as well as the given steroid treatment regimen in the patients who received steroid treatment. Remission was defined as the disappearance of clinical symptoms and resolution of the pancreatic and/or extrapancreatic manifestations on radiological investigations[10-12]. All the patients underwent periodic laboratory tests and imaging studies every 3 mo in the first year after remission resulting from steroid treatment. Relapse was defined as reappearance of symptoms with the development of pancreatic and/or extrapancreatic abnormalities on imaging studies and/or marked elevation of serum IgG or IgG4 levels[11,12]. Relapse also included re-elevation of serological levels alone exclusive of clinical symptoms or abnormal imaging[7].

Statistical analysis

Statistical analysis was performed using Fisher's exact test and Mann-Whitney's *U* test (with commercially available software SPSS version 13.0; SPSS, Inc, Chicago, IL). Data were expressed as mean ± SD or median (range). The period from the start of steroid treatment to relapse was evaluated using the Kaplan-Meier method. Differences with P values < 0.05 were considered significant.

RESULTS

Patient profile

Sixty-eight patients with AIP fulfilling the Asian diagnostic criteria for AIP were included in this study. There were 50 men and 18 women with an average age of 62.5 years [patients with steroid 62 (43-78) years, patients without steroid 64 (47-72) years]. Forty patients underwent various surgical procedures (30 males, 10 females), while the remaining 28 patients received steroid treatment (20 males, 8 females). The surgical procedures included pancreatoduodenectomy, distal pancreatectomy and choledochojejunostomy, radiofrequency ablation for the enlarged pancreatic head, percutaneous transhepatic biliary drainage and endoscopic biliary drainage. The numbers of patients undergoing various surgical procedures are as follow: (1) thirteen underwent pancreatoduodenectomy; (2) nineteen underwent distal pancreatectomy and choledochojejunostomy; (3) three underwent radiofrequency ablation for the enlarged pancreatic head; (4) three underwent percutaneous transhepatic biliary drainage; and (5) two underwent endoscopic biliary drainage. In the current study, no patient received any other immunosuppressive treatments such as azathioprine or ursodeoxycholic acid or mycophenolate mofetil.

Given steroid therapy

Steroid therapy was administered in 28 of the 68 AIP patients who were initially presented with obstructive jaundice, abdominal pain, rapid weight loss (> 5 kg in the past 3 mo), diffuse enlargement of the pancreas, associated extrapancreatic abnormalities such as cholangitis and retroperitoneal fibrosis. The clinical presentations in the 28 patients are shown in Table 1.

Table 1 Clinical presentation of patients with steroid treatment *n* (%)

Clinical presentation	Patients (*n* = 28)
Obstructive jaundice	17 (60.7)
Abdominal pain	9 (32.1)
Rapid weight loss	6 (21.4)
Diffuse enlargement of the pancreas	17 (60.7)
Associated extrapancreatic abnormalities	
Cholangitis	18 (64.3)
Retroperitoneal fibrosis	3 (10.7)

Before steroid therapy, blood glucose levels were controlled using insulin in 6 (21.4%) patients and oral hypoglycemics in AIP patients with diabetes mellitus. Alternatively, for patients showing hyperbilirubinemia > 3 mg/dL, percutaneous transhepatic biliary drainage and endoscopic biliary drainage were performed in 4 and 3 patients, respectively.

Regimens of steroid therapy

Among the 28 AIP patients who received steroid therapy, 18 (64.3%) patients started on oral prednisolone at 30 mg/d, while 10 (35.7%) patients began oral prednisolone at 40 mg/d. The therapy monitoring 3 wk after starting steroid treatment was achieved based on the patient's clinical presentation as well as the biochemical results and the imaging findings. When steroid treatment was effective, the dose was tapered by 5 mg every 1-2 wk until the dose reached a maintenance dose of 5 mg/d. Then, maintenance steroids for at least 6-12 mo were given to complete the treatment course. The maintenance steroid treatment was withdrawn whenever complete radiological and serological improvement was obtained.

Remission rate with and without steroid therapy

Steroid therapy used in this study was very effective in alleviating the clinical presentation of AIP (such as obstructive jaundice and abdominal pain) and induced remission more quickly than other treatments without steroids. Likewise, clinical and radiological responses to steroid therapy were seen in 2-3 wk in this study. We also found that response to steroid therapy was typically rapid with significant radiological improvement at 2-3 wk.

The remission rate of AIP patients with steroid treatment (27/28 patients, 96.4%) was significantly higher ($P < 0.001$) than in those without steroid treatment (30/40 patients, 75%). Furthermore, the period to yield a remission in the patients treated with an initial prednisolone dose of 30 mg/d was not significantly different ($P = 0.273$) from the period in those treated with an initial prednisolone dose of 40 mg/d (6.4 ± 5.72 mo and 6.1 ± 6.05 mo, respectively). At remission, the enlarged pancreas returned to near-normal size in 22 (78.6%) patients. Pancreatic atrophy occurred in 6 (21.4%) patients and persistent focal enlargement was found in 1 (3.6%) patient. Irregularity of the pancreatic ducts and/or some degree of bile duct stenosis remained in 15 (53.6%) patients. Although the elevated serum IgG level declined in all patients after the start of steroid treatment, it failed to normalize (< 751 mg/dL) in 17 (60.7%) patients. Likewise, 15 (53.6%) patients with persistently elevated serum IgG levels showed irregular pancreatic ducts and/or some degree of bile duct stenosis.

Relapse rate with and without steroid therapy

The relapse rate was significantly lower ($P = 0.004$) in AIP patients with steroid treatment than in those without steroid treatment (8/28 patients, 28.6% and 17/40 patients, 42.5%, respectively). The relapse period after starting steroid treatment differed in the 8 patients: within 6 mo in 1 (12.5%) patient, within 1 year in 4 (50%) patients, within 2 years in 6 (75%) patients and within 3 years in all 8 (100%) patients. Multivariate analysis showed that there was no correlation ($P = 0.573$) between the relapse rate and the initial prednisolone dose (20% with prednisolone dose of 40 mg/d and 22.2% with prednisolone dose of 30 mg/d). Furthermore, the relapse occurred in 2 of the 10 patients who received prednisolone at 40 mg/d and in 4 of the 18 patients who received prednisolone at 30 mg/d. The relapse rate of AIP was significantly higher ($P = 0.003$) in patients with persistently elevated serum IgG levels (6/17 patients, 35.3%) than in those with normalized serum IgG levels (1/7 patients, 10%). Relapse occurred in 3 of the 8 (37.5%) patients during the maintenance treatment, whereas it occurred in the rest of the 5 (62.5%) patients when the maintenance treatment was stopped. Steroid re-treatment was effective in all relapsed patients with or without steroid therapy.

Maintenance dose of oral steroids after remission

The maintenance steroid therapy was administered after remission in all 28 AIP patients treated with steroids. After remission, the starting oral prednisolone dose was gradually tapered by 5 mg every 1-2 wk to reach a maintenance dose of 5 mg/d. The maintenance steroids were continued for at least 6-12 mo to complete the treatment course. The maintenance dose of steroid was withdrawn in 7 (25%) patients in whom complete radiological and serological improvement was obtained.

Complications of steroid treatment

There was no death attributable to steroid treatment related complications. As AIP patients were typically elder patients, they were at a high risk of developing steroid related complications. Steroid treatment related complications occurred in 7 of the 28 patients, including pneumonia in 3 patients, avascular necrosis of the femoral head in 1 patient, lumbar vertebral fracture in 1 patient and diabetes mellitus in 2 patients. In these patients, the steroid medication dose was reduced or completely ceased. In addition, the 3 patients with pneumonia were treated with antibiotics.

DISCUSSION

It has been reported that the overall prevalence of AIP was 0.82/100 000 in Japan. Moreover, on the basis of clinical presentation, biochemical results or histological findings, AIP constitutes 5%-11% of all patients with chronic pancreatitis[13,14]. Likewise, Song *et al*[15] stated that AIP patients accounted for 5% of chronic pancreatitis population in China.

In this study, AIP was found predominantly in elderly males. This coincides with the feature that although AIP occurs in both sexes, it is at least twice as common in men as in women. In addition, in spite that AIP varies widely in age, most AIP patients are older than 50 years[16]. The clinical presentation of AIP in the current study was in agreement with the studies by Kim *et al*[12] and Nishimori *et al*[17]. AIP was presented with obstructive jaundice in 49 (72.1%) patients, abdominal pain in 30 (44.1%) patients and rapid body weight loss in 21 (30.9%) patients. Alternatively, diabetes mellitus was present in 40 (58.8%) patients.

Moreover, AIP has frequently been diagnosed or suspected as pancreaticobiliary malignancy, which consequently imposed the patients an unnecessary pancreatic resection[18,19]. This study also showed that 40 (58.8%) patients with AIP underwent unnecessary surgical procedures.

Alternatively, Refaat *et al*[20] showed in their case report of AIP that although the imaging findings were suggestive of AIP, pancreatic biopsy was necessary to differentiate AIP from pancreatic cancer as the management differs significantly. Consequently, clinicians should be conversant with the clinical, radiographic, serologic and histologic evidence of AIP to improve the diagnostic accuracy for AIP.

Although steroid therapy is generally considered to be very effective in the initial inflammatory phase of AIP, its facile use for patients whose diagnosis of AIP is questionable should be prohibited[21]. In this study, steroid therapy was very effective in improving patient's clinical presentation such as obstructive jaundice and abdominal pain. Additionally, it induced remission more quickly than surgical treatments as the clinical presentation, the imaging findings and the laboratory results were improved in all cases in four week after steroid treatment. This result concurs with Hirano *et al*[22]. Therefore, we think that steroid therapy should be considered in all patients with AIP with active disease. This is in agreement with the study by Pannala *et al*[8].

There has been no consensus to date on steroid regimen and duration of treatment in AIP. Mayo Clinic recommended the initial oral prednisolone dose of 40 mg/d for 4 wk. Afterward, laboratory tests and radiological investigation should be performed 4-6 wk after initiating treatment. If there are biochemical and radiological responses, the dose is gradually tapered by 5 mg every week to complete the treatment course of 11 wk[10]. Considering that many complications are related to steroid treatment, maintenance low-dose oral prednisolone treatment was not routinely performed for patients with AIP[8]. In a Japanese consensus statement, Kamisawa *et al*[7] suggested that initiating treatment with prednisolone at 0.6 mg/kg daily should be tapered gradually to reach a maintenance dose of 5 mg/d over 3-6 mo. Finally, maintenance steroids are continued for at least 6 mo and possibly up to 3 years. Although the remission can be achieved in most AIP patients after steroid treatment[9], relapses still occur in 24%-40% of the patients and the starting doses of steroid (typically given for 3-4 wk) are often continued for varying periods of time[8].

In our practice, the starting oral prednisolone dose was 30-40 mg/d for 3 wk. Then, we monitored the patient's clinical presentation, the biochemical and serological results as well as the imaging findings in 3 wk after starting steroid treatment. Consequently, when steroid treatment was effective, we tapered the dose by 5 mg every 1-2 wk to reach a maintenance dose of 5 mg/d. Lastly, maintenance steroids were continued for at least 6-12 mo to complete the given treatment course. The maintenance steroid treatment was withdrawn once complete radiological and serological improvement was achieved. In this study, the response was typically rapid through significant radiological improvement at 2-3 wk in 96.4% of the patients. This coincides with Kamisawa *et al*[23].

Future studies should help to establish an optimal steroid therapy regimen to effectively alleviate the clinical presentation of AIP patients and reduce the risk of steroid-induced complications.

Similar to the previous studies[6,7], we also found that AIP patients were typically elder patients and are at a high risk of developing steroid related complications (diabetes, pneumonia and so forth). Instead of using long-term low-dose steroids, azathioprine, mycophenolate mofetil and other immunosuppresive drugs are used for maintenance of remission in AIP patients who relapse after steroid withdrawal[10]. But, similar to steroids, azathioprine or mycophenolate mofetil also have significant side effects such as increased risk of infection, headache, peripheral edema, hypertension, leucopoenia and lymphoma[8,24]. Thus, the option of long-term treatment for maintenance of AIP remission needs prospective and controlled trials in larger numbers of patients to assess the risk to benefit ratio of each approach.

In conclusion, steroid therapy, usually in a dose range of 30-40 mg/d, is effective in AIP patients and should be considered for all AIP patients in the acute inflammatory phase. The optimal regimen of steroid therapy remains to be determined.

ACKNOWLEDGMENTS

The authors are grateful to the staff of Department of General Surgery, General Hospital of Tianjin Medical University and the staff of Department of General Surgery, West China Hospital, Sichuan University for collecting the clinical materials, and to the staff of De-

partment of Endocrine, General Hospital of Tianjin Medical University for their skillful technical assistance. Special thanks are extended to the staff of Department of Radiology, General Hospital of Tianjin Medical University for collecting the imaging materials and the staff of Department of Clinical Laboratory of General Hospital of Tianjin Medical University for processing the blood samples.

COMMENTS

Background

There is no consensus so far on steroid regimen for patients with autoimmune pancreatitis (AIP).

Research frontiers

Steroid therapy has been accepted as a standard treatment for AIP. But, there is little consensus worldwide on a steroid treatment regimen for patients with AIP.

Innovations and breakthroughs

This is a retrospective study exploring the optimal steroid therapeutic strategy for AIP. The performed radiological investigations and biochemical tests, the regimen of the given steroid treatment, remission and relapse whether with and without steroid therapy were analyzed.

Applications

The study results suggest that steroid therapy should be considered in all patients with active inflammatory phase of AIP.

Terminology

Remission: the disappearance of clinical symptoms and resolution of the pancreatic and/or extrapancreatic manifestations on radiological investigations. Relapse: reappearance of symptoms with the development of pancreatic and/or extrapancreatic abnormalities on imaging studies and/or marked elevation of serum immunoglobulin G (IgG) or IgG4 levels

Peer review

This is a good retrospective study in which authors analyzed the optimal steroid therapeutic strategy for AIP. The results are interesting and suggest that steroid therapy should be considered in all patients with active inflammatory phase of AIP.

REFERENCES

1 **Finkelberg DL**, Sahani D, Deshpande V, Brugge WR. Autoimmune pancreatitis. *N Engl J Med* 2006; **355**: 2670-2676 [PMID: 17182992 DOI: 10.1056/NEJMra061200]

2 **Yoshida K**, Toki F, Takeuchi T, Watanabe S, Shiratori K, Hayashi N. Chronic pancreatitis caused by an autoimmune abnormality. Proposal of the concept of autoimmune pancreatitis. *Dig Dis Sci* 1995; **40**: 1561-1568 [PMID: 7628283]

3 **Giday SA**, Khashab MA, Buscaglia JM, Krishnamurty DM, Chen T, Kalloo AN, Canto MI, Okolo PI, Hruban RH, Jagannath SB. Autoimmune pancreatitis: current diagnostic criteria are suboptimal. *J Gastroenterol Hepatol* 2011; **26**: 970-973 [PMID: 21299615 DOI: 10.1111/j.1440-1746.2011.06683.x]

4 **Kanno A**, Ishida K, Hamada S, Fujishima F, Unno J, Kume K, Kikuta K, Hirota M, Masamune A, Satoh K, Notohara K, Shimosegawa T. Diagnosis of autoimmune pancreatitis by EUS-FNA by using a 22-gauge needle based on the International Consensus Diagnostic Criteria. *Gastrointest Endosc* 2012; **76**: 594-602 [PMID: 22898417 DOI: 10.1016/j.gie.2012.05.014]

5 **Imazu H**, Kanazawa K, Mori N, Ikeda K, Kakutani H, Sumiyama K, Hino S, Ang TL, Omar S, Tajiri H. Novel quantitative perfusion analysis with contrast-enhanced harmonic EUS for differentiation of autoimmune pancreatitis from pancreatic carcinoma. *Scand J Gastroenterol* 2012; **47**: 853-860 [PMID: 22507131 DOI: 10.3109/00365521.2012.679686]

6 **Hirano K**, Tada M, Isayama H, Yagioka H, Sasaki T, Kogure H, Nakai Y, Sasahira N, Tsujino T, Yoshida H, Kawabe T, Omata M. Long-term prognosis of autoimmune pancreatitis with and without corticosteroid treatment. *Gut* 2007; **56**: 1719-1724 [PMID: 17525092 DOI: 10.1136/gut.2006.115246]

7 **Kamisawa T**, Shimosegawa T, Okazaki K, Nishino T, Watanabe H, Kanno A, Okumura F, Nishikawa T, Kobayashi K, Ichiya T, Takatori H, Yamakita K, Kubota K, Hamano H, Okamura K, Hirano K, Ito T, Ko SB, Omata M. Standard steroid treatment for autoimmune pancreatitis. *Gut* 2009; **58**: 1504-1507 [PMID: 19398440 DOI: 10.1136/gut.2008.172908]

8 **Pannala R**, Chari ST. Corticosteroid treatment for autoimmune pancreatitis. *Gut* 2009; **58**: 1438-1439 [PMID: 19834112 DOI: 10.1136/gut.2009.183293]

9 **Park SW**, Chung JB, Otsuki M, Kim MH, Lim JH, Kawa S, Ito T, Nishimori I, Ryu JK, Okazaki K, Lee K, Kamisawa T. Conference report: Korea-Japan symposium on autoimmune pancreatitis. *Gut Liver* 2008; **2**: 81-87 [PMID: 20485615 DOI: 10.5009/gnl.2008.2.2.81]

10 **Agrawal S**, Daruwala C, Khurana J. Distinguishing autoimmune pancreatitis from pancreaticobiliary cancers: current strategy. *Ann Surg* 2012; **255**: 248-258 [PMID: 21997803]

11 **Ghazale A**, Chari ST. Optimising corticosteroid treatment for autoimmune pancreatitis. *Gut* 2007; **56**: 1650-1652 [PMID: 17998320 DOI: 10.1136/gut.2007.129833]

12 **Kim HM**, Chung MJ, Chung JB. Remission and relapse of autoimmune pancreatitis: focusing on corticosteroid treatment. *Pancreas* 2010; **39**: 555-560 [PMID: 20182397 DOI: 10.1097/MPA.0b013e3181c8b4a5]

13 **Nishimori I**, Tamakoshi A, Otsuki M. Prevalence of autoimmune pancreatitis in Japan from a nationwide survey in 2002. *J Gastroenterol* 2007; **42** Suppl 18: 6-8 [PMID: 17520216 DOI: 10.1007/s00535-007-2043-y]

14 **Kim KP**, Kim MH, Song MH, Lee SS, Seo DW, Lee SK. Autoimmune chronic pancreatitis. *Am J Gastroenterol* 2004; **99**: 1605-1616 [PMID: 15307882 DOI: 10.1111/j.1572-0241.2004.30336.x]

15 **Song Y**, Liu QD, Zhou NX, Zhang WZ, Wang DJ. Diagnosis and management of autoimmune pancreatitis: experience from China. *World J Gastroenterol* 2008; **14**: 601-606 [PMID: 18203294 DOI: 10.3748/wjg.14.601]

16 **Okazaki K**, Uchida K, Ohana M, Nakase H, Uose S, Inai M, Matsushima Y, Katamura K, Ohmori K, Chiba T. Autoimmune-related pancreatitis is associated with autoantibodies and a Th1/Th2-type cellular immune response. *Gastroenterology* 2000; **118**: 573-581 [PMID: 10702209]

17 **Nishimori I**, Tamakoshi A, Kawa S, Tanaka S, Takeuchi K, Kamisawa T, Saisho H, Hirano K, Okamura K, Yanagawa N, Otsuki M. Influence of steroid therapy on the course of diabetes mellitus in patients with autoimmune pancreatitis: findings from a nationwide survey in Japan. *Pancreas* 2006; **32**: 244-248 [PMID: 16628078 DOI: 10.1097/01.mpa.0000202950.02988.07]

18 **Hardacre JM**, Iacobuzio-Donahue CA, Sohn TA, Abraham SC, Yeo CJ, Lillemoe KD, Choti MA, Campbell KA, Schulick RD, Hruban RH, Cameron JL, Leach SD. Results of pancreaticoduodenectomy for lymphoplasmacytic sclerosing pancreatitis. *Ann Surg* 2003; **237**: 853-858; discussion 858-859 [PMID: 12796582 DOI: 10.1097/01.SLA.0000071516.54864.C1]

19 **Nakazawa T**, Ohara H, Sano H, Ando T, Imai H, Takada H, Hayashi K, Kitajima Y, Joh T. Difficulty in diagnosing autoimmune pancreatitis by imaging findings. *Gastrointest Endosc* 2007; **65**: 99-108 [PMID: 17185087]

20 **Refaat R**, Harth M, Proschek P, Lindemayr S, Vogl TJ. Autoimmune pancreatitis in an 11-year-old boy. *Pediatr Radiol* 2009; **39**: 389-392 [PMID: 19190899 DOI: 10.1007/s00247-008-1132-2]

21 **Chari ST**. Diagnosis of autoimmune pancreatitis using its five cardinal features: introducing the Mayo Clinic's HI-

SORt criteria. *J Gastroenterol* 2007; **42 Suppl 18**: 39-41 [PMID: 17520222 DOI: 10.1007/s00535-007-2046-8]
22 **Hirano K**, Shiratori Y, Komatsu Y, Yamamoto N, Sasahira N, Toda N, Isayama H, Tada M, Tsujino T, Nakata R, Kawase T, Katamoto T, Kawabe T, Omata M. Involvement of the biliary system in autoimmune pancreatitis: a follow-up study. *Clin Gastroenterol Hepatol* 2003; **1**: 453-464 [PMID: 15017645]
23 **Kamisawa T**, Egawa N, Nakajima H, Tsuruta K, Okamoto A. Morphological changes after steroid therapy in autoimmune pancreatitis. *Scand J Gastroenterol* 2004; **39**: 1154-1158 [PMID: 15545176 DOI: 10.1080/00365520410008033]
24 **Sodikoff JB**, Keilin SA, Cai Q, Bharmal SJ, Lewis MM, Raju GS, Willingham FF. Mycophenolate mofetil for maintenance of remission in steroid-dependent autoimmune pancreatitis. *World J Gastroenterol* 2012; **18**: 2287-2290 [PMID: 22611324 DOI: 10.3748/wjg.v18.i18.2287]

P- Reviewer Refaat R **S- Editor** Wen LL
L- Editor A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i4.575

World J Gastroenterol 2013 January 28; 19(4): 575-580
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Increased expression of tyrosine phosphatase SHP-2 in *Helicobacter pylori*-infected gastric cancer

Jing Jiang, Mei-Shan Jin, Fei Kong, Yin-Ping Wang, Zhi-Fang Jia, Dong-Hui Cao, Hong-Xi Ma, Jian Suo, Xue-Yuan Cao

Jing Jiang, Fei Kong, Zhi-Fang Jia, Dong-Hui Cao, Division of Clinical Epidemiology, Jilin University First Hospital, Changchun 130021, Jilin Province, China
Mei-Shan Jin, Yin-Ping Wang, Hong-Xi Ma, Division of Pathology, Jilin University First Hospital, Changchun 130021, Jilin Province, China
Jian Suo, Xue-Yuan Cao, Department of Gastric and Colorectal Surgery, Jilin University First Hospital, Changchun 130021, Jilin Province, China
Author contributions: Cao XY and Jiang J designed the study and carried out most of the study; Jiang J, Cao XY and Jin MS wrote the first draft of manuscript; Jin MS, Wang YP and Kong F analyzed pathological data; Kong F, Jia ZF, Cao DH collected patients' clinical information; Jiang J performed statistical analysis; Ma HX and Suo J provided valuable suggestions in the preparation of the manuscript.
Supported by National Natural Science Foundation of China, No. 81072369; and Natural Science Foundation of Jilin Province, China, No. 200905131
Correspondence to: Xue-Yuan Cao, MD, PhD, Department of Gastric and Colorectal Surgery, Jilin University First Hospital, 71th Xin Min Street, Changchun 130021, Jilin Province, China. ccmzc32jdyycao@yahoo.com.cn
Telephone: +86-431-88783953 Fax: +86-431-88782889
Received: October 16, 2012 Revised: November 17, 2012
Accepted: December 15, 2012
Published online: January 28, 2013

Abstract

AIM: To explore the alteration of tyrosine phosphatase SHP-2 protein expression in gastric cancer and to assess its prognostic values.

METHODS: Three hundred and five consecutive cases of gastric cancer were enrolled into this study. SHP-2 expression was carried out in 305 gastric cancer specimens, of which 83 were paired adjacent normal gastric mucus samples, using a tissue microarray immunohistochemical method. Correlations were analyzed between expression levels of SHP-2 protein and tumor parameters or clinical outcomes. Serum anti-*Helicobacter pylori* (*H. pylori*) immunoglobulin G was detected with enzyme-linked immunosorbent assay. Cox proportional hazards model was used to evaluate prognostic values by compassion of the expression levels of SHP-2 and disease-specific survivals in patients.

RESULTS: SHP-2 staining was found diffuse mainly in the cytoplasm and the weak staining was also observed in the nucleus in gastric mucosa cells. Thirty-two point five percent of normal epithelial specimen and 62.6% of gastric cancer specimen were identified to stain with SHP-2 antibody positively ($P < 0.001$). Though SHP-2 staining intensities were stronger in the *H. pylori* (+) group than in the *H. pylori* (-) group, no statistically significant difference was found in the expression levels of SHP-2 between *H. pylori* (+) and *H. pylori* (-) gastric cancer ($P = 0.40$). The SHP-2 expression in gastric cancer was not significantly associated with cancer stages, lymph node metastases, and distant metastasis of the tumors ($P = 0.34$, $P = 0.17$, $P = 0.52$). Multivariate analysis demonstrated no correlation between SHP-2 expression and disease-free survival ($P = 0.86$).

CONCLUSION: Increased expression of SHP-2 protein in gastric cancer specimen suggesting the aberrant up-regulation of SHP-2 protein might play an important role in the gastric carcinogenesis.

© 2013 Baishideng. All rights reserved.

Key words: Gastric cancer; SH2-containing protein tyrosine phosphatase 2; Expression; *Helicobacter pylori*

Jiang J, Jin MS, Kong F, Wang YP, Jia ZF, Cao DH, Ma HX, Suo J, Cao XY. Increased expression of tyrosine phosphatase SHP-2 in *Helicobacter pylori*-infected gastric cancer. *World J Gastroenterol* 2013; 19(4): 575-580 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i4/575.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i4.575

INTRODUCTION

Gastric cancer remains the fourth most commonly diagnosed cancer and is the second leading cause of cancer related deaths worldwide[1]. The SHP-2 tyrosine posphatase encoded by tyrosine phosphatase SHP-2 (PTPN11) is an evolutionarily conserved protein, containing two Src homology domains at the N terminus, a central catalytic domain, and a C-terminal tail[2,3]. SHP-2 positively regulates many signaling pathways, such as insulin, epidermal growth factor, platelet-derived growth factor, fibroblast growth factor, interleukin (IL)-1, and IL-6[4-6]. SHP-2 has been identified as an essential component in several oncogenic signaling pathways. Elucidation of the events underlying SHP-2-evoked transformation may provide new insights into oncogenic mechanisms and novel targets for anti-cancer therapy[7-11]. Meanwhile, infection with *Helicobacter pylori* (*H. pylori*) is the leading cause for developing atrophic gastritis and gastric cancer worldwide. Many studies reveal that strains of *H. pylori* carrying the major protein virulence factor, cytotoxin-associated antigen A (CagA), are associated with increased risks of gastric cancer compared to strains of *H. pylori* lacking CagA[12]. *H. pylori* inject CagA protein into gastric epithelial cells *via* the bacterial type Ⅳ secretion system and then CagA localizes to the cell membrane and aberrantly activates SHP-2 and its downstream effectors. However, little effort has been devoted to assess an association of the expression level of SHP-2 with gastric cancer risk[13-15]. To investigate the relationship between *H. pylori* infection and SHP-2 protein production in gastric cancer, the SHP-2 protein expression was investigated in 305 patients with gastric cancer, and paired adjacent normal tissue samples were collected in 83 patients. Associations of the protein expression with patient clinical characteristics and prognostic values were also explored in this study.

MATERIALS AND METHODS

Patients and tissue specimens

Three hundred and five consecutive cases of gastric cancer were enrolled in the study. The patient did not receive any treatment before the surgical operation. Gastric cancers were removed by surgical excisions. Adjacent normal gastric epithelial samples were also collected from 83 patients for comparison. Patient ages ranged from 32 to 87 years, with a median age of 64 years. The diagnosis of gastric cancer was confirmed by two pathologists (Jin MS, Wang YP) independently at First Hospital of Jilin University. Written informed consent was obtained from each patient and the study protocol was approved by the Ethics Committee of the First Hospital of Jilin University.

Immunohistochemistry

The section (4 μm in width) of the archival paraffin-embedded block was excised, deparaffinized and stained using a streptavidin-biotin immunoperoxidase technique. The primary antibody, anti-SHP-2 monoclonal antibody (Santa Cruz Biotec, United States), was used in 1:500 dilution; and 3, 3-diaminobenzidine was employed as a chromogen. The section was counterstained with hematoxylin. The stained slides were evaluated by two independent pathologists (Jin MS, Wang YP), who were blinded to the clinical data. The widely accepted H-score system was used to assess staining intensity and percentage of the cells stained with a specific magnitude of intensity. The H-score was calculated with the following equation: H-score = $\sum$Pi (i) (i = 0, 1, 2, 3, Pi = 0%-100%), where i means the intensity of staining, 0 = no staining, 1 = weak staining, 2 = moderate staining and 3 = strong staining. and Pi represents percentages of stained cells with intensities varying from 0% to 100%. Therefore, the H-score ranged from 0 to 300. The H-score $\geqslant$ 100 is considered as positive staining and < 100 is considered as negative staining.

Determination of H.pylori infection

Among 305 gastric cancer patients, blood samples were collected from 100 patients for the examination of *H. pylori* infection before the surgical operation. Four mL of fasting blood samples were left at room temperature for 30 min and the centrifuged. The serum was drawn and stored at a -80 ℃ freezer. Serum immunoglobulin G (IgG) antibodies to *H. pylori* were detected by *H. pylori*-IgG enzyme-linked immunosorbent assay kit (Biohit, Helsink, Finland). The antibody titers were defined by optical density values according to the manufacturer's protocol, and titers higher than the cut off value of 30EIU were considered as positive for *H. pylori* infection. The kit quality control samples showed CVs of 4.5% for *H. pylori* infection examination.

Statistical analysis

As SHP-2 H-scores and their difference values were not normally distributed, these continuous variables were presented as median (interquartile). The Wilcoxon matched-pairs signed-rank test and Wilcoxon signed-rank test were used when comparing paired groups and two independent groups, respectively. Disease-specific survival analysis was performed using the Kaplan-Meier method with log rank test. Risk ratios and corresponding 95% CIs were calculated by the Cox proportional hazards model after adjusted by age (scale variable), sex (nominal variable), differentiation (nominal variable), lymph-vascular invasion (nominal variable) and tumor node metastasis (TNM) staging (scale variable). All statistical tests were two-tailed and *P* values < 0.05 were considered statistically significant. All analyses were performed using the SPSS software package 18.0 (SPSS Inc., United States).

RESULTS

Expression of SHP-2 in gastric cancer specimen and normal gastric epithelial tissues

SHP-2 staining was found diffuse mainly in the cytoplasm and the weak staining was also observed in the nucleus (Figure 1). SHP-2 positive staining was found in

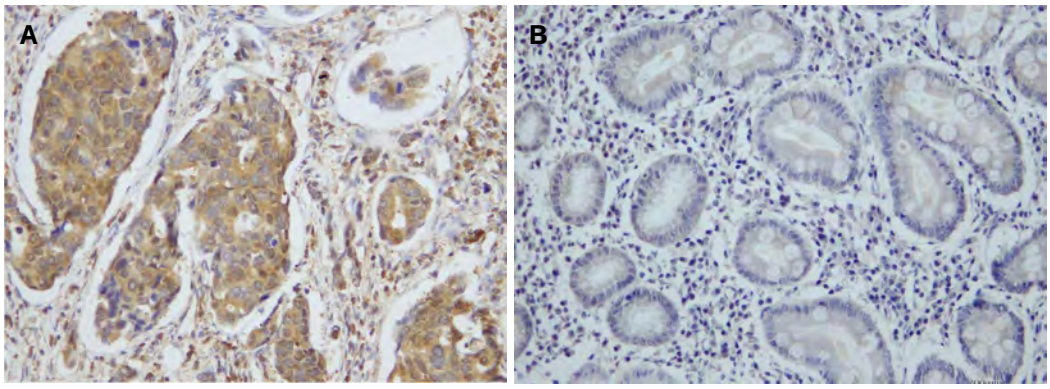


Figure 1 Visualization of SHP-2 expression in malignant gastric epithelial cells and normal mucosal cells by immunohistochemistry (× 200). A: SHP-2 positive immunostaining (2+) in the cytoplasm of gastric carcinoma cells; B: SHP-2 negative immunostaining in glandular cells of normal gastric mucosa.

Table 1 SHP-2 expression in 83 paired gastric cancers and adjacent normal mucus and SHP-2 by *Helicobacter pylori* infection in gastric cancer groups *n* (%)

	H-score				Median H-score (quartile)	*P* value
	0	1-99	100-200	201-300		
Cancer (n = 83)	10 (12.0)	21 (25.3)	27 (32.5)	25 (30.1)	160 (40-210)	< 0.001
Control (n = 83)	41 (49.4)	15 (18.1)	21 (25.3)	6 (7.2)	20 (0-160)	
Positive (n = 67)	8 (11.9)	8 (11.9)	24 (35.8)	27 (40.3)	180 (120-270)	0.401
Negative (n = 33)	1 (3.0)	10 (30.3)	11 (33.3)	11 (33.3)	160 (85-240)	

62/83 (62.6%) gastric cancer samples and in basal cells of 27/83 (32.5%) normal mucosal samples, respectively. There was a significantly increased rate of SHP-2 positive expression in gastric cancer compared to the normal mucosa ($P < 0.001$). In addition, the results showed statistically significant difference in the median H-score of SHP-2 expression between the cancer lesion and normal mucosa (160 *vs* 20, $P < 0.001$; Table 1).

SHP-2 levels and H. pylori infection

Among 100 gastric cancer patients tested for *H. pylori* antibodies, the positive rate of *H. pylori* infection was 67.0%. In the age subgroup analysis, the *H. pylori* infection rate was higher in the group aged between 45-65 years compared to the groups aged younger than 45 years, or older than 65 years. The results showed no correlation between *H. pylori* infection and the positive staining rate of SHP-2 expression ($P = 0.40$). Though SHP-2 staining intensities were stronger in the *H. pylori* (+) group than in the *H. pylori* (-) group, no statistical difference was observed between the two groups (76.1% *vs* 66.6%, $P = 0.09$; Table 1).

SHP-2 expression and clinical characteristics

Statistically, the SHP-2 expression in gastric cancer was not associated with the tumor stage, lymph node metastasis and distant metastasis of the tumors ($P = 0.34$, $P = 0.17$, $P = 0.52$). Kaplan-Meier survival curve was plotted for the 305 patients according to H-score of SHP-2, the overall survival rates of the four groups was not significantly different (log rank test, $P = 0.86$; Figure 2). In addition, the multivariate analysis did not demonstrate an association between SHP-2 expression and disease specific death. There were no differences of SHP-2 levels, age, sex, tumor differentiation, lymph-vascular invasion, TNM staging and survival time between subgroups (83 and 100 cases) and total subjects. The clinical characteristics of subjects are summarized in Tables 2 and 3.

DISCUSSION

Tyrosine phosphorylation and dephosphorylation are coordinated by tyrosine kinases and phosphatases. Recently, SHP-2 has been identified to play a vital role in the pathogenesis of human cancers[10,16,17]. Over-expressions of SHP-2 were reported in infiltrating ductal carcinomas of breast and in gastric cancers as well,[16,18] suggesting that the up-regulation of SHP-2 protein is involved in developing these cancers. In addition, the SHP-2 expression in breast cancers was positively correlated with lymph node metastases and tumor grades, implying that SHP-2 expression is associated with the progression of breast cancers[16]. By contrast, SHP-2 expression in gastric cancers was not found to be associated with lymph node metastases, neither tumor grades in our study (Table 2). The down-regulation in SHP-2 expression was identified to be a better prognostic marker in patients with hepatocellular carcinoma, indicating that SHP-2 plays a role in the tissue specific manner[10].

In general, SHP2 plays multiple roles in tumorigenesis and immune responses[7,8,12]. Activated glycogen synthase kinase-3β, bioactive lipids and their enzymatic generators have been reported to synergistically facilitate inter-

Table 2 SHP-2 expressions and clinical characteristics of 305 gastric cancer patients

	H-score, *n* (%)				Median H-score (quartile)	*P* value
	0	1-99	100-200	201-300		
Sex						
Male (*n* = 233)	16 (6.9)	44 (18.9)	79 (33.9)	94 (40.3)	160 (100-240)	0.82
Female (*n* = 72)	4 (5.6)	8 (11.1)	36 (50.0)	24 (33.3)	160 (140-240)	
Age						
≤ 60 yr (*n* = 129)	9 (7.0)	23 (17.8)	50 (38.8)	47 (36.4)	160 (110-240)	0.92
> 60 yr (*n* = 176)	11 (6.3)	29 (16.5)	65 (36.9)	71 (40.3)	180 (110-240)	
Differentiation						
Well (*n* = 6)	0 (0)	1 (16.7)	1 (16.7)	4 (66.7)	225 (155-270)	0.43
Moderate (*n* = 114)	8 (7.0)	20 (17.5)	41 (36.0)	45 (39.5)	160 (115-240)	
Poor (*n* = 185)	12 (6.5)	31 (16.8)	73 (39.5)	69 (37.3)	160 (120-240)	
Lymph-vascular invasion						
No (*n* = 142)	9 (6.3)	22 (15.5)	54 (38.0)	57 (40.1)	160 (120-240)	0.89
Yes (*n* = 163)	11 (6.7)	30 (18.4)	61 (37.4)	61 (37.4)	160 (100-240)	
Nerve invasion						
No (*n* = 184)	14 (7.6)	30 (16.3)	70 (38.0)	70 (38.0)	160 (120-240)	0.40
Yes (*n* = 121)	6 (5.0)	22 (18.2)	45 (37.2)	48 (39.7)	160 (120-240)	
TNM staging						
Ⅰ (*n* = 25)	0 (0)	3 (12.0)	12 (48.0)	10 (40.0)	180 (140-270)	0.34
Ⅱ (*n* = 50)	4 (8.0)	8 (16.0)	13 (26.0)	25 (50.0)	195 (115-270)	
Ⅲ (*n* = 194)	14 (7.2)	32 (16.5)	77 (39.7)	71 (36.6)	160 (120-240)	
Ⅳ (*n* = 36)	0 (0)	7 (20.6)	14 (41.2)	13 (38.2)	160 (100-242)	
Invasion						
T1 (*n* = 9)	0 (0)	0 (0)	4 (44.4)	5 (55.6)	240 (170-270)	0.34
T2 (*n* = 37)	1 (2.7)	7 (18.9)	18 (48.6)	11 (29.7)	160 (120-225)	
T3 (*n* = 225)	19 (8.4)	38 (16.9)	79 (35.1)	89 (39.6)	160 (100-240)	
T4 (*n* = 34)	0 (0)	7 (20.6)	14 (41.2)	13 (38.2)	160 (120-248)	
Lymph node metastases						
N0 (*n* = 66)	3 (4.5)	10 (15.2)	23 (34.8)	30 (45.5)	180 (140-270)	0.17
N1 (*n* = 94)	7 (7.4)	15 (16.0)	31 (33.0)	41 (43.6)	180 (120-240)	
N2 (*n* = 79)	3 (3.8)	15 (19.0)	39 (49.4)	22 (27.8)	160 (120-210)	
N3 (*n* = 66)	7 (10.6)	12 (18.2)	22 (33.3)	25 (37.9)	160 (100-270)	
Distant metastasis						
No (*n* = 266)	18 (6.8)	43 (16.2)	101(38.0)	104 (39.1)	170 (120-240)	0.52
Yes (*n* = 39)	2 (5.1)	9 (23.1)	14 (35.9)	14 (35.9)	160 (100-240)	
Survival						
Survived (*n* = 180)	12 (6.7)	28 (15.6)	69 (38.3)	71 (39.4)	180 (120-240)	0.77
Died (*n* = 125)	8 (6.4)	24 (19.2)	46 (36.8)	47 (37.6)	160 (100-240)	

TNM: Tumor node metastasis.

Table 3 Multivariate analyses for disease-specific survival

	RR (95% CI)	*P* value
SHP-2 expression		
H-score (201-300) (*n* = 118)		
H-score (100-200) (*n* = 115)	0.99 (0.63-1.56)	0.98
H-score (1-99) (*n* = 52)	1.08 (0.72-1.64)	0.70
Negative (0) (*n* = 20)	1.04 (0.49-1.67)	0.82
Tumor node metastasis staging		
Ⅰ (*n* = 25)		
Ⅱ (*n* = 51)	1.87 (0.52-6.79)	0.34
Ⅲ (*n* = 195)	3.94 (1.22-12.74)	0.02
Ⅳ (*n* = 34)	13.63 (4.04-46.06)	0.00
Lymph-vascular invasion		
Yes (*n* = 142)		
No (*n* = 163)	1.61 (1.09-2.37)	0.02

Calculated by age, sex, differentiation, lymph-vascular invasion and tumor node metastasis staging adjusted Cox regression model. RR: Relative risk.

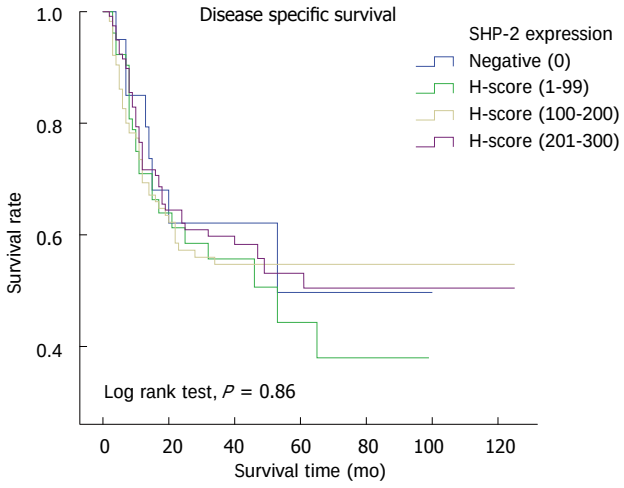


Figure 2 SHP-2 expression and disease specific survival.

ferons (IFN)-γ-induced STAT1 activation by inhibiting SHP-2[19]. Increasing evidences demonstrate that SHP-2 regulates immune responses through its effects on cytokine signaling pathways or inhibits receptor signaling

pathways, such as IFN, tumor necrosis factor-alpha, and JAK/STAT pathways[20-22]. These results support a hypothesis that increased SHP-2 expression would lead to changes in immune responses, eventually resulting in malignant transformation.

H. pylori is a group I carcinogen according to the World Health Organization and International Agency for Research on Cancer[23]. It has been generally accepted that gastric cancer is a multistep process involving a series of events such as atrophic gastritis, intestinal metaplasia, dysplasia and carcinoma. Several studies demonstrated that CagA molecules tethered to the inner surface of plasma membrane of gastric epithelial cells, where they are tyrosine phosphorylated[14,24-26]. SHP-2 is specifically bound by tyrosine phosphorylated CagA, which provokes RAS- and extracellular signal-regulated kinase-dependent signaling cascades. The deregulation of SHP-2 signal transductions leads to loss of epithelial cell polarity, manifested by cell elongation and increased motility, that has been considered to be an important mechanism by which CagA-positive *H. pylori* promotes gastric carcinogenesis[14]. Recently, we reported that rs12423190 polymorphism of the *PTPN11* gene (encoding SHP-2 protein) is significantly associated with an increased risk of gastric atrophy in *H. pylori* infection[27]. To the best of our knowledge, the present study is the first clinical evaluation of SHP-2 expression levels and *H. pylori* infection in gastric cancer. Although SHP-2 expression level was higher in the *H. pylori* (+) group of gastric cancer compared to the *H. pylori* (-) group, no statistical difference was found (76.1% *vs* 66.6%, $P = 0.40$).

In conclusion, we demonstrated that SHP-2 is overexpressed in gastric cancer. However, the level of SHP2 expression was associated with neither *H. pylori* infection nor prognosis of the tumor. Targeting SHP-2 might lead to development of a novel treatment for gastric cancer. The precise mechanisms underlying the effect of SHP-2 on gastric carcinogenesis remains to be further investigated[28-31].

ACKNOWLEDGMENTS

The authors would like to thank those who participated in this study, and particularly thank Mr. Chang-Song Guo and Ms. Ying Song for their technical supports.

COMMENTS

Background

Gastric cancer remains the second leading cause of cancer-related deaths worldwide. Recently, SHP-2 has been identified to play a vital role in the pathogenesis of human cancers.

Research frontiers

SHP-2 has been identified as an essential component of several oncogenic signaling pathways. Mutation in SHP-2 has been confirmed in several types of solid tumors. Elucidation of the events underlying SHP-2-evoked transformation may provide new insights into the novel targets for anti-cancer therapy.

Innovations and breakthroughs

The present study is the first clinical evaluation of SHP-2 expression levels and *Helicobacter pylori* infection in gastric cancer patients.

Applications

Targeting SHP-2 might lead to development of a novel treatment for gastric cancer in the future, such as SHP-2 inhibitors.

Terminology

SHP-2 tyrosine posphatase encoded by tyrosine phosphatase SHP-2 is an evolutionarily conserved protein, containing two Src homology domains at the N terminus, a central catalytic domain, and a C-terminal tail. SHP-2 positively regulates many signaling pathways.

Peer review

The authors showed that SHP-2 expression was higher in cancer specimen compared with normal specimen. However, no association was found between SHP-2 and other clinical parameters.

REFERENCES

1 **Bray F**, Jemal A, Grey N, Ferlay J, Forman D. Global cancer transitions according to the Human Development Index (2008-2030): a population-based study. *Lancet Oncol* 2012; **13**: 790-801 [PMID: 22658655 DOI: 10.1016/S1470-2045(12)70211-5]

2 **Kharitonenkov A**, Chen Z, Sures I, Wang H, Schilling J, Ullrich A. A family of proteins that inhibit signalling through tyrosine kinase receptors. *Nature* 1997; **386**: 181-186 [PMID: 9062191 DOI: 10.1038/386181a0]

3 **Poole AW**, Jones ML. A SHPing tale: perspectives on the regulation of SHP-1 and SHP-2 tyrosine phosphatases by the C-terminal tail. *Cell Signal* 2005; **17**: 1323-1332 [PMID: 16084691 DOI: 10.1016/j.cellsig.2005.05.016]

4 **Qu CK**. Role of the SHP-2 tyrosine phosphatase in cytokine-induced signaling and cellular response. *Biochim Biophys Acta* 2002; **1592**: 297-301 [PMID: 12421673 DOI: 10.1016/S0167-4889(02)00322-1]

5 **You M**, Flick LM, Yu D, Feng GS. Modulation of the nuclear factor kappa B pathway by Shp-2 tyrosine phosphatase in mediating the induction of interleukin (IL)-6 by IL-1 or tumor necrosis factor. *J Exp Med* 2001; **193**: 101-110 [PMID: 11136824 DOI: 10.1084/jem.193.1.101]

6 **Tseng PC**, Huang WC, Chen CL, Sheu BS, Shan YS, Tsai CC, Wang CY, Chen SO, Hsieh CY, Lin CF. Regulation of SHP2 by PTEN/AKT/GSK-3β signaling facilitates IFN-γ resistance in hyperproliferating gastric cancer. *Immunobiology* 2012; **217**: 926-934 [PMID: 22325465 DOI: 10.1016/j.imbio.2012.01.001]

7 **Chan RJ**, Feng GS. PTPN11 is the first identified proto-oncogene that encodes a tyrosine phosphatase. *Blood* 2007; **109**: 862-867 [PMID: 17053061 DOI: 10.1182/blood-2006-07-028829]

8 **Chan G**, Kalaitzidis D, Neel BG. The tyrosine phosphatase Shp2 (PTPN11) in cancer. *Cancer Metastasis Rev* 2008; **27**: 179-192 [PMID: 18286234 DOI: 10.1007/s10555-008-9126-y]

9 **Meng F**, Zhao X, Zhang S. Expression and significance of SHP-2 in human papillomavirus infected cervical cancer. *J Huazhong Univ Sci Technolog Med Sci* 2012; **32**: 247-251 [PMID: 22528229 DOI: 10.1007/s11596-012-0044-4]

10 **Jiang C**, Hu F, Tai Y, Du J, Mao B, Yuan Z, Wang Y, Wei L. The tumor suppressor role of Src homology phosphotyrosine phosphatase 2 in hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2012; **138**: 637-646 [PMID: 22228034 DOI: 10.1007/s00432-011-1143-5]

11 **Bard-Chapeau EA**, Li S, Ding J, Zhang SS, Zhu HH, Princen F, Fang DD, Han T, Bailly-Maitre B, Poli V, Varki NM, Wang H, Feng GS. Ptpn11/Shp2 acts as a tumor suppressor in hepatocellular carcinogenesis. *Cancer Cell* 2011; **19**: 629-639 [PMID: 21575863 DOI: 10.1016/j.ccr.2011.03.023]

12 **Hatakeyama M**. Oncogenic mechanisms of the Helicobacter pylori CagA protein. *Nat Rev Cancer* 2004; **4**: 688-694 [PMID: 15343275 DOI: 10.1038/nrc1433]

13 **Kim JS**, Shin OR, Kim HK, Cho YS, An CH, Lim KW, Kim SS. Overexpression of protein phosphatase non-receptor

type 11 (PTPN11) in gastric carcinomas. *Dig Dis Sci* 2010; **55**: 1565-1569 [PMID: 19690960 DOI: 10.1007/s10620-009-0924-z]
14 **Higashi H**, Tsutsumi R, Muto S, Sugiyama T, Azuma T, Asaka M, Hatakeyama M. SHP-2 tyrosine phosphatase as an intracellular target of Helicobacter pylori CagA protein. *Science* 2002; **295**: 683-686 [PMID: 11743164]
15 **Higashi H**, Nakaya A, Tsutsumi R, Yokoyama K, Fujii Y, Ishikawa S, Higuchi M, Takahashi A, Kurashima Y, Teishikata Y, Tanaka S, Azuma T, Hatakeyama M. Helicobacter pylori CagA induces Ras-independent morphogenetic response through SHP-2 recruitment and activation. *J Biol Chem* 2004; **279**: 17205-17216 [PMID: 14963045 DOI: 10.1074/jbc.M309964200]
16 **Zhou X**, Coad J, Ducatman B, Agazie YM. SHP2 is up-regulated in breast cancer cells and in infiltrating ductal carcinoma of the breast, implying its involvement in breast oncogenesis. *Histopathology* 2008; **53**: 389-402 [PMID: 18643929 DOI: 10.1111/j.1365-2559.2008.03103.x]
17 **Yu SJ**, Yu JK, Ge WT, Hu HG, Yuan Y, Zheng S. SPARCL1, Shp2, MSH2, E-cadherin, p53, ADCY-2 and MAPK are prognosis-related in colorectal cancer. *World J Gastroenterol* 2011; **17**: 2028-2036 [PMID: 21528083 DOI: 10.3748/wjg.v17.i15.2028]
18 **Zhou X**, Agazie YM. Molecular mechanism for SHP2 in promoting HER2-induced signaling and transformation. *J Biol Chem* 2009; **284**: 12226-12234 [PMID: 19261604 DOI: 10.1074/jbc.M900020200]
19 **Tsai CC**, Kai JI, Huang WC, Wang CY, Wang Y, Chen CL, Fang YT, Lin YS, Anderson R, Chen SH, Tsao CW, Lin CF. Glycogen synthase kinase-3beta facilitates IFN-gamma-induced STAT1 activation by regulating Src homology-2 domain-containing phosphatase 2. *J Immunol* 2009; **183**: 856-864 [PMID: 19542364 DOI: 10.4049/jimmunol.0804033]
20 **Du Z**, Shen Y, Yang W, Mecklenbrauker I, Neel BG, Ivashkiv LB. Inhibition of IFN-alpha signaling by a PKC- and protein tyrosine phosphatase SHP-2-dependent pathway. *Proc Natl Acad Sci USA* 2005; **102**: 10267-10272 [PMID: 16000408 DOI: 10.1073/pnas.0408854102]
21 **Kim HY**, Park EJ, Joe EH, Jou I. Curcumin suppresses Janus kinase-STAT inflammatory signaling through activation of Src homology 2 domain-containing tyrosine phosphatase 2 in brain microglia. *J Immunol* 2003; **171**: 6072-6079 [PMID: 14634121]
22 **Hong F**, Nguyen VA, Gao B. Tumor necrosis factor alpha attenuates interferon alpha signaling in the liver: involvement of SOCS3 and SHP2 and implication in resistance to interferon therapy. *FASEB J* 2001; **15**: 1595-1597 [PMID: 11427497]
23 Schistosomes, liver flukes and Helicobacter pylori. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June 1994. *IARC Monogr Eval Carcinog Risks Hum* 1994; **61:** 1-241 [PMID: 7715068]
24 **Tsutsumi R**, Higashi H, Higuchi M, Okada M, Hatakeyama M. Attenuation of Helicobacter pylori CagA x SHP-2 signaling by interaction between CagA and C-terminal Src kinase. *J Biol Chem* 2003; **278**: 3664-3670 [PMID: 12446738 DOI: 10.1074/jbc.M208155200]
25 **Truong BX**, Mai VT, Tanaka H, Ly le T, Thong TM, Hai HH, Van Long D, Furumatsu K, Yoshida M, Kutsumi H, Azuma T. Diverse characteristics of the CagA gene of Helicobacter pylori strains collected from patients from southern vietnam with gastric cancer and peptic ulcer. *J Clin Microbiol* 2009; **47**: 4021-4028 [PMID: 19846630 DOI: 10.1128/JCM.00504-09]
26 **Higuchi M**, Tsutsumi R, Higashi H, Hatakeyama M. Conditional gene silencing utilizing the lac repressor reveals a role of SHP-2 in cagA-positive Helicobacter pylori pathogenicity. *Cancer Sci* 2004; **95**: 442-447 [PMID: 15132773 DOI: 10.1111/j.1349-7006.2004.tb03229.x]
27 **Jiang J**, Jia ZF, Kong F, Jin MS, Wang YP, Tian S, Suo J, Cao X. Association of polymorphism of PTPN 11 encoding SHP-2 with gastric atrophy but not gastric cancer in Helicobacter pylori seropositive Chinese population. *BMC Gastroenterol* 2012; **12**: 89 [PMID: 22788847 DOI: 10.1186/1471-230X-12-89]
28 **Feng H**, Liu KW, Guo P, Zhang P, Cheng T, McNiven MA, Johnson GR, Hu B, Cheng SY. Dynamin 2 mediates PDGFRα-SHP-2-promoted glioblastoma growth and invasion. *Oncogene* 2012; **31**: 2691-2702 [PMID: 21996738 DOI: 10.1038/onc.2011.436]
29 **Aceto N**, Sausgruber N, Brinkhaus H, Gaidatzis D, Martiny-Baron G, Mazzarol G, Confalonieri S, Quarto M, Hu G, Balwierz PJ, Pachkov M, Elledge SJ, van Nimwegen E, Stadler MB, Bentires-Alj M. Tyrosine phosphatase SHP2 promotes breast cancer progression and maintains tumor-initiating cells via activation of key transcription factors and a positive feedback signaling loop. *Nat Med* 2012; **18**: 529-537 [PMID: 22388088 DOI: 10.1038/nm.2645]
30 **Lee KS**, Kalantzis A, Jackson CB, O'Connor L, Murata-Kamiya N, Hatakeyama M, Judd LM, Giraud AS, Menheniott TR. Helicobacter pylori CagA triggers expression of the bactericidal lectin REG3γ via gastric STAT3 activation. *PLoS One* 2012; **7**: e30786 [PMID: 22312430 DOI: 10.1371/journal.pone.0030786]
31 **Miyamoto D**, Miyamoto M, Takahashi A, Yomogita Y, Higashi H, Kondo S, Hatakeyama M. Isolation of a distinct class of gain-of-function SHP-2 mutants with oncogenic RAS-like transforming activity from solid tumors. *Oncogene* 2008; **27**: 3508-3515 [PMID: 18223690 DOI: 10.1038/sj.onc.1211019]

P- Reviewer Hu B **S- Editor** Gou SX
L- Editor Ma JY **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i4.581

World J Gastroenterol 2013 January 28; 19(4): 581-585
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Acute hepatitis C virus infection in a nurse trainee following a needlestick injury

Renzo Scaggiante, Liliana Chemello, Roberto Rinaldi, Giovanni Battista Bartolucci, Andrea Trevisan

Renzo Scaggiante, Roberto Rinaldi, Department of Infectious and Tropical Diseases, Padua Hospital, I-35128 Padua, Italy
Liliana Chemello, Department of Medicine, Padua University, I-35128 Padua, Italy
Giovanni Battista Bartolucci, Andrea Trevisan, Department of Molecular Medicine, Padua University, I-35128 Padua, Italy
Author contributions: Trevisan A, Chemello L and Bartolucci GB interpreted the data and wrote the manuscript; Scaggiante R and Rinaldi R took care of the patient.
Correspondence to: Andrea Trevisan, MD, Professor, Department of Molecular Medicine, University of Padova, Via Giustiniani 2, I-35128 Padova, Italy. andrea.trevisan@unipd.it
Telephone: +39-49-8211362 Fax: +39-49-8763238
Received: May 11, 2012 Revised: July 31, 2012
Accepted: August 8, 2012
Published online: January 28, 2013

Abstract

Hepatitis C virus (HCV) infection after biological accident (needlestick injury) is a rare event. This report describes the first case of acute HCV infection after a needlestick injury in a female nursing student at Padua University Hospital. The student nurse was injured on the second finger of the right hand when recapping a 23-gauge needle after taking a blood sample. The patient who was the source was a 72-year-old female with weakly positive anti-HCV test results. Three months after the injury, at the second step of follow-up, a relevant increase in transaminases with a low viral replication activity (350 IU/mL) was observed in the student, indicating HCV infection. The patient tested positive for the same genotype (1b) of HCV as the injured student. A rapid decline in transaminases, which was not accompanied by viral clearance, and persistently positive HCV-RNA was described 1 mo later. Six months after testing positive for HCV, the student was treated with pegylated interferon plus ribavirin for 24 wk. A rapid virological response was observed after 4 wk of treatment, and a sustained virological response (SVR) was evident 6 mo after therapy withdrawal, confirming that the patient was definitively cured. Despite the favourable *IL28B* gene (rs12979860) CC- polymorphism observed in the patient, which is usually predictive of a spontaneous clearance and SVR, spontaneous viral clearance did not take place; however, infection with this genotype was promising for a sustained virological response after therapy.

© 2013 Baishideng. All rights reserved.

Key words: Biological risk; Acute hepatitis; Hepatitis C infection; Occupational exposure; Antiviral therapy

Scaggiante R, Chemello L, Rinaldi R, Bartolucci GB, Trevisan A. Acute hepatitis C virus infection in a nurse trainee following a needlestick injury. *World J Gastroenterol* 2013; 19(4): 581-585 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i4/581.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i4.581

INTRODUCTION

The incidence rate of acute hepatitis C virus (HCV) infection has been dramatically reduced since the advent of blood products screening and the introduction of single-use medical equipment. However, acute hepatitis C is still present in Western countries. In Italy, the incidence ranges from 1 to 14 cases per 100 000 according to the National Surveillance Agency[1], the Italian Blood Program[2] and by evaluation in the general population[3].

Currently, intravenous (IV) drug use is the most common exposure leading to HCV infection, with HCV prevalence rates of 50%-80% in long-term IV drug abusers. Other possible modes of acquisition are medical procedures, sexual intercourse and needlestick injuries, particularly in health care professionals[4-7]. The average rate of HCV transmission after a single needlestick exposure depends on the amount of inoculated blood, increasing to 0.9% with a hollow needle full of blood and decreas-

ing to 0.3% for conjunctival exposure[8]. In our previous experience at the Padua University Teaching Hospital no cases resulted in seroconversion[9].

In the present report, the first case of HCV seroconversion in our hospital following a needlestick injury is reported. Even though the spontaneous rate of viral clearance after acute hepatitis C ranges from 20% to 50%[5,10] and it can be predicted by measurement of serum viral load[11], which should become negative within 12-14 wk after the exposure, the injured patient developed acute hepatitis without spontaneous viral clearance. The student nurse was treated with pegylated interferon and ribavirin.

CASE REPORT

A 19-year-old female student nurse was injured on the second finger of the right hand when recapping a 23-gauge needle after taking a blood sample. The patient who was the source was a 72-year-old female, weakly positive for anti-HCV by chemiluminescence (CIA) (VITROS, anti-HCV assay, Ortho Clinical Diagnostic, Abbott Laboratories, IL, United States) with an undetermined pattern (only C1+ and C2+) at confirmatory test by immunoblot assay (HCV-RIBA 3.0 assay, Chiron Corporation, United States). Moreover, the patient had a quantitative HCV-RNA level of 14 491 571 IU/mL (COBAS TaqMan, Roche, Basel) and viral genotyping showed HCV-1b (VERSANT® HCV genotype 2.0, INNOLiPA, Innogenetics, Belgium).

The student nurse was checked and followed according to the SIROH recommendations and procedures, which consist of the assessment of hepatitis B surface antigen (HBsAg), hepatitis C antibody and human immunodeficiency virus (HIV) antibody at the time of injury (step 1), 3 mo after the injury (step 2), and 6 mo after the injury (step 3). At the time of the injury she was anti-HBsAg positive (recently vaccinated) and HIV and HCV negative. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were within the reference range.

At the second step (3 mo after injury), a high increase in transaminases with a HCV seroconversion (CIA: anti-HCV positive and RIBA: NS4+, NS3+, C1+++, C2+++, GST-) was detected in the student nurse. The student had a quantitative HCV-RNA level of 330 IU/mL and the HCV genotype was also 1b, as was that of the patient source. Despite the fact that the injured student nurse had no symptoms, she was diagnosed with acute HCV infection. Transaminases were frequently analysed, decreasing until normalization at 4 mo after the injury (Figure 1). HCV-RNA was detectable, but below the lower limit of the linearity range (< 43 IU/mL) and was negative 5 mo after injury. At this time, AST and ALT persisted within the reference range. Unfortunately, 6 mo after injury a recurrence of viraemia (HCV-RNA positive, < 43 IU/mL) together with a slight ALT spike were observed. One month later, further analysis confirmed low viral load and an increase in transaminases.

Due to the chronic profile of the infection, antiviral therapy was prescribed and the subject received a regimen consisting of 180 μg pegylated interferon α-2a subcutaneously once a week and 400 mg ribavirin (adjusted for weight), by mouth twice a day.

During this treatment (Figure 1), a persistent transaminasemia and a slight neutrophilic leukopenia were evident. HCV-RNA analysis showed that the patient remained persistently negative beginning at week 4 of therapy. The patient suffered profound fatigue and musculoskeletal pain, which showed a good response to acetaminophen. During the last month of therapy, she had severe knee pain that was resistant to acetaminophen and only partially mitigated by other non-steroidal anti-inflammatory drugs (NSAID). Therapy lasted 24 wk without any adjustment to the drug dose.

At one month after therapy withdrawal the patient had increasing asthenia and unexplained anaemia (Hb 82 g/L, reference values 123-153 g/L) that resolved with vitamins and iron supplementation, and was probably due to NSAID-induced gastrointestinal bleeding. At 6 mo, HCV-RNA analysis remained negative, transaminases remained within the reference values, the patient felt well and her blood cell counts were restored.

Annual monitoring of HCV-RNA and liver function tests confirmed viral eradication and resolution of the liver disease up to 36 mo from the onset of the infection, thus the patient was defined as "cured".

DISCUSSION

Needle recapping is an unsuitable operation, which is usually performed only by expert physicians[12]. Student nurses should only use standard precautions and procedures and the subject of this case report has been re-educated according to the protocol following her professional exposure.

Because the student had no history of blood transfusion, sexual behaviour or IV drug abuse between the time of injury and the onset of acute HCV, it was highly probable that the lesion with the contaminated source was the cause of seroconversion. Furthermore, the student nurse had the same HCV-1b genotype as the patient; however, sequence analysis comparing the source and student nurse HCV-RNA would be required to eliminate all doubt.

The average rate of HCV transmission after a single needlestick exposure is low[13], and the transmission rate in Italy is as low as 0.4%[8]. The higher seroprevalence in health care workers[2] is probably related to the fact that health care workers are submitted to follow-up after biological contamination, therefore, there is a greater rate of discovery of seroconversions than in other workers not submitted to surveillance for biological risk. The adherence to procedures after biological exposure is important, because patients with acute HCV infection are often asymptomatic and this makes diagnosis difficult and cases remain underestimated[14].

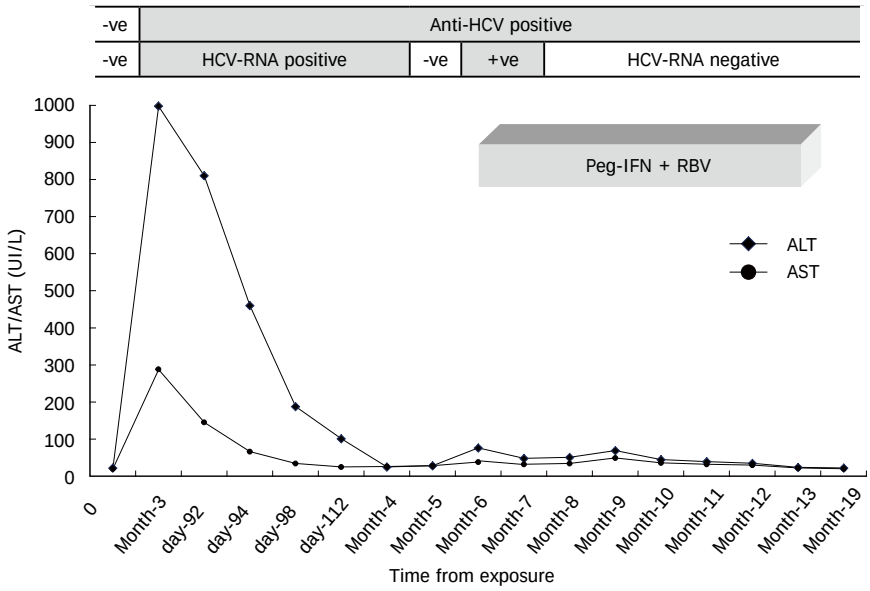


Figure 1 Behaviour of transaminases and hepatitis C virus-RNA after a needle stick injury and during the treatment with pegylated interferon α-2a at 180 μg *s.c.* once a week and ribavirin weight adjusted at 400 mg *p.o.* twice a day are shown. ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; HCV: Hepatitis C virus; PEG-IFN RBV: Pegylated interferon plus ribavirin.

Table 1 Immunologic mechanisms involved in acute phase of hepatitis C virus infection and predictive of viral clearance or persistence and chronicity of infection

	Immunologic marker	Mechanism involved	Ref.
Acute phase of HCV infection and predictive of viral clearance	IFN-α/-β	Vigorous IFN-stimulated genes; inhibition of IL12 expression	[21]
	IFN-γ	HCV core induced generation of reactive oxygen species with stimulation of monocytes and NKs	[22]
	IFN-λ	Host *IL28B* gene polymorphism (CC allele favorable)	[23]
	CD4/CD8	Multispecific intrahepatic Th1 T cells activation	[26]
	HLA-B27/-B57/-A3	Protective HLA alleles on T cell epitopes	[29]
HCV persistence and chronicity of infection	TGF-β/IL10	Inhibition of anti-HCV core/NS2 Th1 response	[24,25]
	CD161	HCV-specific Th17 T cells regulated by TGF-β and IL10	[27]
	HCV HVR-1 specific Abs	Viral escape from Abs mediated neutralization	[28]
	PD1/2B4/CD160	Inhibitory T cell receptors on CD8+ cells	[30]

HCV: Hepatitis C virus; HVR: Hypervariable region; IFN: Interferon; TGF-β: Transforming growth factor-β; IL: Interleukin; HLA: Human leukocyte antigen; NK: Natural killer.

The expectation of a spontaneous viral clearance was initially high in our subject due to the normalization of transaminases and the transient HCV-RNA negativity. Some factors have been proposed as being associated with spontaneous viral clearance including ethnicity[15,16], short incubation period[17], rapid decline in HCV-RNA level[11], and female gender[18]. Unfortunately, despite the age, gender, absence of additional risk factors, rapid normalization of transaminases[19], low viral load (330 IU/mL, approximately 7000 copies/mL) and favourable *IL28B* gene (rs12979860) CC-polymorphism[20] no spontaneous viral clearance was observed in our subject. This was probably due to persistence of the virus (> 3 mo from onset), and particularly to the host complex immunological mechanisms that are associated with spontaneous clearance or to chronic infection (Table 1)[21-30].

In general, following injury with a HCV source, no post-exposure prophylaxis is indicated; on the other hand, whether and how to treat acute HCV infection is still up for debate. The use of α-interferon may be effective in preventing progression from acute to chronic disease[31], but there are no data to indicate that early treatment during the course of chronic infection is less effective than immediate treatment during the acute phase. Certainly, we must consider that in 20%-30% of cases, after an acute infection, spontaneous clearance of HCV occurs and that antiviral therapy is expensive and not well tolerated in the majority of cases[32]. Delaying treatment for 12-16 wk after disease onset permits the identification of cases whose infections spontaneously resolve and whether early initiation of treatment is more appropriate in cases with genotype 1b and/or with asymptomatic disease is still an open question.

The aim of therapy is to achieve a sustained virological response (SVR), defined as the absence of HCV-RNA in serum 6 mo after therapy withdrawal[33]. In acute infection, which has the favourable features of low pretreatment HCV-RNA levels and HCV-genotype non-1 correlate,

SVR varies between 71% and 94%[34-37] and is certainly higher than the rate obtained in cases with long time chronic infection[38].

During therapy, the student nurse showed a rapid virological response (RVR), defined as the absence of HCV-RNA at 4 wk after therapy withdrawal[39], which was maintained at 6 mo after therapy withdrawal, thus, she was defined as having a SVR. These findings confirm that the earlier the virus disappears from serum the higher the probability of achieving a sustained response to treatment.

This case report supports the vigorous application of measures to increase adherence to protocols after biological contamination. We believe that all cases, if there are no contraindications, should be evaluated promptly to prescribe a standard full antiviral schedule of pegylated interferon and weight-based ribavirin, especially in cases like this one in which there is a viral kinetic showing a transient or slow resolution of the active infection. Currently, response-guided therapy helps to determine the duration of treatment, which is cost saving and more advantageous for patients[40]. In this case report, the RVR allowed for the use of a shorter schedule, 24 wk of therapy, which is approximately 12.000 € less than the standard of 48 wk.

In conclusion, the lessons from this case are as follows: (1) health care workers must be counselled and trained to avoid occupational exposure; (2) all injured cases must report the exposure immediately and undergo standard procedures; and (3) a weight-adjusted full schedule of the standard regimen with pegylated interferon and ribavirin should be proposed in all cases with acute HCV infection showing an active and longer viral replication, in particular for cases with more than 6 mo of acquisition. These suggestions might help to avoid the unaware HCV-carrier status, and thus some chronic infections with the potential progression to cirrhosis and to major complications, such as hepatocellular carcinoma.

REFERENCES

1 **Mele A**, Tosti ME, Marzolini A, Moiraghi A, Ragni P, Gallo G, Balocchini E, Santonastasi F, Stroffolini T. Prevention of hepatitis C in Italy: lessons from surveillance of type-specific acute viral hepatitis. SEIEVA collaborating Group. *J Viral Hepat* 2000; **7**: 30-35 [PMID: 10718940 DOI: 10.1046/j.1365-2893.2000.00179.x]

2 **Tosti ME**, Mariano A, Spada E, Pizzuti R, Gallo G, Ragni P, Zotti C, Lopalco P, Curtale F, Graziani G, Mele A, Stroffolini T. Incidence of parenterally transmitted acute viral hepatitis among healthcare workers in Italy. *Infect Control Hosp Epidemiol* 2007; **28**: 629-632 [PMID: 17464931 DOI: 10.1086/513728]

3 **Kondili LA**, Chionne P, Costantino A, Villano U, Lo Noce C, Pannozzo F, Mele A, Giampaoli S, Rapicetta M. Infection rate and spontaneous seroreversion of anti-hepatitis C virus during the natural course of hepatitis C virus infection in the general population. *Gut* 2002; **50**: 693-696 [PMID: 11950818 DOI: 10.1136/gut.50.5.693]

4 **Prati D**. Transmission of hepatitis C virus by blood transfusions and other medical procedures: a global review. *J Hepatol* 2006; **45**: 607-616 [PMID: 16901579 DOI: 10.1016/j.jhep.2006.07.003]

5 **Santantonio T**, Medda E, Ferrari C, Fabris P, Cariti G, Massari M, Babudieri S, Toti M, Francavilla R, Ancarani F, Antonucci G, Scotto G, Di Marco V, Pastore G, Stroffolini T.Risk factors and outcome along a larger patient cohort with community-acquired acute hepatitis C in Italy. *Clin Infect Dis* 2006; **43:** 1154-1159 [PMID: 170291134 DOI: 10.1086/507640]

6 **Deterding K**, Wiegand J, Grüner N, Wedemeyer H. Medical procedures as a risk factor for HCV infection in developed countries: do we neglect a significant problem in medical care? *J Hepatol* 2008; **48**: 1019-1020; author reply 1020-1021 [PMID: 18439703 DOI: 10.1016/j.jhep.2008.03.001]

7 **Martínez-Bauer E**, Forns X, Armelles M, Planas R, Solà R, Vergara M, Fàbregas S, Vega R, Salmerón J, Diago M, Sánchez-Tapias JM, Bruguera M. Hospital admission is a relevant source of hepatitis C virus acquisition in Spain. *J Hepatol* 2008; **48**: 20-27 [PMID: 17998149 DOI: 10.1016/j.jhep.2007.07.031]

8 **Ippolito G**, Puro V, Petrosillo N, De Carli G. Surveillance of occupational exposure to bloodborne pathogens in health care workers: the Italian national programme. *Euro Surveill* 1999; **4**: 33-36 [PMID: 12631910]

9 **Davanzo E**, Frasson C, Morandin M, Trevisan A. Occupational blood and body fluid exposure of university health care workers. *Am J Infect Control* 2008; **36**: 753-756 [PMID: 18945522 DOI: 10.1016/j.ajic.2008.04.254]

10 **Micallef JM**, Kaldor JM, Dore GJ. Spontaneous viral clearance following acute hepatitis C infection: a systematic review of longitudinal studies. *J Viral Hepat* 2006; **13**: 34-41 [PMID: 16364080 DOI: 10.1111/j.1365-2893.2005.00651.x]

11 **Hofer H**, Watkins-Riedel T, Janata O, Penner E, Holzmann H, Steindl-Munda P, Gangl A, Ferenci P. Spontaneous viral clearance in patients with acute hepatitis C can be predicted by repeated measurements of serum viral load. *Hepatology* 2003; **37**: 60-64 [PMID: 12500189 DOI: 10.1053/jhep.2003.50019]

12 **Doebbeling BN**, Vaughn TE, McCoy KD, Beekmann SE, Woolson RF, Ferguson KJ, Torner JC. Percutaneous injury, blood exposure, and adherence to standard precautions: are hospital-based health care providers still at risk? *Clin Infect Dis* 2003; **37**: 1006-1013 [PMID: 14523763 DOI: 10.1086/377535]

13 **Jagger J**, Puro V, De Carli G. Occupational transmission of hepatitis C virus. *JAMA* 2002; **288**: 1469; author reply 1469-1471 [PMID: 12243628 DOI: 10.1001/jama.288.12.1469]

14 **Blackard JT**, Shata MT, Shire NJ, Sherman KE. Acute hepatitis C virus infection: a chronic problem. *Hepatology* 2008; **47**: 321-331 [PMID: 18161707 DOI: 10.1002/hep.21902]

15 **Thomas DL**, Astemborski J, Vlahov D, Strathdee SA, Ray SC, Nelson KE, Galai N, Nolt KR, Laeyendecker O, Todd JA. Determinants of the quantity of hepatitis C virus RNA. *J Infect Dis* 2000; **181**: 844-851 [PMID: 10720503 DOI: 10.1086/315314]

16 **Wang CC**, Krantz E, Klarquist J, Krows M, McBride L, Scott EP, Shaw-Stiffel T, Weston SJ, Thiede H, Wald A, Rosen HR. Acute hepatitis C in a contemporary US cohort: modes of acquisition and factors influencing viral clearance. *J Infect Dis* 2007; **196**: 1474-1482 [PMID: 18008226 DOI: 10.1086/522608]

17 **Hwang SJ**, Lee SD, Lu RH, Chu CW, Wu JC, Lai ST, Chang FY. Hepatitis C viral genotype influences the clinical outcome of patients with acute posttransfusion hepatitis C. *J Med Virol* 2001; **65**: 505-509 [PMID: 11596085 DOI: 10.1002/jmv.2064]

18 **Page K**, Hahn JA, Evans J, Shiboski S, Lum P, Delwart E, Tobler L, Andrews W, Avanesyan L, Cooper S, Busch MP. Acute hepatitis C virus infection in young adult injection drug users: a prospective study of incident infection, resolution, and reinfection. *J Infect Dis* 2009; **200**: 1216-1226 [PMID: 19764883 DOI: 10.1086/605947]

19 **Yeung LT**, To T, King SM, Roberts EA. Spontaneous clearance of childhood hepatitis C virus infection. *J Viral Hepat* 2007; **14**: 797-805 [PMID: 17927616 DOI: 10.1111/j.1365-2893.2007.00873.x]

20 **Ge D**, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, Heinzen EL, Qiu P, Bertelsen AH, Muir AJ, Sulkowski M, McHutchison JG, Goldstein DB. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; **461**: 399-401 [PMID: 19684573 DOI: 10.1038/nature08309]

21 **Klenerman P**, Thimme R. T cell responses in hepatitis C: the good, the bad and the unconventional. *Gut* 2012; **61**: 1226-1234 [PMID: 21873736 DOI: 10.1136/gutjnl-2011-300620]

22 **Thimme R**, Bukh J, Spangenberg HC, Wieland S, Pemberton J, Steiger C, Govindarajan S, Purcell RH, Chisari FV. Viral and immunological determinants of hepatitis C virus clearance, persistence, and disease. *Proc Natl Acad Sci USA* 2002; **99**: 15661-15668 [PMID: 12441397 DOI: 10.1073/pnas.202608299]

23 **Tillmann HL**, Thompson AJ, Patel K, Wiese M, Tenckhoff H, Nischalke HD, Lokhnygina Y, Kullig U, Göbel U, Capka E, Wiegand J, Schiefke I, Güthoff W, Grüngreiff K, König I, Spengler U, McCarthy J, Shianna KV, Goldstein DB, McHutchison JG, Timm J, Nattermann J. A polymorphism near IL28B is associated with spontaneous clearance of acute hepatitis C virus and jaundice. *Gastroenterology* 2010; **139**: 1586-192, 1592.e1 [PMID: 20637200 DOI: 10.1053/j.gastro.2010.07.005]

24 **Brady MT**, MacDonald AJ, Rowan AG, Mills KH. Hepatitis C virus non-structural protein 4 suppresses Th1 responses by stimulating IL-10 production from monocytes. *Eur J Immunol* 2003; **33**: 3448-3457 [PMID: 14635055 DOI: 10.1002/eji.200324251]

25 **Alatrakchi N**, Graham CS, van der Vliet HJ, Sherman KE, Exley MA, Koziel MJ. Hepatitis C virus (HCV)-specific CD8+ cells produce transforming growth factor beta that can suppress HCV-specific T-cell responses. *J Virol* 2007; **81**: 5882-5892 [PMID: 17376924 DOI: 10.1128/JVI.02202-06]

26 **Thimme R**, Oldach D, Chang KM, Steiger C, Ray SC, Chisari FV. Determinants of viral clearance and persistence during acute hepatitis C virus infection. *J Exp Med* 2001; **194**: 1395-1406 [PMID: 11714747 DOI: 10.1084/jem.194.10.1395]

27 **Rowan AG**, Fletcher JM, Ryan EJ, Moran B, Hegarty JE, O'Farrelly C, Mills KH. Hepatitis C virus-specific Th17 cells are suppressed by virus-induced TGF-beta. *J Immunol* 2008; **181**: 4485-4494 [PMID: 18802051]

28 **Pestka JM**, Zeisel MB, Bläser E, Schürmann P, Bartosch B, Cosset FL, Patel AH, Meisel H, Baumert J, Viazov S, Rispeter K, Blum HE, Roggendorf M, Baumert TF. Rapid induction of virus-neutralizing antibodies and viral clearance in a single-source outbreak of hepatitis C. *Proc Natl Acad Sci U S A* 2007; **104**: 6025-6030 [PMID: 17392433 DOI: 10.1073/pnas.0607026104]

29 **Fitzmaurice K**, Petrovic D, Ramamurthy N, Simmons R, Merani S, Gaudieri S, Sims S, Dempsey E, Freitas E, Lea S, McKiernan S, Norris S, Long A, Kelleher D, Klenerman P. Molecular footprints reveal the impact of the protective HLA-A*03 allele in hepatitis C virus infection. *Gut* 2011; **60**: 1563-1571 [PMID: 21551190 DOI: 10.1136/gut.2010.228403]

30 **Nakamoto N**, Kaplan DE, Coleclough J, Li Y, Valiga ME, Kaminski M, Shaked A, Olthoff K, Gostick E, Price DA, Freeman GJ, Wherry EJ, Chang KM. Functional restoration of HCV-specific CD8 T cells by PD-1 blockade is defined by PD-1 expression and compartmentalization. *Gastroenterology* 2008; **134**: 1927-1937 [PMID: 18549878 DOI: 10.1053/j.gastro.2008.02.033]

31 **Noguchi S**, Sata M, Suzuki H, Ohba K, Mizokami M, Tanikawa K. Early therapy with interferon for acute hepatitis C acquired through a needlestick. *Clin Infect Dis* 1997; **24**: 992-994 [PMID: 9142809 DOI: 10.1093/clinids/24.5.992]

32 **Manns MP**, Wedemeyer H, Cornberg M. Treating viral hepatitis C: efficacy, side effects, and complications. *Gut* 2006; **55**: 1350-1359 [PMID: 16905701 DOI: 10.1136/gut.2005.076646]

33 **European Association for the Study of the Liver**. EASL Clinical Practice Guidelines: management of hepatitis C virus infection. *J Hepatol* 2011; **55**: 245-264 [PMID: 21371579 DOI: 10.1016/j.jhep.2011.02.023]

34 **De Rosa FG**, Bargiacchi O, Audagnotto S, Garazzino S, Cariti G, Raiteri R, Di Perri G. Dose-dependent and genotype-independent sustained virological response of a 12 week pegylated interferon alpha-2b treatment for acute hepatitis C. *J Antimicrob Chemother* 2006; **57**: 360-363 [PMID: 16396921 DOI: 10.1093/jac/dki458]

35 **Kamal SM**, Moustafa KN, Chen J, Fehr J, Abdel Moneim A, Khalifa KE, El Gohary LA, Ramy AH, Madwar MA, Rasenack J, Afdhal NH. Duration of peginterferon therapy in acute hepatitis C: a randomized trial. *Hepatology* 2006; **43**: 923-931 [PMID: 16628640 DOI: 10.1002/hep.21197]

36 **Wiegand J**, Buggisch P, Boecher W, Zeuzem S, Gelbmann CM, Berg T, Kauffmann W, Kallinowski B, Cornberg M, Jaeckel E, Wedemeyer H, Manns MP. Early monotherapy with pegylated interferon alpha-2b for acute hepatitis C infection: the HEP-NET acute-HCV-II study. *Hepatology* 2006; **43**: 250-256 [PMID: 16440367 DOI: 10.1002/hep.21043]

37 **Calleri G**, Cariti G, Gaiottino F, De Rosa FG, Bargiacchi O, Audagnotto S, Quaglia S, De Blasi T, Romano P, Traverso A, Leo G, Carbone R, Del Mastro B, Tinelli M, Caramello P, Di Perri G. A short course of pegylated interferon-alpha in acute HCV hepatitis. *J Viral Hepat* 2007; **14**: 116-121 [PMID: 17244251 DOI: 10.1111/j.1365-2893.2006.00802.x]

38 **McHutchison JG**, Lawitz EJ, Shiffman ML, Muir AJ, Galler GW, McCone J, Nyberg LM, Lee WM, Ghalib RH, Schiff ER, Galati JS, Bacon BR, Davis MN, Mukhopadhyay P, Koury K, Noviello S, Pedicone LD, Brass CA, Albrecht JK, Sulkowski MS. Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. *N Engl J Med* 2009; **361**: 580-593 [PMID: 19625712 DOI: 10.1056/NEJMoa0808010]

39 **Yasin T**, Riley TR, Schreibman IR. Current treatment of choice for chronic hepatitis C infection. *Infect Drug Resist* 2011; **4**: 11-18 [PMID: 21694905 DOI: 10.2147/IDR.S4827]

40 **Di Martino V**, Richou C, Cervoni JP, Sanchez-Tapias JM, Jensen DM, Mangia A, Buti M, Sheppard F, Ferenci P, Thévenot T. Response-guided peg-interferon plus ribavirin treatment duration in chronic hepatitis C: meta-analyses of randomized, controlled trials and implications for the future. *Hepatology* 2011; **54**: 789-800 [PMID: 21674553 DOI: 10.1002/hep.24480]

P- Reviewers Schvoerer E, Wang JT **S- Editor** Lv S
L- Editor Webster JR **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i4.586

World J Gastroenterol 2013 January 28; 19(4): 586-589
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Duplication of the transverse colon in an adult: Case report and review

Filippo Banchini, Rocco Delfanti, Evelina Begnini, Maria Candida Tripodi, Patrizio Capelli

Filippo Banchini, Rocco Delfanti, Evelina Begnini, Patrizio Capelli, Department of General Surgery, Ospedale Guglielmo da Saliceto, 29100 Piacenza, Italy
Maria Candida Tripodi, UO Fibrosi Cistica Dipartimento Materno Infantile Azienda Ospedaliera Universitaria di Parma, 43126 Parma, Italy
Author contributions: Banchini F, Delfanti R, Begnini E, Tripodi MC and Capelli P contributed to the manuscript writing and revision.
Correspondence to: Filippo Banchini, MD, Department of General Surgery, Ospedale Guglielmo da Saliceto, Via Taverna 49, 29100 Piacenza, Italy. filippobanchini@virgilio.it
Telephone: +39-523-303160 Fax: +39-523-303173
Received: August 30, 2012 Revised: November 19, 2012
Accepted: November 24, 2012
Published online: January 28, 2013

Abstract

Tubular duplication of the colon is very rare especially in adulthood, because it is frequently symptomatic earlier in newborn life, so only few cases are reported in literature. Several theories are proposed to explain the onset and the evolution of gut malformations as the aberrant lumen recanalization or the diverticular theory, the alteration of the lateral closure of the embryonal disk or finally the dorsal protrusion of the yolk-sac for herniation or adhesion to the ectoderm for an abnormality of the longitudinal line, but none clarifies the exact genesis of duplication. We present a case of "Y-shaped" tubular duplication of the transverse colon in a 21-year-old adult, with a history of chronic pain and constipation, referred to our department for abdominal pain with retrosternal irradiation, treated with the resection of the aberrant bowel.

© 2013 Baishideng. All rights reserved.

Key words: Colonic diseases; Colorectal duplication; Neogenesis; Colonic duplication; Transverse duplication

Banchini F, Delfanti R, Begnini E, Tripodi MC, Capelli P. Duplication of the transverse colon in an adult: Case report and review. *World J Gastroenterol* 2013; 19(4): 586-589 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i4/586.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i4.586

INTRODUCTION

Duplications of gastrointestinal tract are infrequent in general population with an incidence of two or three cases per year in referred pediatric center[1]. The most frequent localization is the ileum 30% and the ileocecal valve 30%, followed by the jejunum with 8%, the colon 6%-7% and the rectum 5%[2,3]. Since 1950, less than 50 colonic duplication have been reported in literature[4] and they were more often discovered in the first two years of life (80%)[5]. If no associated malformation is present, they remain frequently hidden for several years until a complication occurs[6]. The symptoms are unspecific and depend on the type of duplication and on the associated abnormality. More often they are abdominal mass, chronic pain, constipation, occlusion, and less frequently volvulus[7] intussusception, bleeding[8] or perforation (especially in sigmoid colon mimicking diverticulitis). Also the arising of carcinoma is observed: the most frequent is adenocarcinoma[9-13] followed by squamous carcinoma and carcinoid tumors[14-16]. The carcinoembryonic antigen expression can be observed in association with the arising of adenocarcinoma[17], but the presence of the carbohydrate antigen 19-9 is not well defined yet.

CASE REPORT

A 21-year-old man with a history of chronic constipation was referred to our department for abdominal pain with retrosternal irradiation. During childhood he was hospitalized for the same symptoms without a clear diagnosis:



Figure 1 Computed tomography scan. The arrows indicate the duplication.

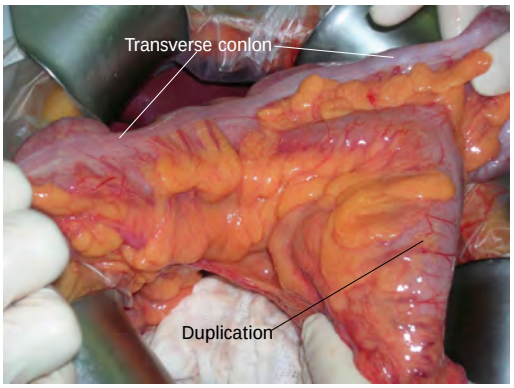


Figure 2 Intraoperative dissection.

alimentary intolerance or celiac disease were excluded, and all the abdominal ultrasounds were always negative. The mother had no particular problems during pregnancy and labour, she had no exposition to pollution, but the patient was an eight months premature birth. At the hospitalization the blood samples showed mild leukocytosis and the abdominal Rx plane was normal. Abdominal sonography was performed but it was not diagnostic, because of air hiding; an abdominal computed tomography (CT) scan (Figure 1) revealed an oval mass with wall enhancement and similar stool material inside, suggestive for intestinal duplication. A small-bowel contrast study was performed but no alteration could be demonstrated. The patient was treated with antibiotics and was discharged asymptomatic with a planned intervention for the following week. At the explorative laparotomy a transverse colon Y-type duplication was shown (Figure 2); the duplication was bent down at the origin of the transverse mesocolon and an own mesocolon was seen. After dissection from the mesocolic stamp, the duplication was unbent revealing that the blood supply origin from a distal division of the middle colic artery with a trifurcation for right and left transverse colon and one branch for the duplication. The upper part of the common mesocolon presented a crabbed fusion of small vessels. To preserve the left marginal arcade, we dissected the artery for the duplication with conservation of the two branches for the native transverse colon and with resection of the duplicated bowel and 5 cm of the native transverse colon. A colo-colic anastomosis was performed. The post operative period was uneventful and the patient was discharged; at the moment he lies symptoms free. Histopathology confirmed the tubular duplication with multiple mucosal ulceration.

DISCUSSION

Digestive duplication is defined by the three Rowling's criteria[18]: (1) the wall of the duplication is in continuity with one of the duplicated organ; (2) the cyst is surrounded by a smooth muscular layer; and (3) a layer of digestive mucosa is present, more often typical or heterotopic as gastric mucosa, colonic mucosa, bronchial or pancreatic structure[19,20]. Smith[21] well defined the term of duplication as "these formations, either tubular or spherical, which lie in contiguity with normal bowel and which share with it a common blood supply and usually a common muscle coat. There may or may not be a communication between the normal and anomalous lumina and the dividing septum may be muscular or merely a double layer of epithelium". Gross *et al*[22] described four variations in the shape of duplications: (1) a tubular structure that branches out from the intestine and extends for some distance between the mesenteric leaves; (2) a double-barreled structure communicating with the intestinal lumen at one or both ends; (3) a cystic structure lying in the peritoneal cavity, attached by a mesenteric stalk; and (4) a spherical lesion contiguous with some part of the bowel, particularly along the ileum. Cystic types are the most frequent with 90%-95% of the cases and the tubular type 5%-10%; the latter can be distinguished in double barreled (80%) or Y-shaped form (20%)[23-26]. For colonic duplication McPherson makes another classification as type I (simple cysts), type II (diverticula) or the most common type III (tubular colonic duplication)[27]. Several theories have been proposed to describe this abnormality in which environmental factors, such as trauma or hypoxia, can play a role[28]. The aberrant lumen recanalization theory exposed by Bremer[29] seems to be the most comprehensive. As described by Johnson[30] in 1910 the gut grows and obliterates the lumen at the sixth week with subsequent internal vacuolization and coalescence of vacuoles with the formation of the single lumen. The aberrant vacuolization well explains that vacuoles can remain separated and create one or more duplication that can be separated or communicating with the original gut. This theory can be easily associated to the double barrel duplication. Another theory is the diverticular one, described by Lewis *et al*[31], criticized by some authors[1] because it doesn't explain the forms of entire duplication of the colon (for example) with the presence of circular and longitudinal muscle coats. Blinder describes more simply that the tubular forms can be caused by an alteration of the lateral closure of the embryonal disk at the stage 9 of Steeter for an abnormality of the longitudinal line, instead of

cystic forms that originate from diverticulation later in the evolution[32,33]. Another explanation, well described by Smith[21], is the dorsal protrusion of the yolk-sac caused by its herniation or adhesion to the ectoderm in same phase of presomite development taking to a "dorsal remnants" structure, that can be divided into: congenital dorsal enteric fistula, congenital dorsal enteric sinus, congenital dorsal enteric cyst, congenital dorsal enteric divericulum[34]. This last theory can also explain the association with other abnormalities, especially the vertebral alteration. The association of other malformations, as vertebral or genitourinary alterations, can be classified in the notochordodysraphies: duplication or alteration of the hindgut is associated with genitourinary problem in 60% of cases[35,36] and high malformation with vertebral problems[37-40]. In particular the hindgut duplication can be explained by an insult to the caudal cell mass at the days 23-25 resulting in the "split notochord syndrome[41,42]. Until 2011 only 18 cases of transverse colon duplication have been reported. The particular localisation sometimes can give problems in diagnosis, like mismatching with pancreatic cyst[5,43]. In tubular type, CT scan, contrast enema and colonoscopy, could be the best diagnostic tools: in fact the presence of air and stool can facilitate the diagnosis; in ultrasonography the presence of air doesn't actually allow a good vision. Probably sonography, instead of CT scan, can be more performant in the cystic type, where the diagnosis is more difficult as described above. In fact in the cystic type of the transverse colon, the lesion have fluid inside and it could be adherent to the mesocolon or the pancreas, mimicking other alterations. The small-bowel contrast study, as in our case, is not so performant also in tubular type. The management of colonic duplication consists in the resection of the duplicated bowel with an extension of 2 cm in the normal bowel, due to the common blood supply at the two organs, the presence of fibrosis in the conjunction area[6,44] and the probability of arising tumor in the duplication. Our report shows that the symptomatology could be hidden for a long time, especially in absence of other anatomical alterations; probably for the presence of a big communication with the native bowel. The presence of stool inside the duplication helped us in the diagnosis, but it remains not easy to do, as reported by some authors[43]. In conclusion the colon duplication is not frequent especially in adulthood; the differential diagnosis is not easy; it must be resected even if asymptomatic, because of the risk of arising tumor.

REFERENCES

1 **Blickman JG**, Rieu PH, Buonomo C, Hoogeveen YL, Boetes C. Colonic duplications: clinical presentation and radiologic features of five cases. *Eur J Radiol* 2006; **59**: 14-19 [PMID: 16781838]

2 **Kekez T**, Augustin G, Hrstic I, Smud D, Majerovic M, Jelincic Z, Kinda E. Colonic duplication in an adult who presented with chronic constipation attributed to hypothyroidism. *World J Gastroenterol* 2008; **14**: 644-646 [PMID: 18203304 DOI: 10.3748/wjg.13.644]

3 **Puligandla PS**, Nguyen LT, St-Vil D, Flageole H, Bensoussan AL, Nguyen VH, Laberge JM. Gastrointestinal duplications. *J Pediatr Surg* 2003; **38**: 740-744 [PMID: 12720184 DOI: 10.1016/jpsu.2003.50197]

4 **Vargas-de la Llata RG**, García-Huerta G, Gutiérrez-Mata F, Noriega-Maldonado O, Dorantes-González HJ, López-Giacoman C. [Sigmoid colon duplication: an adult case report.]. *Rev Gastroenterol Mex* 2009; **74**: 259-262 [PMID: 19858020]

5 **Mourra N**, Chafai N, Bessoud B, Reveri V, Werbrouck A, Tiret E. Colorectal duplication in adults: report of seven cases and review of the literature. *J Clin Pathol* 2010; **63**: 1080-1083 [PMID: 20924093 DOI: 10.1136/jcp.2010.083238]

6 **Correia-Pinto J**, Romero R, Carvalho JL, Silva G, Guimarães H, Estevão-Costa J. Neonatal perforation of a Y-shaped sigmoid duplication. *J Pediatr Surg* 2001; **36**: 1422-1424 [PMID: 11528620 DOI: 10.1053/jpsu.2001.26390]

7 **Salvador II**, Modelli ME, Pereira CR. [Tubular duplication of the colon: a case report and review of the literature]. *J Pediatr* (Rio J) 1996; **72**: 254-257 [PMID: 14688938]

8 **Fotiadis C**, Genetzakis M, Papandreou I, Misiakos EP, Agapitos E, Zografos GC. Colonic duplication in adults: report of two cases presenting with rectal bleeding. *World J Gastroenterol* 2005; **11**: 5072-5074 [PMID: 16124070]

9 **Hattori H**. Adenocarcinoma occurring just at the attached site of colonic duplication in an adult man. *Dig Dis Sci* 2005; **50**: 1754 [PMID: 16133984]

10 **Delladetsima J**, Papachristodoulou A, Zografos G. Carcinoma arising in a duplicated colon. *Am Surg* 1992; **58**: 782-783 [PMID: 1456608]

11 **Arkema KK**, Calenoff L. Adenocarcinoma in tubular duplication of the sigmoid colon. *Gastrointest Radiol* 1977; **2**: 137-139 [PMID: 210081]

12 **Hsu H**, Gueng MK, Tseng YH, Wu CC, Liu PH, Chen CC. Adenocarcinoma arising from colonic duplication cyst with metastasis to omentum: A case report. *J Clin Ultrasound* 2011; **39**: 41-43 [PMID: 20812340]

13 **Letarte F**, Sideris L, Leblanc G, Leclerc YE, Dubé P. Pseudomyxoma peritonei arising from intestinal duplication. *Am Surg* 2011; **77**: 233-234 [PMID: 21337887]

14 **Hickey WF**, Corson JM. Squamous cell carcinoma arising in a duplication of the colon: case report and literature review of squamous cell carcinoma of the colon and of malignancy complicating colonic duplication. *Cancer* 1981; **47**: 602-609 [PMID: 7226009 DOI: 10.1002/1097-0142(19810201)47]

15 **Horie H**, Iwasaki I, Takahashi H. Carcinoid in a gastrointestinal duplication. *J Pediatr Surg* 1986; **21**: 902-904 [PMID: 3783381 DOI: 10.1016/S0022-3468(86)80021-5]

16 **Lee J**, Jeon YH, Lee S. Papillary adenocarcinoma arising in a duplication of the cecum. *Abdom Imaging* 2008; **33**: 601-603 [PMID: 17912582 DOI: 10.1007/s00261-007-9330-1]

17 **Inoue Y**, Nakamura H. Adenocarcinoma arising in colonic duplication cysts with calcification: CT findings of two cases. *Abdom Imaging* 1998; **23**: 135-137 [PMID: 9516499 DOI: 10.1007/s002619900305]

18 **Rowling JT**. Some observations on gastric cysts. *Br J Surg* 1959; **46**: 441-445 [PMID: 13651616]

19 **Glaser C**, Kuzinkovas V, Maurer C, Glättli A, Mouton WG, Baer HU. A large duplication cyst of the stomach in an adult presenting as pancreatic pseudocyst. *Dig Surg* 1998; **15**: 703-706 [PMID: 9845641 DOI: 10.1159/000018662]

20 **Godlewski G**, Bossy J, Baumel H. [Anatomical and embryological aspects of gastric duplications]. *Bull Assoc Anat* (Nancy) 1980; **64**: 229-241 [PMID: 6779879]

21 **Smith JR**. Accessory enteric formations: a classification and nomenclature. *Arch Dis Child* 1960; **35**: 87-89 [PMID: 13832044 DOI: 10.1136/adc.35.179.87]

22 **Gross RE**, Holcomb GW, Farber S. Duplications of the alimentary tract. *Pediatrics* 1952; **9**: 448-468 [PMID: 14920118]

23 **Bona R**, Costamagna D, Gentilli S, Mioli PR, Pinna Pintor M, Spampinato L. Diagnosis and surgical treatment of colonic

duplication in a young woman. *Tech Coloproctol* 2005; **9**: 169 [PMID: 16292619 DOI: 10.1007/s10151-005-0221-6]
24 **Haouimi A,** Ouslimane D, Berair R. Duplications coliques. Paris, France: Encycl Med Chir Radiodiagnostic-Appareil digestif, 1999: 4
25 **Iyer CP**, Mahour GH. Duplications of the alimentary tract in infants and children. *J Pediatr Surg* 1995; **30**: 1267-1270 [PMID: 8523222 DOI: 10.1016/0022-3468(95)90482-4]
26 **Pintér AB**, Schubert W, Szemlédy F, Göbel P, Schäfer J, Kustos G. Alimentary tract duplications in infants and children. *Eur J Pediatr Surg* 1992; **2**: 8-12 [PMID: 1571331 DOI: 10.1055/s-2008-1063390]
27 **McPherson AG**, Trapnell JE, Airth GR. Duplication of the colon. *Br J Surg* 1969; **56**: 138-142 [PMID: 5765395 DOI: 10.1002/bjs.1800560214]
28 **Bishop HC**, Koop CE. Surgical management of duplications of the alimentary tract. *Am J Surg* 1964; **107**: 434-442 [PMID: 14129317 DOI: 10.1016/0002-9610(64)90210-7]
29 **Bremer JL.** Diverticula and duplications of the intestinal tract. *Arch Pathol* 1944; **38:** 132-140
30 **Johnson FP.** The development of the mucous membrane of the œsophagus, stomach and small intestine in the human embryo. *Am J Anat* 1910; **10:** 521–575 [DOI: 10.1002/aja.1000100116]
31 **Lewis FT,** Thyng FW. Regular occurrence of intestinal diverticula in embryos of pig, rabbit, and man. *Am J Anat* 1908; **7:** 505-519 [DOI: 10.1002/aja.1000070406]
32 **Blinder G**, Hiller N, Adler SN. A double stomach in an adult. *Am J Gastroenterol* 1999; **94**: 1100-1102 [PMID: 10201493]
33 **Baumel H**, Godlewski G, Pignodel C. [Gastric duplications. A case report (author's transl)]. *J Chir* (Paris) 1980; **117**: 629-633 [PMID: 7451576]
34 **Bentley JF**, Smith JR. Developmental posterior enteric remnants and spinal malformations: the split notochord syndrome. *Arch Dis Child* 1960; **35**: 76-86 [PMID: 13799033 DOI: 10.1136/adc.35.179.76]
35 **Craigie RJ**, Abbaraju JS, Ba'ath ME, Turnock RR, Baillie CT. Anorectal malformation with tubular hindgut duplication. *J Pediatr Surg* 2006; **41**: e31-e34 [PMID: 16769326]
36 **Yousefzadeh DK**, Bickers GH, Jackson JH, Benton C. Tubular colonic duplication--review of 1876-1981 literature. *Pediatr Radiol* 1983; **13**: 65-71 [PMID: 6856378]
37 **Scotte M**, Testart J. [Cystic duplications of the esophagus in adults. Report of 2 cases]. *J Chir* (Paris) 1989; **126**: 583-585 [PMID: 2584287]
38 **Anderson MC**, Silberman WW, Shields TW. Duplications of the alimentary tract in the adult. *Arch Surg* 1962; **85**: 94-108 [PMID: 13861258 DOI: 10.1001/archsurg.1962.01310010098014]
39 **Guivarc'h M**, Granveau C. [Esophageal duplication in the adult. A case detected by x-ray computed tomography and review of the literature]. *Chirurgie* 1987; **113**: 154-165 [PMID: 3622065]
40 **Ildstad ST**, Tollerud DJ, Weiss RG, Ryan DP, McGowan MA, Martin LW. Duplications of the alimentary tract. Clinical characteristics, preferred treatment, and associated malformations. *Ann Surg* 1988; **208**: 184-189 [PMID: 3401062]
41 **Dominguez R**, Rott J, Castillo M, Pittaluga RR, Corriere JN. Caudal duplication syndrome. *Am J Dis Child* 1993; **147**: 1048-1052 [PMID: 8213674]
42 **Stern LE**, Warner BW. Gastrointestinal duplications. *Semin Pediatr Surg* 2000; **9**: 135-140 [PMID: 10949423 DOI: 10.1053/spsu.2000.7565]
43 **Carneiro FP**, de Nazareth Sobreira M, de Azevedo AE, Alves AP, Campos KM. Colonic duplication in an adult mimicking a tumor of pancreas. *World J Gastroenterol* 2008; **14**: 966-968 [PMID: 18240361]
44 **Holcomb GW**, Gheissari A, O'Neill JA, Shorter NA, Bishop HC. Surgical management of alimentary tract duplications. *Ann Surg* 1989; **209**: 167-174 [PMID: 2916861]

P- Reviewer Augustin G **S- Editor** Zhai HH
L- Editor A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i4.590

World J Gastroenterol 2013 January 28; 19(4): 590-593
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Steroid-refractory ulcerative colitis and associated primary sclerosing cholangitis treated with infliximab

Ileana Duca, Patricia Ramírez de la Piscina, Silvia Estrada, Rosario Calderón, Katerina Spicakova, Leire Urtasun, Carlos Marra-López, Salvador Zabaleta, Raquel Bengoa, María Asunción Marcaide, Francisco García-Campos

Ileana Duca, Patricia Ramírez de la Piscina, Silvia Estrada, Rosario Calderón, Katerina Spicakova, Leire Urtasun, Carlos Marra-López, Salvador Zabaleta, Raquel Bengoa, María Asunción Marcaide, Francisco García-Campos, Service of Gastroenterology, Hospital Universitario de Álava-Sede Txagorritxu, Universidad del País Vasco, E-01009 Vitoria-Gasteiz, Spain

Author contributions: Duca I and Ramírez de la Piscina P reviewed the literature, design the study, collected information, provided care to the patient, and wrote the manuscript; Estrada S, Calderón R, Spicakova K, Urtasun L, Zabaleta S, Marra-López C, Bengoa R, Marcaide MA and García-Campos F were involved in the different stages of the diagnosis, care, and management of the patient, provided constructive criticisms, revised and approved the final draft; García-Campos F also supervised the entire process from drafting the paper to the final manuscript submitted to the journal.

Correspondence to: Patrícia Ramírez de la Piscina, MD, Service of Gastroenterology, Hospital Universitario de Álava-Sede Txagorritxu, Universidad del País Vasco, C/ José Achótegui s/n, E-01009 Vitoria-Gasteiz,
Spain. patricia.ramirezdelapiscinaurraca@osakidetza.net
Telephone: +34-945-007301 Fax: +34-945-007359
Received: October 18, 2012 Revised: December 9, 2012
Accepted: December 15, 2012
Published online: January 28, 2013

Abstract

Primary sclerosing cholangitis is an infrequent extraintestinal manifestation of ulcerative colitis. Damage to bile ducts is irreversible and medical therapies to prevent progression of the disease are usually ineffective. We describe a patient with long-standing ulcerative colitis, which was refractory to corticosteroid therapy who developed primary sclerosing cholangitis (biochemical stage Ⅱ/Ⅳ) in the course of his pancolitis. Treatment with infliximab (5 mg/kg as an induction dose followed by maintenance doses every two months) was indicated because of steroid-dependent disease associated to primary sclerosing cholangitis as well as sacroiliitis and uveitis and previous episode of severe azathioprine-related hepatic toxicity. At present, after two years of follow-up, the patient is asymptomatic with normal liver tests and complete resumption of daily life activities. This case draws attention to the usefulness of anti-tumor necrosis factor-alpha therapy for the management of primary sclerosing cholangitis as extraintestinal manifestation of inflammatory bowel disease.

© 2013 Baishideng. All rights reserved.

Key words: Ulcerative colitis; Infliximab; Monoclonal antibodies; Sclerosing cholangitis; Bile duct diseases; Tumor necrosis factor-alpha

Duca I, Ramírez de la Piscina P, Estrada S, Calderón R, Spicakova K, Urtasun L, Marra-López C, Zabaleta S, Bengoa R, Marcaide MA, García-Campos F. Steroid-refractory ulcerative colitis and associated primary sclerosing cholangitis treated with infliximab. *World J Gastroenterol* 2013; 19(4): 590-593 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i4/590.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i4.590

INTRODUCTION

Inflammatory bowel diseases are associated with extraintestinal manifestations involving almost every organ system in the body, including the musculoskeletal, dermatologic, hepatic, pancreatic, biliary, ocular, renal and pulmonary systems and can cause a significant challenge to physicians managing patients with Crohn's disease and ulcerative colitis[1-3]. Primary sclerosing cholangitis, a chronic, progressive disorder of unknown etiology that manifests as inflammation, stricturing, and fibrosis of medium and large intra- and extrahepatic bile ducts, is one of the most serious complications of inflammatory bowel disease, with an established strong relationship with ulcerative colitis[4]. At least 75% of patients with primary sclerosing

cholangitis have coexisting ulcerative colitis[2]. However, only 5% of patients with ulcerative colitis develop primary sclerosing cholangitis. The clinical course of sclerosing cholangitis bears no relationship with the underlying inflammatory bowel disease but damage to bile ducts is irreversible, no medical therapies have been shown to be effective at preventing the progression of the disease, and orthotopic liver transplantation is the only curative treatment.

The advent of biologic response modifiers, e.g., tumor necrosis factor-alpha (TNF-α) inhibitors, has improved the treatment of inflammatory bowel disease and its associated extraintestinal manifestations[5], such as arthritis and uveitis[6,7]. Current data suggest that infliximab is an effective alternative treatment option for patients with moderate to severe ulcerative colitis with an inadequate response to conventional glucocorticoid treatment[8]. However, the efficacy of infliximab in primary sclerosing cholangitis in patients with ulcerative colitis has not been previously assessed.

We report the case of a patient with steroid-refractory ulcerative colitis with intolerance to thiopurines and various extraintestinal manifestations, including ankylosing spondylitis and primary sclerosing cholangitis with favorable response to infliximab therapy.

CASE REPORT

We report here on a 68-year-old man in whom positive HLA B27 spondyloarthropathy and uveitis in the left eye were diagnosed at the age of 54. He was referred to the hospital because of an episode of subacute diarrhea with blood and mucous associated with mild iron-deficiency anemia. Also, he was diagnosed endoscopically and histologically of mild to moderate ulcerative rectosigmoiditis. He received oral and topical 5-aminosalicylic acid with good initial response. During the course of the disease, he presented multiple episodes of steroid-dependent ulcerative colitis and treatment with azathioprine was started 6 years after diagnosis. After 4 mo, azathioprine was discontinued due to severe hepatic cytolysis. Four years later, he was readmitted to the hospital because of bloody diarrhea and mucus (12-14 bowel movements daily, with generalized abdominal pain, proctalgia, fecal urgency and tenesmus without fever, and weight loss of 6 kg. Physical examination showed marked impairment of the patient's general condition, diffuse abdominal pain on palpation and functional limitation secondary to ankylosing spondylitis. Blood tests showed serum hemoglobin 10.5 g/dL, C-reactive protein 154 mg/dL and cholestasis hepatitis with bilirubin 1.2 mg/dL, aspartate aminotransferase (AST) 112 IU/L, alanine aminotransferase (ALT) 162 IU/L, alkaline phosphatase 281 IU/L, gamma-glutamyl transpeptidase (γ-GGT) 913 IU/L, and positive p-antinuclear antibodies 1/80. A diagnosis of moderate to severe episode of pancolitis was established. A cholangio-magnetic resonance imaging (MRI) study disclosed stenosis of the proximal common bile duct probably related to cholangitis. Causes of secondary sclerosing cholangitis were excluded as shown by the lack of abnormally elevated immunoglobulin G4 (IgG4) levels as shown in autoimmune pancreatitis or IgG4-related sclerosing cholangitis (IgG4 levels were < 100 mg/dL). Also, the patient showed increased values serum bilirubin and alkaline phosphatase of a lower magnitude that those suggestive of autoimmune pancreatitis, and radioimaging findings for enlargement of the pancreatic gland were absent. Also, the patient did not complain of abdominal symptoms suggestive of pancreatitis. All these data together with the presence of ulcerative pancolitis directed us to confirm the diagnosis of primary sclerosing cholangitis associated to inflammatory bowel disease and to exclude the diagnosis of autoimmune pancreatitis.

The patient was diagnosed of primary sclerosing cholangitis (biochemical stage Ⅱ/Ⅳ). He was treated with full doses of *i.v.* corticosteroids and urodesoxycholic acid 15 mg/kg body weight, without improvement. The patient received full doses of methylprednisolone, 1 mg/kg per day, with subsequent dose reductions at least on seven occasions over the course of 6 years. The situation of the patient was re-assessed and decided to start treatment with infliximab (5 mg/kg as an induction dose followed by maintenance doses every two months) because of steroid-dependent disease associated to primary sclerosing cholangitis, sacroiliitis and uveitis and previous history of an episode of severe azathioprine-related hepatic toxicity. At present, after two years of follow-up, the patient is still on treatment with infliximab and has remained asymptomatic, with improvement of anemia (hemoglobin 13.8 mg/dL) and biochemical evidence of cholestasis (bilirubin 0.4 mg/dL, AST 16 IU/L, ALT 20 IU/L, alkaline phosphatase 59 IU/L, γ-GGT 149 IU/L) and complete restoration of the quality of life. Annual endoscopic assessment did not show signs of dysplasia. Repeated cholangio-MRI performed during the follow-up was also unrevealing.

DISCUSSION

The incidence of primary sclerosing cholangitis varies between 0.9 to 1.6 per 100 000 persons/year[9]. More than two-thirds of patients are males and the most commonly associated condition is an inflammatory bowel disease which occurs in up to 70% of affected subjects. Inflammatory bowel disease in primary sclerosing cholangitis patients represents a distinct phenotype in that pancolitis is observed in 94% of patients with ulcerative colitis and in 96% of patients with Chron's disease[10]. It has been shown large differences between primary sclerosing cholangitis patients with and without concurrent inflammatory bowel disease. Patients with inflammatory bowel disease showed earlier appearance of primary sclerosing cholangitis than those without inflammatory bowel disease and are more likely to develop serious malignant complications and more likely to require liver transplantation[11].

In our patient, distal colitis was the initial manifestation of inflammatory bowel disease and later evolving to pancolitis, the time at which primary sclerosing cholangitis developed. Primary sclerosing cholangitis has been shown to be associated with greater anatomic extent of colitis[12-14]. It has been reported that development of primary sclerosing cholangitis in patients with ulcerative colitis may have a positive effect on colonic disease[15,16] with reduced disease activity and less use of steroids, azathioprine and surgery[17]. In other studies, ulcerative colitis associated with primary sclerosing cholangitis showed unique colonoscopic features (pancolitis, rectal sparing and backwash ileitis) with more frequent colorectal neoplasia development and worse prognosis than ulcerative colitis patients without primary sclerosing cholangitis[18]. In our patient the clinical course was characterized by severe ulcerative colitis refractory to steroid therapy. For this reason and taking into account the presence of other concomitant manifestations (uveitis, sacroiliitis) treatment with infliximab was started. However, indications of anti-TNF-α in well established primary sclerosing cholangitis should be carefully balanced due to immunosuppression and the risk of potentially fatal cholangitis.

The use of infliximab was followed by marked improvement in the patient's clinical condition, including the extraintestinal manifestations of ulcerative colitis and a favorable clinical and biochemical remission of primary sclerosing cholangitis. This suggests a direct effect of infliximab on hepatic inflammation. However, in a double-blind, placebo-controlled study of 24 patients with primary sclerosing cholangitis, no significant treatment benefit of infliximab was demonstrated[19], although in patients with primary sclerosing cholangitis and Crohn's disease treatment with infliximab was associated with improvement of liver function tests[20].

Currently, the use of anti-TNF-α treatment in primary sclerosing cholangitis is not well established. The observation that reduced T cell reactivity in liver infiltrating cells obtained from patients with primary sclerosing cholangitis was due to high local production of TNF-α provides support for the use of anti-TNF antibodies as an alternative treatment for these patients[21]. Contrarily, in the experience of Epstein *et al*[22] etanercept was well tolerated but not effective in a clinical series of 10 patients with clinically active primary sclerosing cholangitis. It is unknown whether early treatment with anti-TNF-α drugs may change the natural history of primary sclerosing cholangitis. Also, the use of this medication over years may raise financial concerns and secondary effects of this prolonged use. In the case here presented, although treatment with infliximab is expensive, the patient did not present recurrent episodes of colitis, uveitis, sacroiliitis or new episodes of cholangitis, allowing prompt resumption of work and social activities with an excellent quality of life and without further admissions to the hospital or the need of surgical operations, as a result of which direct and indirect costs have been markedly reduced in this particular case.

In conclusion, our report on a patient with steroid-refractory ulcerative colitis developing primary sclerosing cholangitis with favorable and maintained response to infliximab therapy draws attention to the usefulness of anti-TNF-α therapy for management of primary sclerosing cholangitis as extraintestinal manifestation of inflammatory bowel disease.

REFERENCES

1 **Bernstein CN**, Blanchard JF, Rawsthorne P, Yu N. The prevalence of extraintestinal diseases in inflammatory bowel disease: a population-based study. *Am J Gastroenterol* 2001; **96**: 1116-1122 [PMID: 11316157 DOI: 10.1111/j.1572-0241.2001.03756.x]

2 **Levine JS**, Burakoff R. Extraintestinal manifestations of inflammatory bowel disease. *Gastroenterol Hepatol* (NY) 2011; **7**: 235-241 [PMID: 21857821]

3 **Veloso FT**. Extraintestinal manifestations of inflammatory bowel disease: do they influence treatment and outcome? *World J Gastroenterol* 2011; **17**: 2702-2707 [PMID: 21734777 DOI: 10.3748/wjg.v17.i22.2702]

4 **Williams H**, Walker D, Orchard TR. Extraintestinal manifestations of inflammatory bowel disease. *Curr Gastroenterol Rep* 2008; **10**: 597-605 [PMID: 19006617]

5 **Barrie A**, Regueiro M. Biologic therapy in the management of extraintestinal manifestations of inflammatory bowel disease. *Inflamm Bowel Dis* 2007; **13**: 1424-1429 [PMID: 17567879 DOI: 10.1002/ibd.20196]

6 **Siemanowski B**, Regueiro M. Efficacy of infliximab for extraintestinal manifestations of inflammatory bowel disease. *Curr Treat Options Gastroenterol* 2007; **10**: 178-184 [PMID: 17547856]

7 **Suhler EB**, Smith JR, Wertheim MS, Lauer AK, Kurz DE, Pickard TD, Rosenbaum JT. A prospective trial of infliximab therapy for refractory uveitis: preliminary safety and efficacy outcomes. *Arch Ophthalmol* 2005; **123**: 903-912 [PMID: 16009830 DOI: 10.1001/archopht.123.7.903]

8 **Wilhelm SM**, McKenney KA, Rivait KN, Kale-Pradhan PB. A review of infliximab use in ulcerative colitis. *Clin Ther* 2008; **30**: 223-230 [PMID: 18343261 DOI: 10.1016/j.clinthera.2008.02.014]

9 **Milkiewicz P**, Wunsch E. Primary sclerosing cholangitis. *Recent Results Cancer Res* 2011; **185**: 117-133 [PMID: 21822823]

10 **Boonstra K**, van Erpecum KJ, van Nieuwkerk KM, Drenth JP, Poen AC, Witteman BJ, Tuynman HA, Beuers U, Ponsioen CY. Primary sclerosing cholangitis is associated with a distinct phenotype of inflammatory bowel disease. *Inflamm Bowel Dis* 2012; **18**: 2270-2276 [PMID: 22407885 DOI: 10.1002/ibd.22938]

11 **Ngu JH**, Gearry RB, Wright AJ, Stedman CA. Inflammatory bowel disease is associated with poor outcomes of patients with primary sclerosing cholangitis. *Clin Gastroenterol Hepatol* 2011; **9**: 1092-1097; quiz e135 [PMID: 21893134 DOI: 10.1016/j.cgh.2011.08.027]

12 **Loftus EV**, Harewood GC, Loftus CG, Tremaine WJ, Harmsen WS, Zinsmeister AR, Jewell DA, Sandborn WJ. PSC-IBD: a unique form of inflammatory bowel disease associated with primary sclerosing cholangitis. *Gut* 2005; **54**: 91-96 [PMID: 15591511 DOI: 10.1136/gut.2004.046615]

13 **Chapman RW**, Arborgh BA, Rhodes JM, Summerfield JA, Dick R, Scheuer PJ, Sherlock S. Primary sclerosing cholangitis: a review of its clinical features, cholangiography, and hepatic histology. *Gut* 1980; **21**: 870-877 [PMID: 7439807]

14 **Schrumpf E**, Elgjo K, Fausa O, Gjone E, Kolmannskog F, Ritland S. Sclerosing cholangitis in ulcerative colitis. *Scand J Gastroenterol* 1980; **15**: 689-697 [PMID: 7209379]

15 **Moayyeri A**, Daryani NE, Bahrami H, Haghpanah B, Nayyer-Habibi A, Sadatsafavi M. Clinical course of ulcerative coli-

tis in patients with and without primary sclerosing cholangitis. *J Gastroenterol Hepatol* 2005; **20**: 366-370 [PMID: 15740478 DOI: 10.1111/j.1440-1746.2005.03727.x]

16 **Lundqvist K**, Broomé U. Differences in colonic disease activity in patients with ulcerative colitis with and without primary sclerosing cholangitis: a case control study. *Dis Colon Rectum* 1997; **40**: 451-456 [PMID: 9106695]

17 **Marelli L**, Xirouchakis E, Kalambokis G, Cholongitas E, Hamilton MI, Burroughs AK. Does the severity of primary sclerosing cholangitis influence the clinical course of associated ulcerative colitis? *Gut* 2011; **60**: 1224-1228 [PMID: 21402617 DOI: 10.1136/gut.2010.235408]

18 **Ye BD**, Yang SK, Boo SJ, Cho YK, Yang DH, Yoon SM, Kim KJ, Byeon JS, Myung SJ, Yu CS, Yun SC, Kim JH. Clinical characteristics of ulcerative colitis associated with primary sclerosing cholangitis in Korea. *Inflamm Bowel Dis* 2011; **17**: 1901-1906 [PMID: 21830268 DOI: 10.1002/ibd.21569]

19 **Hommes DW**, Erkelens W, Ponsioen C, Stokkers P, Rauws E, van der Spek M, ten Kate F, van Deventer SJ. A double-blind, placebo-controlled, randomized study of infliximab in primary sclerosing cholangitis. *J Clin Gastroenterol* 2008; **42**: 522-526 [PMID: 18344886]

20 **Regueiro M,** Chopra K, Slivka A. Improvement of liver function tests in patients with primary sclerosing cholangitis and Crohn's disease treated with infliximab. *Gastroenterology* 2003; **124:** A526

21 **Bo X**, Broome U, Remberger M, Sumitran-Holgersson S. Tumour necrosis factor alpha impairs function of liver derived T lymphocytes and natural killer cells in patients with primary sclerosing cholangitis. *Gut* 2001; **49**: 131-141 [PMID: 11413121 DOI: 10.1136/gut.49.1.131]

22 **Epstein MP**, Kaplan MM. A pilot study of etanercept in the treatment of primary sclerosing cholangitis. *Dig Dis Sci* 2004; **49**: 1-4 [PMID: 14992426]

P- Reviewers Vargas AA, Kinnunen U
S- Editor Gou SX **L- Editor** A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i4.594

World J Gastroenterol 2013 January 28; 19(4): 594-596
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Iatrogenic esophago-tracheal fistula: Challenges in diagnosis and management

Øistein Hovde, Øyvind Haugen Lie, Per Arthur Johansson, Øystein Stubhaug, Egil Johnson, Bjørn Hofstad, Truls Hauge

Øistein Hovde, Øyvind Haugen Lie, Per Arthur Johansson, Øystein Stubhaug, Department of Gastroenterology/Internal Medicine, Innlandet Hospital Trust, 2819 Gjøvik, Norway
Egil Johnson, Department of Pediatric and Gastroenterological Surgery, Oslo University Hospital Ullevål, 0450 Oslo, Norway
Egil Johnson, Faculty of Medicine, University of Oslo, 0318 Oslo, Norway
Bjørn Hofstad, Truls Hauge, Department of Gastroenterology, Oslo University Hospital Ullevål, 0450 Oslo, Norway
Author contributions: All authors gave substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data, drafting the article or revising it critically for important intellectual content, and final approval of the version to be published.
Correspondence to: Øistein Hovde, MD, Department of Gastroenterology/Internal Medicine, Innlandet Hospital Trust, Gjøvik, Kyrre Grepps gt. 11, 2819 Gjøvik, Norway. oistein.hovde@sykehuset-innlandet.no
Telephone: +47-611-57002 Fax: +47- 611-57439
Received: April 12, 2012 Revised: July 3, 2012
Accepted: November 24, 2012
Published online: January 28, 2013

Abstract

Esophageo-tracheal fistula is a rare condition, and in most cases such fistulas are caused by malignant disease or emergency endotracheal intubation. A case where a wrapped tablet produced a fistula between the esophagus and trachea is described. The patient is a male born in 1938 who swallowed a tablet without unwrapping it. The patient was treated with self-expanding metal stents (SEMS), but closure of the fistula was not achieved. Different examinations and treatment options are discussed. Surgical treatment for this condition has demonstrated considerable mortality and morbidity. In some cases closure of the fistula can be achieved by use of SEMS. Although we advise treatment of such cases with SEMS, in some cases treatment with stents will prove troublesome and the risk/benefit analysis will have to be reevaluated.

© 2013 Baishideng. All rights reserved.

Key words: Esophageo-tracheal fistula; Stenting; Iatrogenic; Percutaneous endoscopic gastrotomy; Surgery

Hovde Ø, Lie ØH, Johansson PA, Stubhaug Ø, Johnson E, Hofstad B, Hauge T. Iatrogenic esophago-tracheal fistula: Challenges in diagnosis and management. *World J Gastroenterol* 2013; 19(4): 594-596 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i4/594.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i4.594

INTRODUCTION

Esophagotracheal fistula is a rare condition, and in most cases such fistulas are caused by malignant disease or emergency endotracheal intubation. We describe a case with a unique etiology where a wrapped tablet produced a fistula between the esophagus and trachea. Challenges in diagnosis and treatment are described.

CASE REPORT

The patient is a male born in 1938. In 1997 he had prosthetic hip surgery, for which he was re-operated in 2008. Parkinsons disease was diagnosed in 2009, and he suffers from benign prostatic hyperplasia.

In 2008, during hospitalization for the hip-reoperation, he was given four tablets by a staff nurse as ordinated by a physician. Upon swallowing the tablets, the patient felt intense chest discomfort, and it was speculated whether he had swallowed one of the tablets without unwrapping it. No further investigation was done at this point.

In 2010 the patient was referred to the Department



Figure 1 Foreign body measured 2 cm x 2 cm and the tablet remained intact inside the wrapping.

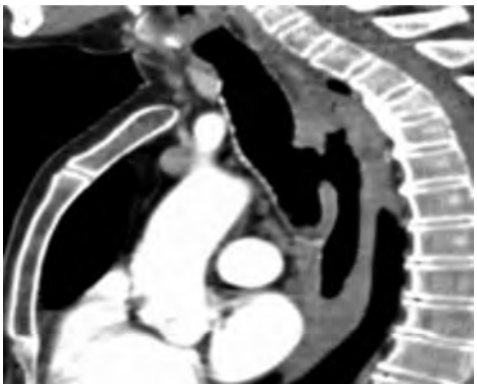


Figure 2 Computed tomography confirmed a fistula, 20 mm x 6-7 mm, at aortic arch level.

of Gastroenterology because of dysphagia.

An upper endoscopy was performed and at 25 cm from the teeth the endoscopist discovered a stenosing process with mucosal "irritation" and signs of ulceration. Biopsies from the area revealed ulceration, but no signs of malignancy. Distally, the esophagus was normal. He had signs of gastritis with a positive *Helicobacter pylori*-test. The duodenum was normal.

The initial computer tomography (CT) raised suspicion of malignancy, as it described a 4-5 cm tumor of the esophageal wall and adjacent pathological lymph nodes in the mediastinum.

Three weeks later a new endoscopy was performed, and the suspicion of a foreign body was raised. The structure in the esophagus had a blueish discoloration, and the consistency was "hard as a rock".

The patient was referred to a tertiary centre for possible endoscopic removal of a foreign body but, due to the risk for perforation, instead of trying to remove the foreign body a self-expanding, fully covered esophageal stent was placed. The patient now was able to eat.

Five months later the patient came back after having coughed up a foreign body. He brought the item to the outpatient clinic. Upon inspection it measured 2 cm × 2 cm and the tablet remained intact inside the wrapping (Figure 1). The patient reported chest discomfort, but no respiratory symptoms. Another endoscopy was performed. The stent was in place, and there was no visible fistula. Biopsies showed inflammation and proton pump inhibitor treatment was initiated.

Again a CT was performed, describing fibrotic changes in the right lung, and low-grade brochiectasia. No contrast leakage between the esophagus and the respiratory tract was seen.

The patient was in good health, but felt some discomfort having a stented esophagus. An informed decision was made to attempt stent removal under anaesthesia.

The stent was removed endoscopically, and 15 min after the procedure bleeding from the endotracheal tube was noticed. There was no subcutaneous emphysema. The patient was extubated and was able to breathe normally. The suspicion of a esophago-tracheal fistula was raised and antibiotic therapy was initiated. CT confirmed a fistula, 20 mm × 6-7 mm, at aortic arch level (Figure 2).

In order to - as far as possible - prevent aspiration from the esophagus to trachea, and to see if a spontanous closure of the fistula was achievable, a percutaneous endoscopic gastrostomy (PEG) was placed. This also ensured nutritional adequacy.

The patient was observed as an outpatient, and attended two control CTs. The fistula persisted unchanged.

After another three months it was decided to place a new esophageal stent. Upon esophagoscopy the fistula still measured 20 mm × 7 mm, and there was direct visability into the carina and the left and right main bronchus. The PEG was removed, and a fully covered, self-expanding stent was placed to cover the fistula. We chose a stent with a diameter of 28 mm at the two ends in an attempt to avoid food/liquids from getting access from the esophagus to the trachea. The length of the stent was 12.5 cm and the diameter in the center of the stent was 23 mm. A bronchoscopy was performed, during which the stent was directly visible from the tracheal side (Figure 3). A CT was performed, showing the stent in place (Figure 4).

Two months after the last stent placement the patient is asymptomatic, and is obtaining natural nutrition. A follow-up CT is scheduled.

DISCUSSION

A report on surgical correction in two cases of iatrogenic esophago-tracheal fistulas is available[1]. These fistulas were secondary to traumatic emergency endotracheal intubation. Another report on four patients with iatrogenic fistulas demonstrated considerable mortality and morbidity in relation to emergent surgery[2]. Two patients were under long term treatment in the ICU, one suffered a stroke, and one died. A study on four patients with esophago-tracheal fistula being treated with self-expanding oesophageal stents reported fistula closure in two patients[3]. A review on surgical techniques is available[4], outlining the invasiveness of the procedures. Management of an iatrogenic tracheo-esophageal fistula

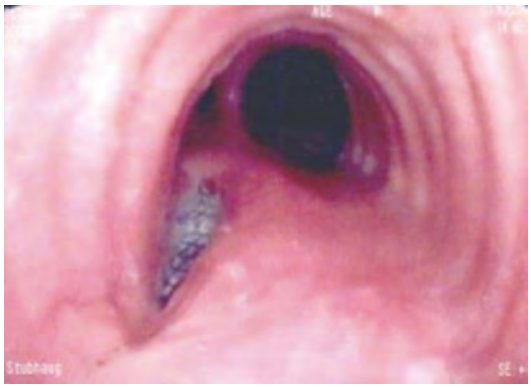


Figure 3 Bronchoscopy was performed, during which the stent was directly visible from the tracheal side.

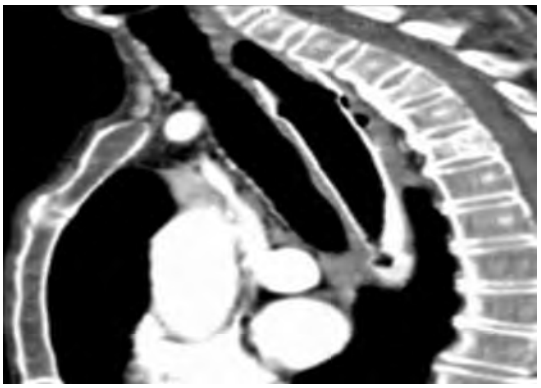


Figure 4 Computed tomography was performed, showing the stent in place.

using a Y-stent on the tracheal side is described[5]. A self-expanding metal stent is, according to one study, safe and effective in palliation of malignant fistulae in selected patients[6]. However, this study was conducted on 90 patients with malignant changes in the esophagus, of which only 5 patients had a fistula.

Our case illustrates a unique etiology of an esophago-tracheal fistula. The patient presents a continuous challenge in treatment. Discussions and considerations with thoracic surgeons have so far concluded with stenting as the treatment of choice. The patient's age and comorbidity is being taken into account in this decision. However, if the management with esophageal stents proves troublesome, the risk/benefit analysis will have to be re-evaluated.

The wrapped tablet was forced from the esophageal wall to the trachea, probably powered by the self-expanding stent. Long-term stenting has so far given no reduction in the size of the defect.

REFERENCES

1 **Balázs A**, Kupcsulik P. [Surgical repair of postintubation esophago-tracheal fistulas: report of two cases surgically repaired]. *Orv Hetil* 2011; **152**: 1618-1622 [PMID: 21945871]

2 **Harenberg T**, Menenakos C, Jacobi CA, Braumann C. [Distal trachea and bronchial large lesions and suture reinforcement with Polyglicol Acid (PGA) patch. First clinical experience]. *G Chir* 2010; **31**: 10-15 [PMID: 20298659]

3 **Blackmon SH**, Santora R, Schwarz P, Barroso A, Dunkin BJ. Utility of removable esophageal covered self-expanding metal stents for leak and fistula management. *Ann Thorac Surg* 2010; **89**: 931-936; discussion 936-937 [PMID: 20172156]

4 **Chappell VL**, Heck HA. Repair of large, iatrogenic, tracheo-esophageal fistulae. *Ann Thorac Surg* 2007; **83**: 705-706 [PMID: 17258028]

5 **Shaida N**, Raj V, Gopalan D. Iatrogenic tracheoesophageal fistula. *J Thorac Oncol* 2009; **4**: 1572 [PMID: 20009912]

6 **Dobrucali A**, Caglar E. Palliation of malignant esophageal obstruction and fistulas with self expandable metallic stents. *World J Gastroenterol* 2010; **16**: 5739-5745 [PMID: 21128325 DOI: 10.3748/wjg.v16.i45.5739]

P- Reviewers Schuchert MJ, Fisichella PM **S- Editor** Wang JL
L- Editor O'Neill M **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i4.597

World J Gastroenterol 2013 January 28; 19(4): 597-603
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Fulminant gastrointestinal graft-versus-host disease concomitant with cytomegalovirus infection: Case report and literature review

Hidetaka Okubo, Naoyoshi Nagata, Naomi Uemura

Hidetaka Okubo, Naoyoshi Nagata, Department of Gastroenterology and Hepatology, National Center for Global Health and Medicine, Tokyo 162-8655, Japan
Naomi Uemura, Department of Gastroenterology and Hepatology, Kohnodai Hospital, Ichikawa 272-0827, Chiba, Japan
Author contributions: Okubo H and Nagata N contributed equally to this work; Okubo H, Nagata N and Uemura N designed the research; Okubo H, Nagata N provided the discussion of the pathology and clinical features and wrote the paper.
Supported by Grant from the National Center for Global Health and Medicine
Correspondence to: Naoyoshi Nagata, MD, Department of Gastroenterology and Hepatology, National Center for Global Health and Medicine, 1-21-1 Toyama, Shinjuku-ku, Tokyo 162-8655, Japan. nnagata_ncgm@yahoo.co.jp
Telephone: +81-3-32027181 Fax: +81-3-32071038
Received: October 25, 2012 Revised: December 2, 2012
Accepted: December 15, 2012
Published online: January 28, 2013

Abstract

Here, we report a case of fulminant gastrointestinal graft-versus-host disease (GI-GVHD) with cytomegalovirus (CMV) infection in 44-year-old woman. Despite the difficulties associated with the treatment of GI-GVHD and GI-CMV disease, the mucosal findings and the clinical course showed marked improvements during long-term clinical observation. The endoscopic findings were remarkable, with diffuse sloughing mucosa in the stomach and highly active inflammation and deep discrete ulcers throughout the colon. Changes in the CMV quantitative polymerase chain reaction results were correlated with the endoscopic mucosal findings and were useful for assessing the efficacy of the treatment. Although a definite diagnosis of GI-GVHD is generally made by endoscopy with biopsy, the gross appearance of this disease can vary depending on the endoscopy. In this paper, we also conduct a literature review of patients with GI-GVHD.

© 2013 Baishideng. All rights reserved.

Key words: Acute gastrointestinal graft-versus-host disease; Allogenic stem-cell transplantation; Cytomegalovirus gastrointestinal disease; Cytomegalovirus-polymerase chain reaction; Endoscopy

Okubo H, Nagata N, Uemura N. Fulminant gastrointestinal graft-versus-host disease concomitant with cytomegalovirus infection: Case report and literature review. *World J Gastroenterol* 2013; 19(4): 597-603 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i4/597.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i4.597

INTRODUCTION

Graft-versus-host disease (GVHD) is a serious complication of allogeneic hematopoietic stem cell transplantation (HSCT) and mainly attacks the skin, gastrointestinal (GI) tract and liver[1-5]. GI-GVHD, in particular, can cause life-threatening complications, such as massive diarrhea, hemorrhage, paralytic ileus, and perforation[6,7]; therefore, the accurate diagnosis of GI-GVHD is essential. Although endoscopic observation is an indispensable diagnostic tool, various mucosal patterns are observed on endoscopy[8]. In addition, testing for cytomegalovirus (CMV) infection is necessary because the complication of GI-GVHD with CMV infection will reduce the quality of life (QoL) of the patients[9]. This reduction in the QoL occurs because CMV-related GI disease is exacerbated by the administration of steroids for the treatment of GI-GVHD when antiviral drugs are not administered[10]. Here, we report a rare case of fulminant GI-GVHD detected by endoscopy and its clinical course, and we review related literature on the endoscopic findings of GI-GVHD.

CASE REPORT

The patient, a 44-year-old woman with acute myeloid leukemia who was diagnosed 18 mo earlier, underwent

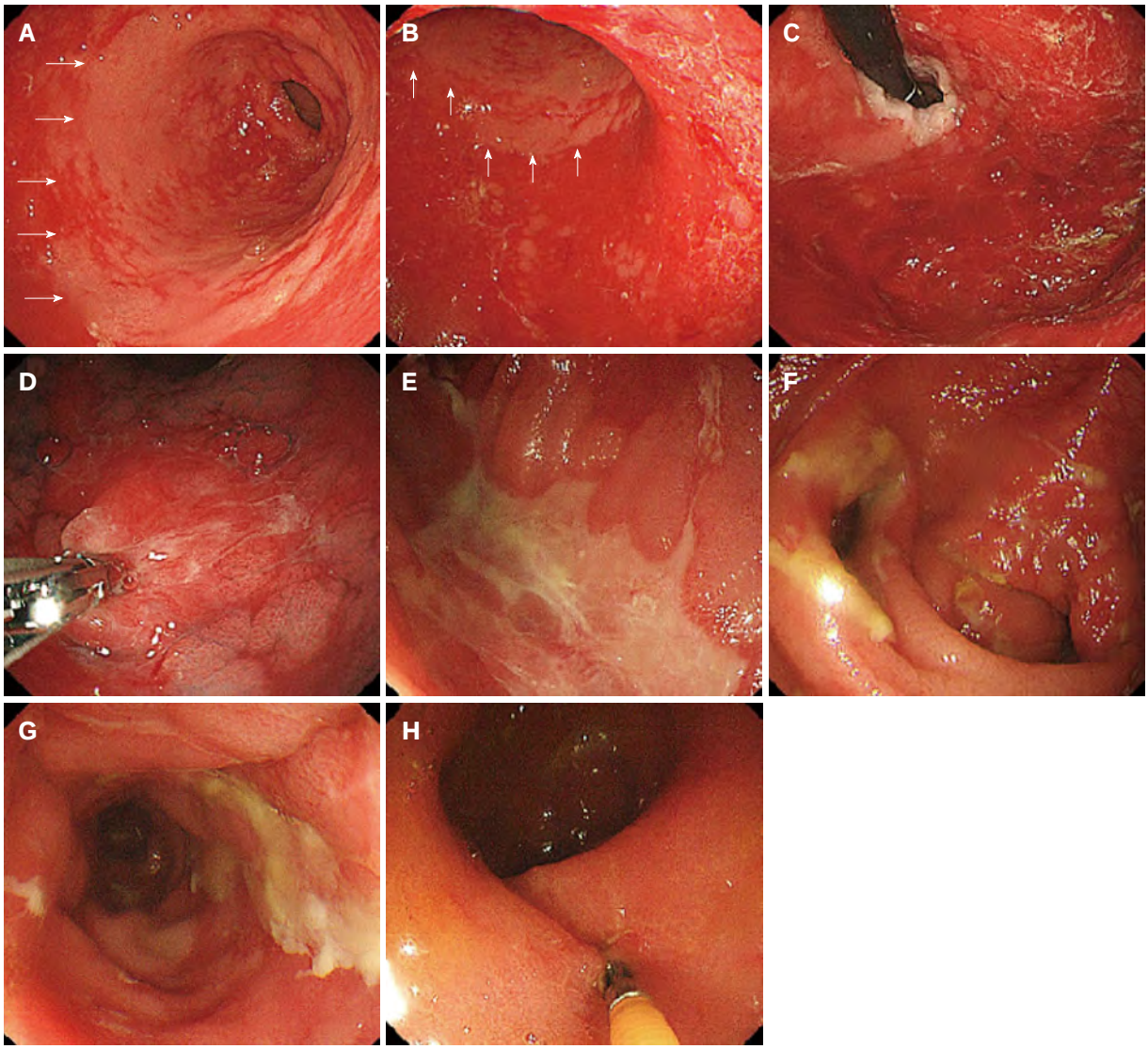


Figure 1 Upper and lower gastrointestinal endoscopic findings. A: The gastric mucosa in the antrum of the stomach. The antrum is the only segment exhibiting normal mucosa (arrows); B: The gastric mucosa in the lower segments of the corpus of the stomach. This mucosa demonstrates diffuse sloughing, whereas the antrum is the only segment exhibiting normal mucosa (arrows); C: The gastric mucosa in the upper segments of the corpus of the stomach; D: The greater curvature of the stomach near the center. A biopsy was taken from the edematous and sloughing mucosa of the greater curvature of the upper corpus; E: Shallow ulcer with fur at the cecal valve and cecum; F: Discrete longitudinal ulcer in the terminal ileum; G: Longitudinal ulcer with fur surrounded by the inflamed edematous mucosa of the sigmoid colon; H: Edematous mucosa of the rectum.

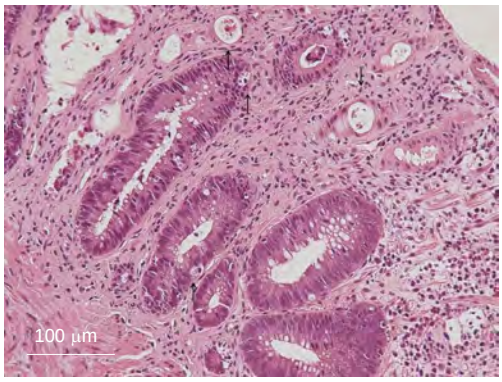


Figure 2 Histological findings. Biopsy specimen from the sigmoid colon revealing a crypt with multiple apoptotic cells (arrows) with severe lymphocytes corresponding to acute gastrointestinal graft-versus-host disease (GVHD). The presence of gland apoptosis is histological evidence of acute GVHD (hematoxylin and eosin stain, × 40).

allogeneic bone marrow transplantation 3 mo prior to the examination. A full-body skin rash appeared on day 26 after the transplantation. On day 85 after the transplantation, she experienced watery diarrhea more than 10 times per day and developed abdominal pain. Upper GI endoscopy showed diffuse sloughing of the mucosa with diffuse erythema and hemorrhage in the antrum and corpus of the stomach (Figure 1A-D). Lower GI endoscopy revealed multiple deep discrete ulcers with exudative and mucosal oozing in the terminal ileum end and throughout the entire colon (Figure 1E and F). Biopsy specimens from the upper and lower GI tract revealed diffuse erythema, erosions, and sloughing mucosa with active bleeding in the stomach, as well as multiple erosions and a small discrete ulcer with active bleeding in the lower GI tract. Gland apoptosis showed histological evidence of acute GVHD (Figure 2). Moreover, the

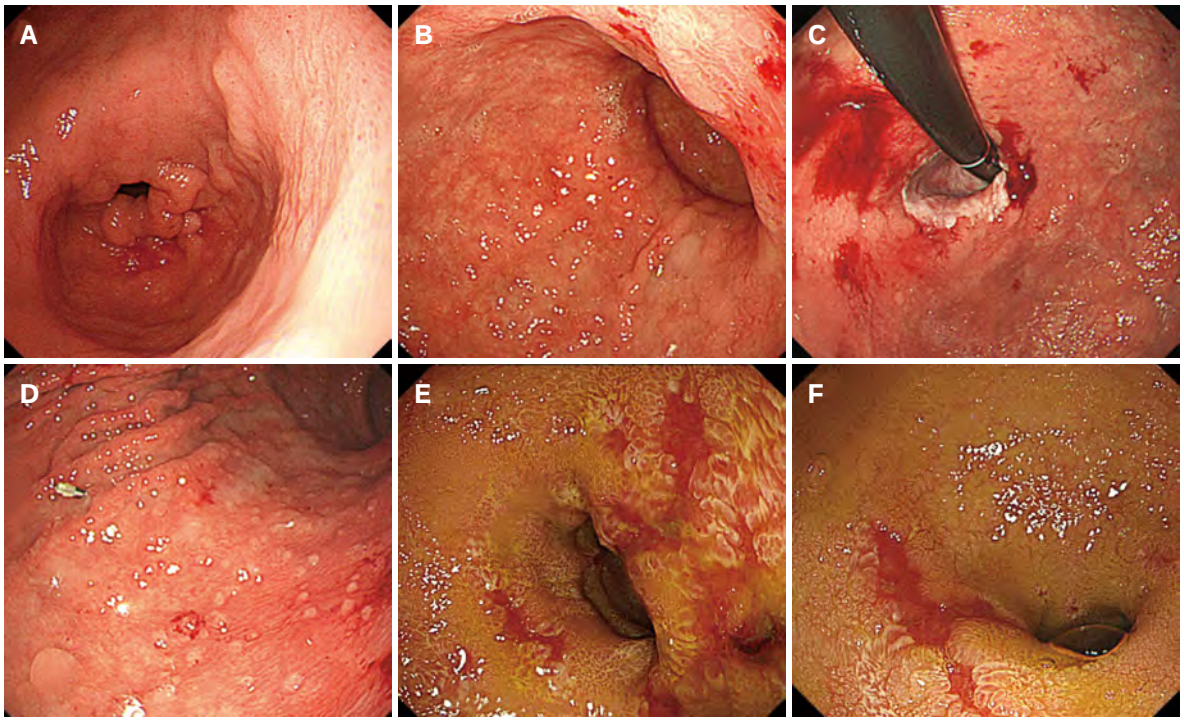


Figure 3 Upper and lower gastrointestinal endoscopic findings 70 d after the steroid therapy. A: The gastric mucosa in the antrum of the stomach. The mucosa is normal but somewhat edematous; B: The gastric mucosa in the lower segments of the corpus of the stomach, which has improved even though the rough gastric mucosal patterns and edematous changes remained; C: The gastric mucosa in the upper segments of the corpus of the stomach, which has also improved; D: The greater curvature of the stomach near the center. The endoscopic clipping has remained; E: Sigmoidoscopy revealing multiple discrete ulcers in the sigmoid colon; F: Disappearance of the visible vascular pattern in the sigmoid colon.

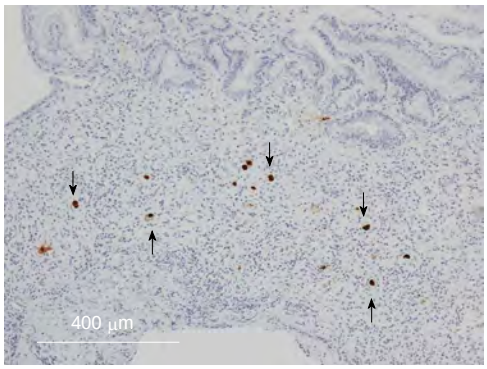


Figure 4 Immunostaining of the biopsy specimen. Immunohistochemically stained biopsy specimen from an ulcer of the sigmoid colon showing multiple cytomegalovirus-positive cells (arrows) (immunohistochemical stain, × 40).

biopsy specimens showed CMV infection detected by polymerase chain reaction (PCR) at remarkably elevated concentrations of 4.0×10^4 copies/μg DNA, but immunochemical staining for the CMV antibody was negative. Finally, the patient was diagnosed with severe GI-GVHD concomitant with a CMV infection. Other organ involvement included a whole-body skin rash and slight liver dysfunction.

The treatment for acute GVHD was started with an initial dose of prednisolone 1 mg/kg per day (50 mg/d) and for CMV infection with an initial dose of ganciclovir (GCV) 10 mg/kg per day. After 40 d of treatment, the patient improved clinically. Then, GCV was discontinued because of thrombocytopenia that appeared 60 d after the treatment. After 64 d, however, CMV was detected in the blood by a CMV-antigenemia assay, and the maintenance dose of GCV (5 mg/kg per day) was resumed. After 70 d, upper and lower endoscopy showed that the endoscopic findings of mucosal sloughing had improved (Figure 3A-D) but that the colonic mucosa remained ulcerative and inflamed (Figure 3E and F). Both upper and lower GI tract biopsies revealed CMV-infected cells in immunohistochemically stained tissue specimens (Figure 4), and the CMV-PCR from the GI tract biopsy was 2.0×10^5 copies/μg DNA. Intravenous GCV at 5 mg/kg every 12 h as the induction therapy was re-started and then changed to the maintenance therapy, and the prednisolone dose was tapered. Although the treatment continued for an additional 102 d, the CMV-PCR from the GI tract biopsy was 1.0×10^3 copies/μg DNA. Oral valganciclovir at 900 mg/d was started, with oral prednisolone 10 mg administered every other day. After 162 d, the upper and lower endoscopy showed notable improvements in the mucosal findings (Figure 5), and the CMV-PCR count from a GI tract biopsy was within the normal range of $< 4.0 \times 10$ copies/μg DNA.

DISCUSSION

Nausea and vomiting, appetite loss, abdominal pain, and watery diarrhea are common symptoms of GI-GVHD. Watery diarrhea appears in almost all cases of GI-GVHD and occasionally becomes chronic or causes bleeding. Therefore, this symptom is a major factor that reduces

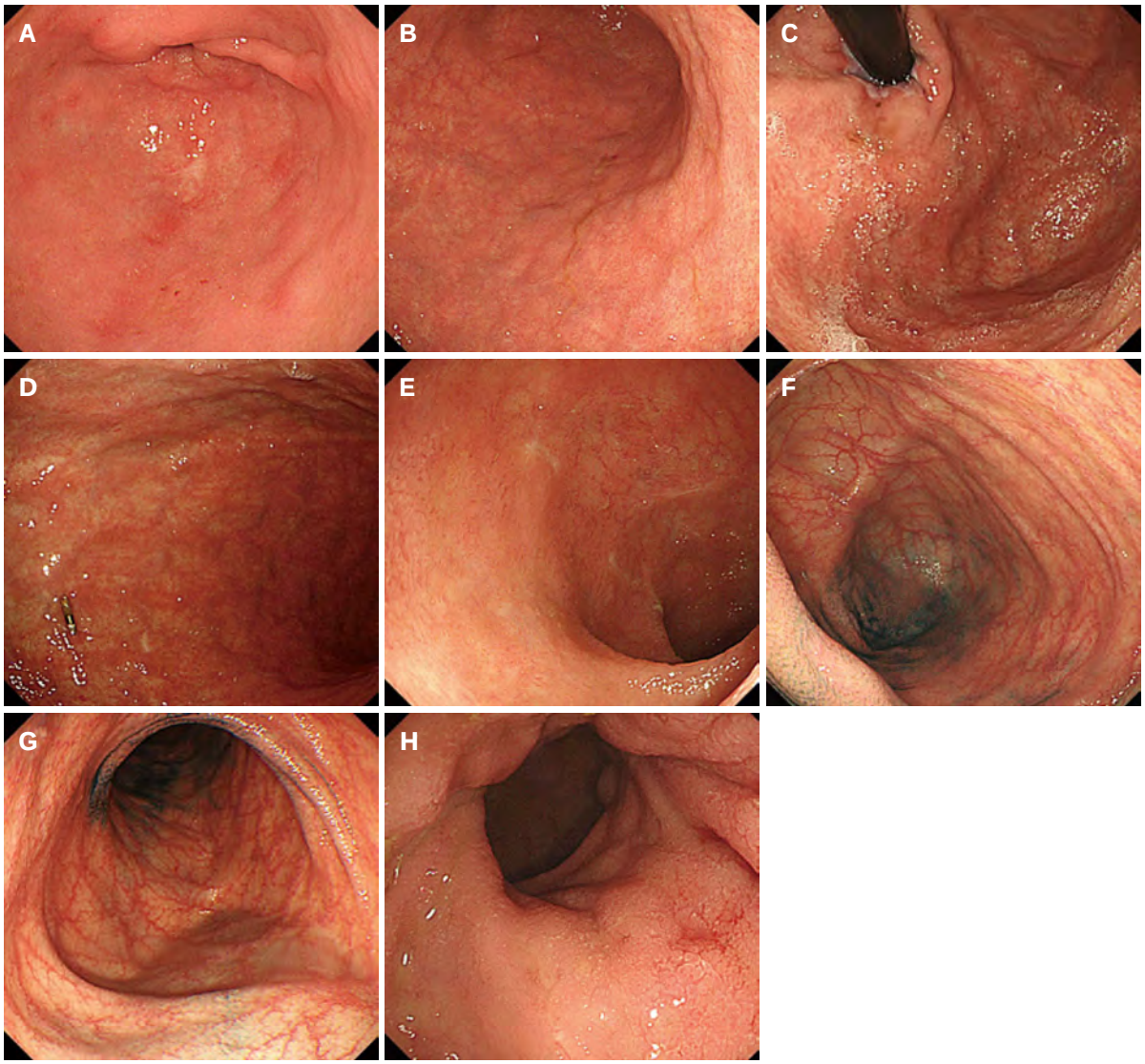


Figure 5 Upper and lower gastrointestinal endoscopic findings 162 d after the steroid and anti-cytomegalovirus therapies. A: The gastric mucosa in the antrum of the stomach; B: The gastric mucosa in the lower segments of the corpus of the stomach; C: The gastric mucosa in the upper segments of the corpus of the stomach; D: The greater curvature of the stomach near the center. The endoscopic clipping has remained, and nearly the entire mucosa of the stomach has improved to a normal state; E: Normal mucosa in the cecal valve and cecum; F: Normal mucosa in the terminal ileum; G: Marked improvement of the inflammation of the colonic mucosa, and healed ulcers in the sigmoid colon; H: The vascular patterns are now visible, indicating that the normal mucosa has returned in the rectum.

patient QoL[11]. Moreover, after HSCT, CMV infection of the GI mucosa and GI-GVHD can both cause diarrhea[12,13].

Diseases characterized by the development of GI symptoms after HSCT include not only CMV and GI-GVHD infection but also virus infections caused by enterovirus, adenovirus, rotavirus, and Epstein-Barr virus, complications with bacterial and fungus infections, and colitis associated with thrombotic microangiopathy and regimen-related toxicity. In routine clinical examination, differentiating among these diseases is difficult[14], particularly because GI-GVHD is frequently associated with CMV infection[15,16]. In this case, because of the severe GI-GVHD accompanied by CMV infection, the patient developed frequent and long-term bloody diarrhea and abdominal pain, which appeared to have led to serious symptoms such as anemia, malnutrition, and dehydration.

The endoscopic findings reflected the severity of the clinical symptoms. The lower GI tract exhibited severe signs of multiple deep ulcers, edema, and erythema, with no normal mucosa observed. The upper GI endoscopy revealed diffuse sloughing of the mucosa in the stomach, except for the antrum. Although the endoscopic findings of chronic GI-GVHD may include the characteristic esophageal web and strictures[2], various endoscopic findings are associated with acute GI-GVHD depending on the severity of the inflammation. We reviewed the English literature in the MEDLINE database by searching with "gastrointestinal", "GVHD", and "endoscopy" as key words (Table 1). Although there are some reports of normal mucosal findings, a large number of studies reported findings of erythema, erosions, and edema. Because the symptoms were generally mild in the upper GI tract, the lower GI tract often had relatively severe inflammatory symptoms, with occasional actively bleeding ulcers. In addition, although tortoise shell-like mu-

Table 1 Literature review of the endoscopic findings in gastrointestinal graft-versus-host disease

No.	Ref.	Patients	Involved GI tract	Endoscopic findings
1	Sultan *et al*[23]	Acute GVHD patients less than 18 yr old (n = 40)	Stomach, duodenum, sigmoid and rectum	Normal, erosions, ulcer
2	Kreisel *et al*[24]	GI-GVHD patients (n = 175)	Stomach, duodenum, colon and rectum	Normal, edema, erosions, aptha, ulcer, nonspecific
3	Aslanian *et al*[25]	Acute GVHD patients (n = 18)	Stomach, duodenum, total colon and rectum	Normal, erythema, edema, erosions, ulcer
4	Martínez *et al*[26]	GI-GVHD patients (n = 20)	Stomach, duodenum, colon and rectum	Normal, erythema, edema, erosions, ulcer, bleeding
5	Krishna *et al*[27]	A 49-yr-old man	Stomach, duodenum, colon and rectum	Normal, erosions
6	Al Ashgar *et al*[28]	A 23-yr-old male who had undergone allogeneic HSCT	Sigmoid colon and rectum	Erythema, edema
7	Varadarajan *et al*[29]	Acute GVHD patients (n = 11)	Stomach, duodenum, small bowel, colon and rectum	Normal, erythema, edema, erosions, ulcer, bleeding
8	He *et al*[17]	GI-GVHD patients (n = 32)	Colon and rectum	Erythema, edema, erosions, ulcer, bleeding, tortoise shell-like mucosa, pseudomembrane mucosa
9	Xu *et al*[8]	GI-GVHD patients (n = 8)	Stomach, duodenum, colon and rectum	Normal, erythema, edema, erosions, ulcer, bleeding
10	Cheung *et al*[30]	GI-GVHD patients (n = 44)	Stomach, duodenum, colon and rectum	Normal, erythema, edema, erosions, ulcer, nonspecific, bleeding
11	Ross *et al*[31]	GI-GVHD patients (n = 92)	Stomach, duodenum and rectum	Normal, erythema, edema, erosion, ulcer, nonspecific
12	Harada *et al*[32]	GI-GVHD patients (n = 15)	Colon and rectum	Erythema, edema, erosion, nonspecific
13	Khan *et al*[33]	Acute GVHD patients (n = 38)	Stomach, duodenum, colon and rectum	Edema, erosion, ulcer, bleeding, nonspecific, pseudomembrane mucosa
14	Silbermintz *et al*[34]	An 8-yr-old child	Small bowel	Erosion, bleeding
15	Holmberg *et al*[35]	GI-GVHD patients (n = 90)	Stomach, duodenum, colon and rectum	Normal, erythema, edema, ulcer
16	Onozawa *et al*[36]	An 18-yr-old male	Stomach, duodenum, colon and rectum	Normal, nonspecific
17	Yeh *et al*[37]	GI-GVHD patients (n = 4)	Stomach, duodenum, small bowel, colon and rectum	Erythema, edema, erosions, ulcer, bleeding, nonspecific
18	Schulenburg *et al*[38]	GI-GVHD patients (n = 29)	Stomach, duodenum, colon and rectum	Ulcer, erythema, erosion, normal
19	Cruz-Correa *et al*[39]	GI-GVHD patients (n = 44)	Stomach, duodenum, colon and rectum	Normal, erythema, edema, erosions, ulcer, bleeding, nonspecific
20	Wakui *et al*[40]	GI-GVHD patients (n = 44)	Stomach, duodenum, colon and rectum	Erythema, erosion, ulcer
21	Terdiman *et al*[41]	Acute GI-GVHD patients (n = 12)	Stomach, duodenum, colon and rectum	Normal, nonspecific

GI-GVHD: Gastrointestinal graft-versus-host disease; HSCT: Hematopoietic stem cell transplantation.

cosa and pseudomembrane formation are occasionally observed, no particular findings are reportedly associated with the colorectal mucosa. Moreover, even though some papers report mucosal sloughing and ulceration[8], a wide area of sloughing mucosa along with ulcers and inflamed mucosa, as observed in the present case, has never been reported. We believe that a combination of severe GVHD and CMV infection is the pathogenesis responsible for these symptoms[13]. According to He *et al*[17], GI-GVHD accompanied by CMV infection causes deep, discrete ulcers. The ischemic consequences of occluded blood vessels caused by enlarged vascular endothelial cells due to CMV infection are thought to be the mechanism underlying the GI mucosal damage[18]. Similarly, ischemic alteration, in addition to the cytotoxicity caused by GVHD[19], is a likely cause of the severe symptoms in our patient.

The early and accurate diagnosis of CMV infection in the GI tract is the key to preventing severe symptoms, such as perforation and bleeding[20]. In this case, even though immunostaining with an anti-CMV antibody was negative, the quantitative PCR results of the biopsy specimens were positive, with a high value of 1.0×10^4 copies/μg DNA, which enabled the diagnosis of CMV-GID. The diagnostic accuracy of the quantitative PCR method using biopsy samples is reportedly superior to that of immunostaining to determine the involvement of CMV in the GI symptoms[21]. This case showed that the results of the CMV quantitative PCR were closely correlated with the post-treatment improvement of the mucosa, suggesting the usefulness of the technique for evaluating the effects of CMV treatment.

In conclusion, when endoscopic observation is performed on HSCT patients with postoperative GI symptoms, it is necessary to look for signs of mucosal sloughing and ulcers. In addition to a detailed endoscopic observation, biopsy samples should be examined for the characteristic pathological features of GVHD[22] and for signs of CMV infection. The course of this case suggests that the quantitative PCR of biopsy samples is useful for revealing CMV infection in the GI tract.

ACKNOWLEDGMENTS

We thank Hisae Kawashiro, the clinical research coordinator, for helping with the data collection.

REFERENCES

1 **Filipovich AH**, Weisdorf D, Pavletic S, Socie G, Wingard JR, Lee SJ, Martin P, Chien J, Przepiorka D, Couriel D, Cowen EW, Dinndorf P, Farrell A, Hartzman R, Henslee-Downey J, Jacobsohn D, McDonald G, Mittleman B, Rizzo JD, Robinson M, Schubert M, Schultz K, Shulman H, Turner M, Vogelsang G, Flowers ME. National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: I. Diagnosis and staging working group report. *Biol Blood Marrow Transplant* 2005; **11**: 945-956 [PMID: 16338616 DOI: 10.1016/j.bbmt.2005.09.004]

2 **Shulman HM**, Kleiner D, Lee SJ, Morton T, Pavletic SZ, Farmer E, Moresi JM, Greenson J, Janin A, Martin PJ, McDonald G, Flowers ME, Turner M, Atkinson J, Lefkowitch J, Washington MK, Prieto VG, Kim SK, Argenyi Z, Diwan AH, Rashid A, Hiatt K, Couriel D, Schultz K, Hymes S, Vogelsang GB. Histopathologic diagnosis of chronic graft-versus-host disease: National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: II. Pathology Working Group Report. *Biol Blood Marrow Transplant* 2006; **12**: 31-47 [PMID: 16399567 DOI: 10.1016/j.bbmt.2005.10.023]

3 **Pavletic SZ**, Martin P, Lee SJ, Mitchell S, Jacobsohn D, Cowen EW, Turner ML, Akpek G, Gilman A, McDonald G, Schubert M, Berger A, Bross P, Chien JW, Couriel D, Dunn JP, Fall-Dickson J, Farrell A, Flowers ME, Greinix H, Hirschfeld S, Gerber L, Kim S, Knobler R, Lachenbruch PA, Miller FW, Mittleman B, Papadopoulos E, Parsons SK, Przepiorka D, Robinson M, Ward M, Reeve B, Rider LG, Shulman H, Schultz KR, Weisdorf D, Vogelsang GB. Measuring therapeutic response in chronic graft-versus-host disease: National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: IV. Response Criteria Working Group report. *Biol Blood Marrow Transplant* 2006; **12**: 252-266 [PMID: 16503494 DOI: 10.1016/j.bbmt.2006.01.008]

4 **Schultz KR**, Miklos DB, Fowler D, Cooke K, Shizuru J, Zorn E, Holler E, Ferrara J, Shulman H, Lee SJ, Martin P, Filipovich AH, Flowers ME, Weisdorf D, Couriel D, Lachenbruch PA, Mittleman B, Vogelsang GB, Pavletic SZ. Toward biomarkers for chronic graft-versus-host disease: National Institutes of Health consensus development project on criteria for clinical trials in chronic graft-versus-host disease: III. Biomarker Working Group Report. *Biol Blood Marrow Transplant* 2006; **12**: 126-137 [PMID: 16443511 DOI: 10.1016/j.bbmt.2005.11.010]

5 **Martin PJ**, Weisdorf D, Przepiorka D, Hirschfeld S, Farrell A, Rizzo JD, Foley R, Socie G, Carter S, Couriel D, Schultz KR, Flowers ME, Filipovich AH, Saliba R, Vogelsang GB, Pavletic SZ, Lee SJ. National Institutes of Health Consensus Development Project on Criteria for Clinical Trials in Chronic Graft-versus-Host Disease: VI. Design of Clinical Trials Working Group report. *Biol Blood Marrow Transplant* 2006; **12**: 491-505 [PMID: 16635784 DOI: 10.1016/j.bbmt.2006.03.004]

6 **Couriel D**, Carpenter PA, Cutler C, Bolaños-Meade J, Treister NS, Gea-Banacloche J, Shaughnessy P, Hymes S, Kim S, Wayne AS, Chien JW, Neumann J, Mitchell S, Syrjala K, Moravec CK, Abramovitz L, Liebermann J, Berger A, Gerber L, Schubert M, Filipovich AH, Weisdorf D, Schubert MM, Shulman H, Schultz K, Mittelman B, Pavletic S, Vogelsang GB, Martin PJ, Lee SJ, Flowers ME. Ancillary therapy and supportive care of chronic graft-versus-host disease: national institutes of health consensus development project on criteria for clinical trials in chronic Graft-versus-host disease: V. Ancillary Therapy and Supportive Care Working Group Report. *Biol Blood Marrow Transplant* 2006; **12**: 375-396 [PMID: 16545722 DOI: 10.1016/j.bbmt.2006.02.003]

7 **Irani JL**, Cutler CS, Whang EE, Clancy TE, Russell S, Swanson RS, Ashley SW, Zinner MJ, Raut CP. Severe acute gastrointestinal graft-vs-host disease: an emerging surgical dilemma in contemporary cancer care. *Arch Surg* 2008; **143**: 1041-1045; discussion 1046 [PMID: 19015460 DOI: 10.1001/archsurg.143.11.1041]

8 **Xu CF**, Zhu LX, Xu XM, Chen WC, Wu DP. Endoscopic diagnosis of gastrointestinal graft-versus-host disease. *World J Gastroenterol* 2008; **14**: 2262-2267 [PMID: 18407606]

9 **Ljungman P**, Griffiths P, Paya C. Definitions of cytomegalovirus infection and disease in transplant recipients. *Clin Infect Dis* 2002; **34**: 1094-1097 [PMID: 11914998 DOI: 10.1086/339329]

10 **Beader N**, Kaleniċ S, Labar B. [Diagnostic approach and therapy for cytomegalovirus (CMV) infection following allogeneic stem cell transplantation]. *Lijec Vjesn* 2011; **133**: 389-396 [PMID: 22329295]

11 **Glucksberg H**, Storb R, Fefer A, Buckner CD, Neiman PE, Clift RA, Lerner KG, Thomas ED. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation* 1974; **18**: 295-304 [PMID: 4153799]

12 **Rosen P**, Armstrong D, Rice N. Gastrontestinal cytomegalovirus infection. *Arch Intern Med* 1973; **132**: 274-276 [PMID: 4352550]

13 **Hoshino K**, Shibata D, Miyagi T, Yamamoto Y, Arakaki S, Maeshiro T, Hokama A, Kinjo F, Takahashi K, Fujita J. Cytomegalovirus-associated gastric ulcers in a patient with dermatomyositis treated with steroid and cyclophosphamide pulse therapy. *Endoscopy* 2011; **43** Suppl 2 UCTN: E277-E278 [PMID: 21915824 DOI: 10.1055/s-0030-1256422]

14 **Jacobsohn DA**, Vogelsang GB. Acute graft versus host disease. *Orphanet J Rare Dis* 2007; **2**: 35 [PMID: 17784964 DOI: 10.1186/1750-1172-2-35]

15 **Nachbaur D**, Larcher C, Kircher B, Eibl G, Nussbaumer W, Gunsilius E, Haun M, Grünewald K, Gastl G. Risk for cytomegalovirus infection following reduced intensity allogeneic stem cell transplantation. *Ann Hematol* 2003; **82**: 621-627 [PMID: 12920570 DOI: 10.1007/s00277-003-0706-1]

16 **Einsele H**, Ehninger G, Hebart H, Weber P, Dette S, Link H, Horny HP, Meuter V, Wagner S, Waller HD. Incidence of local CMV infection and acute intestinal GVHD in marrow transplant recipients with severe diarrhoea. *Bone Marrow Transplant* 1994; **14**: 955-963 [PMID: 7711674]

17 **He JD**, Liu YL, Wang ZF, Liu DH, Chen H, Chen YH. Colonoscopy in the diagnosis of intestinal graft versus host disease and cytomegalovirus enteritis following allogeneic haematopoietic stem cell transplantation. *Chin Med J* (Engl) 2008; **121**: 1285-1289 [PMID: 18713549]

18 **Chong KT**, Gresser I, Mims CA. Interferon as a defence mechanism in mouse cytomegalovirus infection. *J Gen Virol* 1983; **64** (Pt 2): 461-464 [PMID: 6187894]

19 **Goker H**, Haznedaroglu IC, Chao NJ. Acute graft-vs-host disease: pathobiology and management. *Exp Hematol* 2001; **29**: 259-277 [PMID: 11274753]

20 **Söderberg-Nauclér C**, Fish KN, Nelson JA. Reactivation of latent human cytomegalovirus by allogeneic stimulation of blood cells from healthy donors. *Cell* 1997; **91**: 119-126 [PMID: 9335340]

21 **Yoshino T**, Nakase H, Ueno S, Uza N, Inoue S, Mikami S, Matsuura M, Ohmori K, Sakurai T, Nagayama S, Hasegawa S, Sakai Y, Chiba T. Usefulness of quantitative real-time PCR assay for early detection of cytomegalovirus infection in patients with ulcerative colitis refractory to immunosuppressive therapies. *Inflamm Bowel Dis* 2007; **13**: 1516-1521 [PMID: 17828781 DOI: 10.1002/ibd.20253]

22 **Shidham VB**, Chang CC, Shidham G, Ghazala F, Lindholm PF, Kampalath B, George V, Komorowski R. Colon biopsies for evaluation of acute graft-versus-host disease (A-GVHD) in allogeneic bone marrow transplant patients. *BMC Gastroenterol* 2003; **3**: 5 [PMID: 12697049]

23 **Sultan M**, Ramprasad J, Jensen MK, Margolis D, Werlin

S. Endoscopic diagnosis of pediatric acute gastrointestinal graft-versus-host disease. *J Pediatr Gastroenterol Nutr* 2012; **55**: 417-420 [PMID: 22367336 DOI: 10.1097/MPG.0b013e31825048eb]

24 **Kreisel W**, Dahlberg M, Bertz H, Harder J, Potthoff K, Deibert P, Schmitt-Graeff A, Finke J. Endoscopic diagnosis of acute intestinal GVHD following allogeneic hematopoietic SCT: a retrospective analysis in 175 patients. *Bone Marrow Transplant* 2012; **47**: 430-438 [PMID: 21706064 DOI: 10.1038/bmt.2011.137]

25 **Aslanian H**, Chander B, Robert M, Cooper D, Proctor D, Seropian S, Jain D. Prospective evaluation of acute graft-versus-host disease. *Dig Dis Sci* 2012; **57**: 720-725 [PMID: 22011927 DOI: 10.1007/s10620-011-1938-x]

26 **Martínez C**, Rosales M, Calvo X, Cuatrecasas M, Rodriguez-Carunchio L, Llach J, Fernández-Avilés F, Rosiñol L, Rovira M, Carreras E, Urbano-Ispízua A. Serial intestinal endoscopic examinations of patients with persistent diarrhea after allo-SCT. *Bone Marrow Transplant* 2012; **47**: 694-699 [PMID: 21743500 DOI: 10.1038/bmt.2011.143]

27 **Krishna SG**, Barlogie B, Lamps LW, Krishna K, Aduli F, Anaissie E. Recurrent spontaneous gastrointestinal graft-versus-host disease in autologous hematopoietic stem cell transplantation. *Clin Lymphoma Myeloma Leuk* 2010; **10**: E17-E21 [PMID: 20223723 DOI: 10.3816/CLML.2010.n.012]

28 **Al Ashgar H**, Peedikayil M, Chaudhri N, Al-Ghamdi A. Defecation of a "colon cast" as a rare presentation of acute graft-versus-host disease. *Ann Saudi Med* 2009; **29**: 231-233 [PMID: 19448368]

29 **Varadarajan P**, Dunford LM, Thomas JA, Brown K, Paplham P, Syta M, Schiff M, Padmanabhan S, Battiwalla M, Smiley S, Hahn T, McCarthy PL. Seeing what's out of sight: wireless capsule endoscopy's unique ability to visualize and accurately assess the severity of gastrointestinal graft-versus-host-disease. *Biol Blood Marrow Transplant* 2009; **15**: 643-648 [PMID: 19361758 DOI: 10.1016/j.bbmt.2009.02.002]

30 **Cheung DY**, Kim JI, Kim SS, Sung HY, Cho SH, Park SH, Han JY, Kim JK, Lee JW, Min WS, Park GS, Kang CS. Endoscopic evaluation in gastrointestinal graft-versus-host disease: comparisons with histological findings. *Dig Dis Sci* 2008; **53**: 2947-2954 [PMID: 18401714 DOI: 10.1007/s10620-008-0262-6]

31 **Ross WA**, Ghosh S, Dekovich AA, Liu S, Ayers GD, Cleary KR, Lee JH, Couriel D. Endoscopic biopsy diagnosis of acute gastrointestinal graft-versus-host disease: rectosigmoid biopsies are more sensitive than upper gastrointestinal biopsies. *Am J Gastroenterol* 2008; **103**: 982-989 [PMID: 18028511 DOI: 10.1111/j.1572-0241.2007.01639.x]

32 **Harada K**, Higaki S, Hashimoto K, Hashimoto S, Oga A, Gondo T, Sakaida I. Study on the colonoscopic features of GVHD enteritis that developed after hematopoietic stem cell transplantation. *Hepatogastroenterology* 2007; **54**: 2221-2227 [PMID: 18265637]

33 **Khan K**, Schwarzenberg SJ, Sharp H, Jessurun J, Gulbahce HE, Defor T, Nagarajan R. Diagnostic endoscopy in children after hematopoietic stem cell transplantation. *Gastrointest Endosc* 2006; **64**: 379-385; quiz 389-392 [PMID: 16923486 DOI: 10.1016/j.gie.2005.08.040]

34 **Silbermintz A**, Sahdev I, Moy L, Vlachos A, Lipton J, Levine J. Capsule endoscopy as a diagnostic tool in the evaluation of graft-vs.-host disease. *Pediatr Transplant* 2006; **10**: 252-254 [PMID: 16573617 DOI: 10.1111/j.1399-3046.2005.00454.x]

35 **Holmberg L**, Kikuchi K, Gooley TA, Adams KM, Hockenbery DM, Flowers ME, Schoch HG, Bensinger W, McDonald GB. Gastrointestinal graft-versus-host disease in recipients of autologous hematopoietic stem cells: incidence, risk factors, and outcome. *Biol Blood Marrow Transplant* 2006; **12**: 226-234 [PMID: 16443520 DOI: 10.1016/j.bbmt.2005.10.011]

36 **Onozawa M**, Yonezumi M, Kawarazaki M, Izumiyama K, Chiba K, Kondo T, Ota S, Kobayashi S, Kato M, Hashino S, Tanaka J, Imamura M, Asaka M. Usefulness of magnifying endoscopic evaluation of the terminal ileum for a patient with graft-versus-host disease after allogeneic hematopoietic stem cell transplantation. *Ann Hematol* 2005; **84**: 106-109 [PMID: 15300409 DOI: 10.1007/s00277-004-0915-2]

37 **Yeh SP**, Liao YM, Hsu CH, Chen CL, Shen YC, Hsueh CT, Huang HH, Chiu CF. Gastric bleeding due to graft-vs-host disease: discrepancy between endoscopic and histologic assessment. *Am J Clin Pathol* 2004; **122**: 919-925 [PMID: 15539384 DOI: 10.1309/23DA-L9F6-P74X-WJHL]

38 **Schulenburg A**, Turetschek K, Wrba F, Vogelsang H, Greinix HT, Keil F, Mitterbauer M, Kalhs P. Early and late gastrointestinal complications after myeloablative and nonmyeloablative allogeneic stem cell transplantation. *Ann Hematol* 2004; **83**: 101-106 [PMID: 14615909 DOI: 10.1007/s00277-003-0756-4]

39 **Cruz-Correa M**, Poonawala A, Abraham SC, Wu TT, Zahurak M, Vogelsang G, Kalloo AN, Lee LA. Endoscopic findings predict the histologic diagnosis in gastrointestinal graft-versus-host disease. *Endoscopy* 2002; **34**: 808-813 [PMID: 12244503 DOI: 10.1055/s-2002-34257]

40 **Wakui M**, Okamoto S, Ishida A, Kobayashi H, Watanabe R, Yajima T, Iwao Y, Hisamatsu T, Hibi T, Ikeda Y. Prospective evaluation for upper gastrointestinal tract acute graft-versus-host disease after hematopoietic stem cell transplantation. *Bone Marrow Transplant* 1999; **23**: 573-578 [PMID: 10217188 DOI: 10.1038/sj.bmt.1701613]

41 **Terdiman JP**, Linker CA, Ries CA, Damon LE, Rugo HS, Ostroff JW. The role of endoscopic evaluation in patients with suspected intestinal graft-versus-host disease after allogeneic bone-marrow transplantation. *Endoscopy* 1996; **28**: 680-685 [PMID: 8934085 DOI: 10.1055/s-2007-1005576]

P- Reviewers Kiyan M, Yilmaz M
S- Editor Gou SX **L- Editor** A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i4.604

World J Gastroenterol 2013 January 28; 19(4): 604-606
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Emphysematous cholecystitis with massive gas in the abdominal cavity

Hiroyuki Miyahara, Dai Shida, Hiroki Matsunaga, Yukiko Takahama, Sachio Miyamoto

Hiroyuki Miyahara, Hiroki Matsunaga, Yukiko Takahama, Sachio Miyamoto, Department of Surgery, Tokyo Metropolitan Bokutoh Hospital, Tokyo 1308575, Japan
Dai Shida, Colorectal Surgery Division, National Cancer Center Hospital, Tokyo 1040045, Japan
Author contributions: Miyahara H, Matsunaga H, Takahama Y and Miyamoto S collected the data, performed the treatment and wrote the paper; Shida D was responsible for writing the paper and its supervision.
Correspondence to: Dai Shida, MD, PhD, Colorectal Surgery Division, National Cancer Center Hospital, 5-1-1 Tsukiji, Chuo-ku, Tokyo 1040045, Japan. dshida-tky@umin.ac.jp
Telephone: +81-3-35422511 Fax: +81-3-35453567
Received: November 20, 2012 Revised: December 14, 2012
Accepted: December 25, 2012
Published online: January 28, 2013

Abstract

Emphysematous cholecystitis is a rare variant of acute cholecystitis with a high mortality rate. The combination of emphysematous cholecystitis and pneumoperitoneum is also rare. We herein describe a case of emphysematous cholecystitis with massive gas in the abdominal cavity. A 77-year-old male presented with epigastric pain and lassitude lasting for one week. A computed tomography scan demonstrated massive gas in the abdominal cavity. Gas was also detectable inside the gallbladder. Massive ascites as well as a pleural effusion were also detected. Under the diagnosis of perforation of the digestive tract, we performed emergency surgery. Beyond our expectations, the perforation site was not in the alimentary tract, but rather in the gallbladder. We then diagnosed the patient with emphysematous cholecystitis with perforation, and performed cholecystectomy. A pathological examination of the resected gallbladder revealed necrosis in the mucosa and thinning of the wall. Cultures of the ascites detected *Clostridium perfringens*, a gas-producing microorganism.

© 2013 Baishideng. All rights reserved.

Key words: Emphysematous cholecystitis; Pneumoperitoneum; *Clostridium perfringens*

Miyahara H, Shida D, Matsunaga H, Takahama Y, Miyamoto S. Emphysematous cholecystitis with massive gas in the abdominal cavity. *World J Gastroenterol* 2013; 19(4): 604-606 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i4/604.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i4.604

INTRODUCTION

Emphysematous cholecystitis is a type of acute cholecystitis characterized by the presence of intramural and/or intraluminal gas that may develop into gangrene or perforation of the gallbladder. The morbidity and mortality rates of emphysematous cholecystitis are considerable[1]. The disease begins with acute cholecystitis followed by ischemia or gangrene of the gallbladder wall and an infection caused by gas-producing bacteria.

Emphysematous cholecystitis is an uncommon variant of acute cholecystitis. Emphysematous cholecystitis occurring in association with a pneumoperitoneum is very rare. Modini *et al*[2] reported the 16th case of emphysematous cholecystitis with a pneumoperitoneum in the English-language literature in 2008. Thereafter, only one case was reported[3]. We herein report the 18th known case. What is of much note is that, among these cases, the finding of macroscopic perforation of the gallbladder was made in only eight patients[4].

This report presents a case of emphysematous cholecystitis causing a pneumoperitoneum with the finding of macroscopic perforation of the gallbladder.

CASE REPORT

A 77-year-old male was transported to our hospital in September 2011 because he was found falling down in his home with epigastric pain and lassitude lasting for one week. There was nothing particular in the patient's

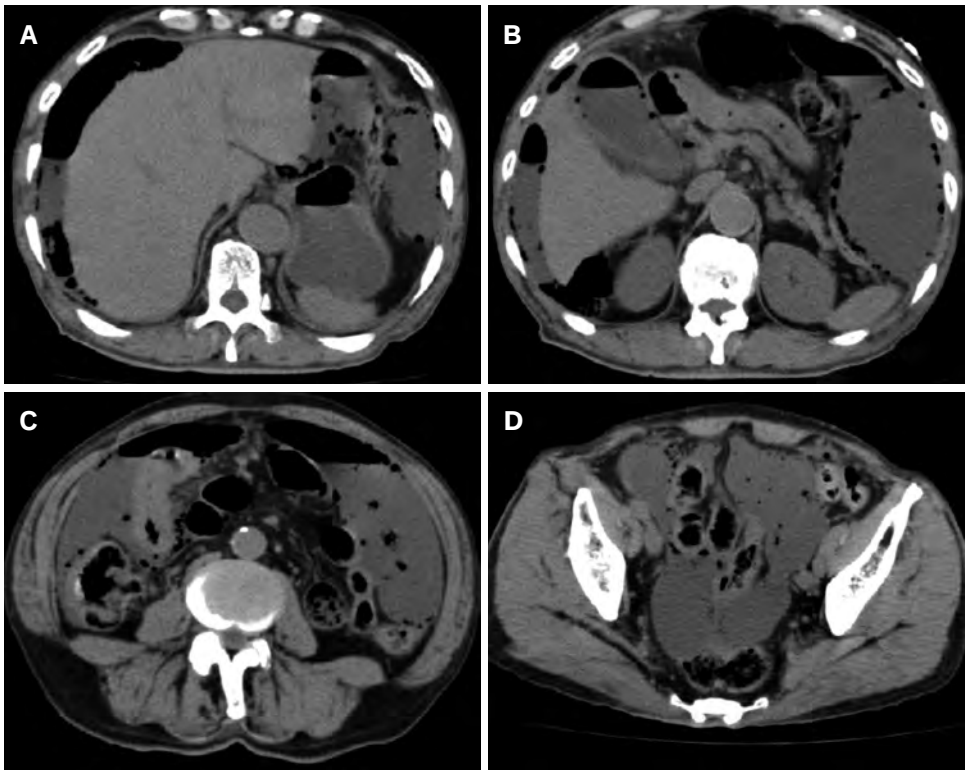


Figure 1 Computed tomography scan (axial axis). A: Demonstrating massive gas in the abdominal cavity; B: Demonstrating gas inside the gallbladder; C, D: Demonstrating massive ascites as well as a pleural effusion.

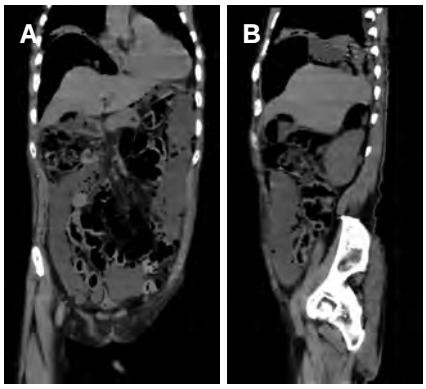


Figure 2 Computed tomography scan clearly demonstrated a massive collection of gas inside the gallbladder. A: The coronal axis; B: Sagittal axis.

history. His vital signs were as follows: pulse: 115 beats/min, blood pressure: 151/86 mmHg, respirations: 36 breaths/min, saturation: 92% on room air, and temperature: 37.7 ℃. The patient's abdomen was distended, and there was local tenderness in the upper abdomen without muscular defense. A laboratory examination showed the following values: leukocyte count: 16.6 × 10^3/μL, hemoglobin: 14.4 g/dL, hematocrit: 42.4%, platelet count: 16.5 ×10^4/μL; serum values: sodium: 130 mEq/L, potassium: 3.8 mEq/L, blood urea nitrogen: 88 mg/dL, creatinine: 3.6 mg/dL, alkaline phosphatase: 251 U/L, lactic dehydrogenase: 371 U/L, aspirate aminotransferase: 32 U/L, alanine aminotransferase: 25 U/L, total bilirubin: 0.4 mg/dL, γ-glutamyl trans ferase: 28 U/L, glucose: 207 mg/dL. Plain abdominal radiography showed the presence of intestinal gas. A computed tomography (CT) scan demonstrated massive gas in the abdominal cavity (Figure 1). Gas was also detectable inside the gallbladder (Figures 1B and 2). Massive ascites as well as a pleural effusion were also detected (Figure 1C and D). Under the diagnosis of perforation of the digestive tract, emergency surgery was performed. In the abdominal cavity, there was massive yellow-brown purulent ascites that did not include any saburra. Contrary to our expectations, the perforation site was not in the alimentary tract, but rather in the gallbladder (Figure 3). We therefore diagnosed the patient to have emphysematous cholecystitis with perforation, and performed cholecystectomy. No gallstones were detected in the gallbladder. Tazobactam/piperacillin was given pre- and post-operation. The patient's postoperative course was uneventful, and he was discharged healthy 28 d after undergoing surgery. Cultures of the ascites detected *Clostridium perfringens*. A pathological examination of the resected gallbladder revealed necrosis in the mucosa and thinning of the wall.

DISCUSSION

Emphysematous cholecystitis is a type of acute cholecystitis characterized by the presence of gas in the gallbladder wall. The disease begins with acute cholecystitis followed by ischemia or gangrene of the gallbladder wall and infection caused by gas-producing bacteria. Whereas the mortality rate of uncomplicated acute cholecystitis is

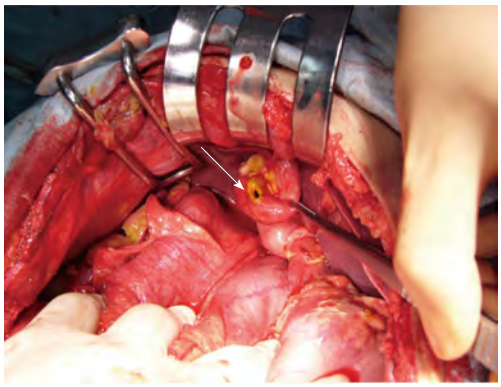


Figure 3 An 8 mm hole in the gallbladder was detected (arrow).

approximately 1.4%, that of acute emphysematous cholecystitis is 15%-20% due to the increased incidence of gallbladder wall gangrene and perforation[5]. Therefore, prompt diagnosis and treatment are essential. The most common symptoms are right upper quadrant pain, low-grade fever, nausea and vomiting. Peritoneal signs may be present, and masses in the right upper quadrant may be palpated in as many as half of patients. CT scanning is the most sensitive test for detecting emphysematous cholecystitis. The presence of gas within the gallbladder wall and lumen is easily confirmed on CT scans.

Emphysematous cholecystitis is more common in males than females (7:3), and 40% of affected patients have diabetes mellitus. In our case, there was no history of diabetes mellitus. After the operation, his blood sugar was decreased down to a normal level, and HbA1c was within normal range.

The presence of a concomitant pneumoperitoneum, which may occur following gallbladder perforation, is rarely found. Most patients with a concomitant pneumoperitoneum are in unstable condition. Therefore, the first choice of treatment in such cases is emergency exploratory laparotomy, followed by cholecystectomy, under a correct intraoperative diagnosis. Another method of treatment, involves initial percutaneous cholecystostomy with a strict intravenous antibiotics regimen, followed by subsequent cholecystectomy during a second stage[4]. In severely ill patients in particular, percutaneous cholecystostomy with broad-spectrum antibiotics may be an alternative choice of treatment[5]. In our case, we did not diagnose the patient with emphysematous cholecystitis preoperatively due to the huge amounts of gas in the abdominal cavity. Compared with previous cases, the amount of gas in our case was very large, thus suggesting not an acute stage, but a sub-acute stage and continuous infection with gas-producing bacteria. If the correct diagnosis could be done preoperatively, laparoscopic surgery may be the alternative treatment.

The bacteria most frequently cultured in this setting include anaerobes, such as *Clostridia welchii* or *Clostridia perfringens*. The bacteria second most frequently cultured include aerobes, such as *Escherichia coli*[5]. In our case, cultures of the ascites detected *Clostridium perfringens*. Zeebregts *et al*[4] reported that, in six of 14 cases, *Clostridium perfringens* was detected on cultures.

In conclusion, we herein reported a case presenting with a very large amount of gas in the abdominal cavity due to gallbladder perforation. We first suspected the possibility of upper gastrointestinal perforation and performed emergency surgery. However, we found a perforation not in the gastrointestinal tract, but rather in the gallbladder. Pneumoperitoneums are nearly always due to perforation of the gastrointestinal tract, however, although unusual, they may also be caused by emphysematous cholecystitis.

REFERENCES

1 **Shrestha Y**, Trottier S. Images in clinical medicine. Emphysematous cholecystitis. *N Engl J Med* 2007; **357**: 1238 [PMID: 17881755]

2 **Modini C**, Clementi I, Simonelli L, Antoniozzi A, Assenza M, Ciccarone F, Bartolucci P, Ricci G, Petroni R. Acute emphysematous cholecystitis as a cause of pneumoperitoneum. *Chir Ital* 2008; **60**: 315-318 [PMID: 18689185]

3 **Kanehiro T**, Tsumura H, Ichikawa T, Hino Y, Murakami Y, Sueda T. Patient with perforation caused by emphysematous cholecystitis who showed flare on the skin of the right dorsal lumbar region and intraperitoneal free gas. *J Hepatobiliary Pancreat Surg* 2008; **15**: 204-208 [PMID: 18392716 DOI: 10.1007/s00534-007-1224-7]

4 **Zeebregts CJ**, Wijffels RT, de Jong KP, Peeters PM, Slooff MJ. Percutaneous drainage of emphysematous cholecystitis associated with pneumoperitoneum. *Hepatogastroenterology* 1999; **46**: 771-774 [PMID: 10370609]

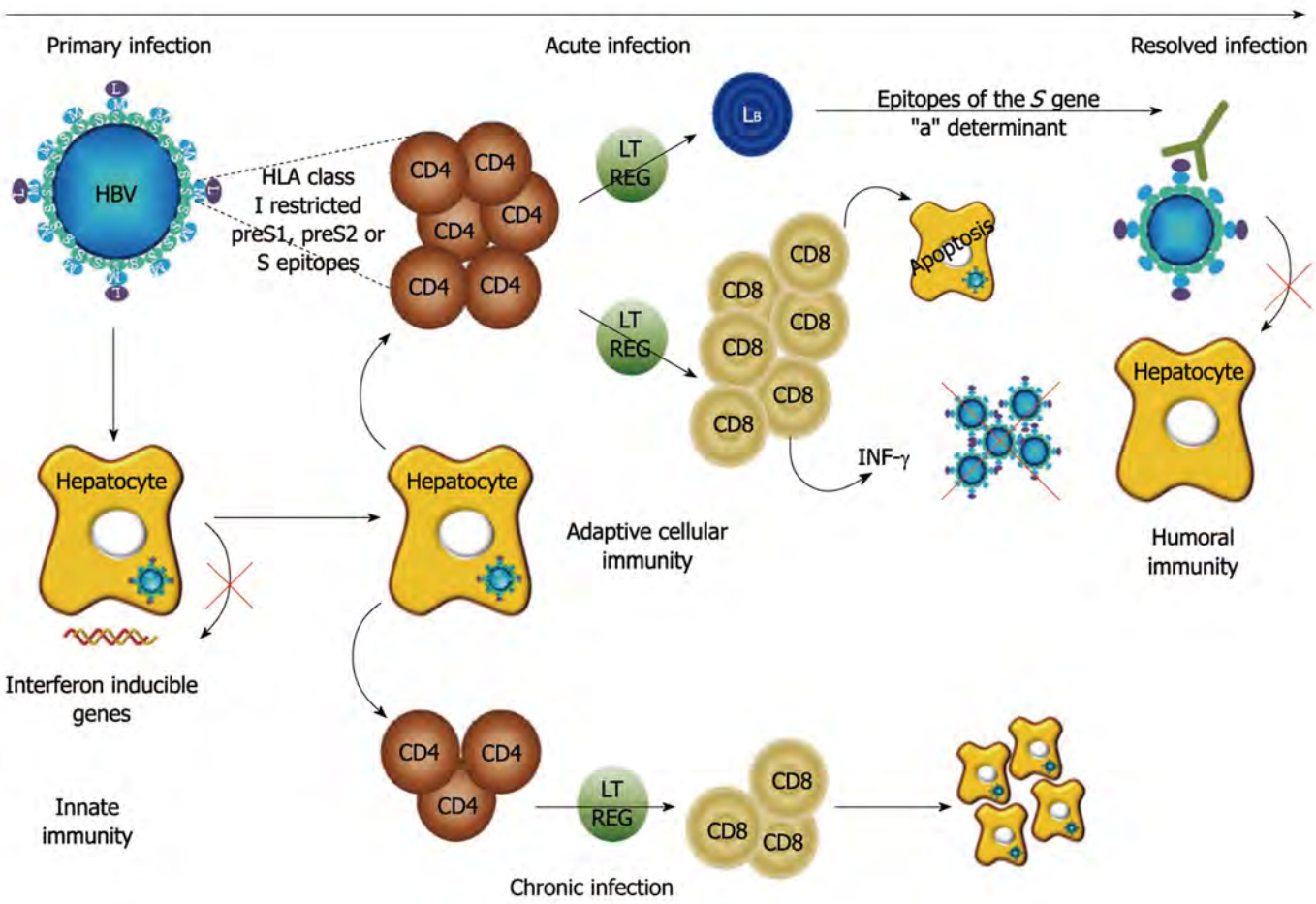
5 **Wu JM**, Lee CY, Wu YM. Emphysematous cholecystitis. *Am J Surg* 2010; **200**: e53-e54 [PMID: 20887835 DOI: 10.1016/j.amjsurg.2010.01.027]

P- Reviewers Aurello P, Toso C, Foelsch UR
S- Editor Gou SX **L- Editor** A **E- Editor** Xiong L

ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

World J Gastroenterol 2013 February 7; 19(5): 607-785



Baishideng Publishing Group
www.wjgnet.com

World Journal of Gastroenterology

Contents

Weekly Volume 19 Number 5 February 7, 2013

EDITORIAL

607 Serrated pathway: Alternative route to colorectal cancer
Patai ÁV, Molnár B, Tulassay Z, Sipos F

REVIEW

616 Controversial role of toll-like receptors in acute pancreatitis
Vaz J, Akbarshahi H, Andersson R

FIELD OF VISION

631 Prophecy about post-endoscopic retrograde cholangiopancreatography pancreatitis: From divination to science
Moon SH, Kim MH

TOPIC HIGHLIGHT

638 Alcohol consumption on pancreatic diseases
Herreros-Villanueva M, Hijona E, Bañales JM, Cosme A, Bujanda L

GUIDELINES FOR CLINICAL PRACTICE

648 Use of antibiotics in the treatment of Crohn's disease
Scribano ML, Prantera C

MINIREVIEWS

654 Clinical impact of hepatitis B and C virus envelope glycoproteins
Jeulin H, Velay A, Murray J, Schvoerer E

665 Burning mouth syndrome
Gurvits GE, Tan A

ORIGINAL ARTICLE

673 Desferrioxamine in warm reperfusion media decreases liver injury aggravated by cold storage
Arthur PG, Niu XW, Huang WH, DeBoer B, Lai CT, Rossi E, Joseph J, Jeffrey GP

682 Interleukin-17 plays a critical role in the acute rejection of intestinal transplantation
Yang JJ, Feng F, Hong L, Sun L, Li MB, Zhuang R, Pan F, Wang YM, Wang WZ, Wu GS, Zhang HW

692 Comparison of different methods of intestinal obstruction in a rat model
Yuan ML, Yang Z, Li YC, Shi LL, Guo JL, Huang YQ, Kang X, Cheng JJ, Chen Y, Yu T, Cao DQ, Pang H, Zhang X

706 Comparison of modified percutaneous transhepatic variceal embolization and endoscopic cyanoacrylate injection for gastric variceal rebleeding
Wang J, Tian XG, Li Y, Zhang CQ, Liu FL, Cui Y, Liu JY

Contents

World Journal of Gastroenterology
Volume 19 Number 5 February 7, 2013

BRIEF ARTICLE

715 Endoscopic stent therapy in patients with chronic pancreatitis: A 5-year follow-up study
Weber A, Schneider J, Neu B, Meining A, Born P, von Delius S, Bajbouj M, Schmid RM, Algül H, Prinz C

721 Australian tertiary care outcomes of entecavir monotherapy in treatment naive patients with chronic hepatitis B
Fahrtash-Bahin F, Kariyawasam VC, Gray T, Byth K, George J, Douglas MW

727 Can mosapride citrate reduce the volume of lavage solution for colonoscopy preparation?
Tajika M, Niwa Y, Bhatia V, Kondo S, Tanaka T, Mizuno N, Hara K, Hijioka S, Imaoka H, Komori K, Yamao K

736 Effectiveness of gastric cancer screening programs in South Korea: Organized *vs* opportunistic models
Kim BJ, Heo C, Kim BK, Kim JY, Kim JG

742 Probiotics increase T regulatory cells and reduce severity of experimental colitis in mice
Zhao HM, Huang XY, Zuo ZQ, Pan QH, Ao MY, Zhou F, Liu HN, Liu ZY, Liu DY

750 Laparoscopic total mesorectal excision with natural orifice specimen extraction
Wang Q, Wang C, Sun DH, Kharbuja P, Cao XY

755 Laparoscopy-assisted total gastrectomy with trans-orally inserted anvil (OrVil™): A single institution experience
Liao GQ, Ou XW, Liu SQ, Zhang SR, Huang W

META-ANALYSIS

761 Preoperative administration of bevacizumab is safe for patients with colorectal liver metastasis
Li DB, Ye F, Wu XR, Wu LP, Chen JX, Li B, Zhou YM

CASE REPORT

769 Degarelix as a new antiangiogenic agent for metastatic colon cancer?
Ghiringhelli F, Isambert N, Ladoire S

773 Internal carotid thrombus in patients with inflammatory bowel disease: Two cases
Richard S, Mione G, Perrin J, Toussaint-Hacquard M, Lacour JC, Ducrocq X

776 Strangulated ileal trans-coloanal-anastomotic hernia: A complication of Altemeier's procedure previously never reported
Di Lena M, Angarano E, Giannini I, Guglielmi A, Altomare DF

778 Ectopic adrenal cortical adenoma in the gastric wall: Case report
Ren PT, Fu H, He XW

781 Splenic lymphangioma that manifested as a solid-cystic mass: A case report
Yang F, Chen WX

LETTERS TO THE EDITOR **784** Severe gastrointestinal mucositis following high dose melphalan therapy for multiple myeloma
Sharma SK, Handoo A, Choudhary D, Dhamija G, Gupta N

World Journal of Gastroenterology
Volume 19 Number 5 February 7, 2013

Contents

APPENDIX	I-VI	Instructions to authors
ABOUT COVER		Jeulin H, Velay A, Murray J, Schvoerer E. Clinical impact of hepatitis B and C virus envelope glycoproteins. *World Journal of Gastroenterology* 2013; 19(5): 654-664 http://www.wjgnet.com/1007-9327/full/v19/i5/654.htm

AIMS AND SCOPE

World Journal of Gastroenterology (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. *WJG* was established on October 1, 1995. It is published weekly on the 7th, 14th, 21st, and 28th each month. The *WJG* Editorial Board consists of 1352 experts in gastroenterology and hepatology from 64 countries.

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

INDEXING/ABSTRACTING

World Journal of Gastroenterology is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, Digital Object Identifier, and Directory of Open Access Journals. ISI, Journal Citation Reports®, Gastroenterology and Hepatology, 2011 Impact Factor: 2.471 (32/74); Total Cites: 16951 (7/74); Current Articles: 677 (1/74); and Eigenfactor® Score: 0.06035 (5/74).

FLYLEAF	I-IX	Editorial Board

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Shuai Ma*
Responsible Science Editor: *Ling-Ling Wen*
Responsible Electronic Editor: *Dan-Ni Zhang*
Proofing Editorial Office Director: *Jin-Lei Wang*
Proofing Editor-in-Chief: *Lian-Sheng Ma*

NAME OF JOURNAL
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

EDITOR-IN-CHIEF
Ferruccio Bonino, MD, PhD, Professor of Gastroenterology, Director of Liver and Digestive Disease Division, Department of Internal Medicine, University of Pisa, Director of General Medicine 2 Unit University Hospital of Pisa, Via Roma 67, 56124 Pisa, Italy

Myung-Hwan Kim, MD, PhD, Professor, Head, Department of Gastroenterology, Director, Center for Biliary Diseases, University of Ulsan College of Medicine, Asan Medical Center, 388-1 Pungnap-2dong, Songpa-gu, Seoul 138-736, South Korea

Kjell Öberg, MD, PhD, Professor, Department of Endocrine Oncology, Uppsala University Hospital, SE-751 85 Uppsala, Sweden

Matt D Rutter, MBBS, MD, FRCP, Consultant Gastroenterologist, Senior Lecturer, Director, Tees Bowel Cancer Screening Centre, University Hospital of North Tees, Durham University, Stockton-on-Tees, Cleveland TS19 8PE, United Kingdom

Andrzej S Tarnawski, MD, PhD, DSc (Med), Professor of Medicine, Chief Gastroenterology, VA Long Beach Health Care System, University of California, Irvine, CA, 5901 E. Seventh Str., Long Beach, CA 90822, United States

EDITORIAL OFFICE
Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
World Journal of Gastroenterology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Co., Limited
Room 1701, 17/F, Henan Building,
No.90 Jaffe Road, Wanchai, Hong Kong, China
Fax: +852-31158812
Telephone: +852-58042046
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

PUBLICATION DATE
February 7, 2013

COPYRIGHT
© 2013 Baishideng. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT
All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i5.607

World J Gastroenterol 2013 February 7; 19(5): 607-615
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

EDITORIAL

Serrated pathway: Alternative route to colorectal cancer

Árpád V Patai, Béla Molnár, Zsolt Tulassay, Ferenc Sipos

Árpád V Patai, Ferenc Sipos, 2nd Department of Internal Medicine, Semmelweis University, 1088 Budapest, Hungary
Béla Molnár, Zsolt Tulassay, Molecular Medicine Research Group, Hungarian Academy of Sciences, 1051 Budapest, Hungary
Author contributions: Patai ÁV and Sipos F contributed to writing and editing; Molnár B and Tulassay Z revised the manuscript.
Correspondence to: Árpád V Patai, MD, 2nd Department of Internal Medicine, Semmelweis University, 1088 Budapest, Hungary. arpad.patai@gmail.com
Telephone: +36-20-4869503 Fax: +36-1-2660816
Received: May 4, 2012 Revised: September 3, 2012
Accepted: September 12, 2012
Published online: February 7, 2013

Abstract

Serrated polyps have been an area of intense focus for gastroenterologists over the past several years. Contrary to what was thought before, a growing body of literature indicates that these polyps can be precursors of colorectal cancer (CRC). Most of these lesions, particularly those in the proximal colon, have so far been under-recognized and missed during colonoscopy, qualifying these lesions to be the main cause of interval cancers. It is estimated that 10%-20% of CRCs evolve through this alternative, serrated pathway, with a distinct genetic and epigenetic profile. Aberrant DNA methylation plays a central role in the development of this CRC subtype. This characteristic molecular background is reflected in a unique pathological and clinical manifestation different from cancers arising *via* the traditional pathway. In this review we would like to highlight morphological, molecular and clinical features of this emerging pathway that are essential for gastroenterologists and may influence their everyday practice.

© 2013 Baishideng. All rights reserved.

Key words: Serrated pathway; DNA methylation; Hyperplastic polyps; Serrated adenomas; Colorectal cancer; Endoscopic surveillance

Patai ÁV, Molnár B, Tulassay Z, Sipos F. Serrated pathway: Alternative route to colorectal cancer. *World J Gastroenterol* 2013; 19(5): 607-615 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i5/607.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i5.607

INTRODUCTION

It is being increasingly recognized that colorectal cancer (CRC) is not a single disease, but rather a heterogeneous disorder including a collection of many distinct diseases with diverse molecular background and clinicopathological manifestations. According to the adenoma-carcinoma sequence proposed by Vogelstein *et al*[1] adenomatous polyps have long been considered as the sole preneoplastic lesions leading to CRC. On the other hand, hyperplastic polyps (HP) often found in the distal colon, until recently have been considered innocuous lesions, despite some contradictory opinions[2,3]. This common view has recently been challenged, as it turned out that these polyps along with other similar lesions commonly termed "serrated polyps" can be precursors to CRC[4,5].

The aim of this article is to provide a thorough clinicopathologic overview of this emerging pathway in colorectal carcinogenesis and help to understand how this accumulating data can be translated into clinical management strategies and better clinical outcomes.

CLASSIFICATION OF SERRATED POLYPS

General features of serrated polyps

The term "serrated polyp" contains a wide variety of colonic lesions and broadly refers to HP and different serrated adenomas. The main histological feature of serrated polyps is the infolding of the crypt epithelium[5], that is represented as a serrated or saw-toothed appearance in longitudinal section and a stellate or starlike appearance on cross section (Figure 1). The molecular basis for this histological feature has been attributed to decreased apoptosis[6-8] that is caused by the activated

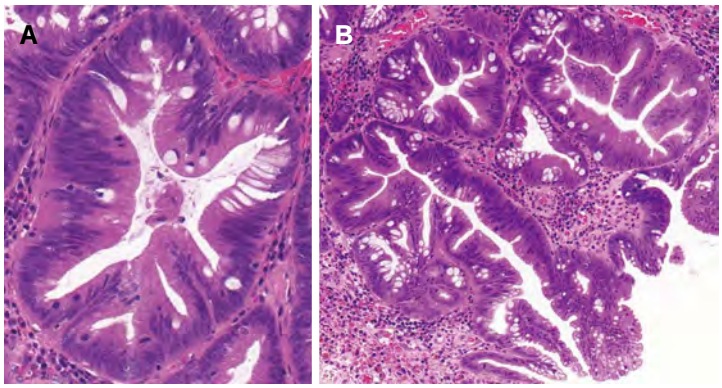


Figure 1 Microscopic features of serrated polyps. A: On cross section serrated crypt shows a stellate or starlike appearance; B: In longitudinal section a characteristic serrated or saw-toothed appearance can be seen.

mitogen activated protein kinase (MAPK)-ERK pathway that is induced by either *BRAF* or *KRAS* mutation (Figure 2). Inhibited apoptosis leads to the accumulation of non-proliferating cells. Serration is a general characteristics of this pathway from HP all the way to serrated adenocarcinoma (SAC)[9].

Hyperplastic (non-dysplastic) aberrant crypt foci

The earliest known microscopical precursors to CRC are mucosal abnormalities termed aberrant crypt foci (ACF). ACF can be further subclassified into two categories: dysplastic and hyperplastic[10,11] (also called as heteroplastic or non-dysplastic). Dysplastic ACFs, often termed as microadenomas, have been associated with sporadic adenomas arising *via* the traditional pathway[12,13]. Hyperplastic ACF may be serrated or non-serrated[11]. They are very frequent; almost every individual over 50 has at least one ACF in the distal colorectum[14]. Serrated hyperplastic ACF has a higher frequency of *BRAF* mutations, than non-serrated ACF, whereas non-serrated ACF has a higher frequency of *KRAS* mutations, than serrated ACF[11,15]. This finding supports the idea that these lesions are potentially initiating step on the serrated pathway to CRC[15], however their high frequency imply that only a small fragment progresses to HP or more advanced lesions of the serrated pathway[9]. *BRAF* and *KRAS* (mutually exclusive) mutations induce the activation of the MAPK-ERK pathway leading to decreased apoptosis and an initial burst of MAPK-ERK-dependent proliferation, leading to the formation of hyperplastic crypts. This uncontrolled proliferation is counteracted by a protective phenomenon called oncogene-induced senescence that is driven by telomere attrition, that triggers the induction of tumor suppressors including *p16*[16] or *IGFBP7*[17] (insulin-like growth factor binding protein 7), similarly as it was described in melanocytes. Hyperplastic crypts may remain dormant for prolonged periods due to the induction of crypt senescence[8] (Figure 2).

HP

HPs are the most common (80%-90%) and the best described serrated polyps. They occur most frequently in the distal colon and the rectum; they are usually slightly elevated, diminutive polyps, less than 5 mm in size. Key morphological features include elongated crypts with serration limited to the upper half of the crypt, with lack of cytologic or architectural dysplasia. These alterations can be seen only in the upper third or only on the surface of the crypts[18]. The proliferative zone may be expanded, but usually confined to the crypt base. The nuclei are small, uniform and basally placed[18], the cytoplasm is eosinophilic. If surface epithelium is not present for histological evaluation, a thickened basal membrane and muscularis mucosae with short smooth muscle extensions into the basal part of the mucosa ("comb-like" appearance) can be helpful hints to identify HP[19] (Table 1).

HPs usually occur a decade earlier (in the fifth and the sixth decade) than adenomatous polyps[20]. Several risk factors have been linked with the prevalence of serrated polyps including cigarette smoking, alcohol consumption, obesity and low-folate intake[20,21], whereas regular nonsteroidal anti-inflammatory drug use, hormone replacement therapy, and high calcium intake have been associated with reduced risk[20]. It is of note that besides smoking, all other factors have also been linked to adenoma formation *via* the traditional pathway[9,20]. This observation gained further importance when it was discovered that smoking is only a strong risk factor for those CRCs that exhibit a unique molecular phenotype [CpG island methylator phenotype (CIMP)] linked to sessile serrated pathway[22,23].

Subclassification of HP

Based on the epithelial mucin content, Torlakovic *et al*[19] histologically subclassified HPs into three categories: goblet cell-rich, microvesicular, and mucin-poor.

Microvesicular hyperplastic polyp (MVHP), also called type 2 HP, is the most common type and the typical representation of HPs encountered in the distal colon. It is characterized by large microvesicular mucin-containing epithelial cells in the upper half of the crypt, reduced goblet cells compared to normal colonic mucosa, and goblet cell abnormalities. MVHP shows prominent serration mostly in the upper half of the crypt and it has a large proliferative zone, which may take up the basal half of the mucosa. Nuclear stratification is present, but it is not prominent. The overall architecture is slightly distorted and minimal to mild crypt dilatation is present. Almost all MVHPs are slightly thicker than surrounding normal colonic mucosa. At the molecular level, MVHPs

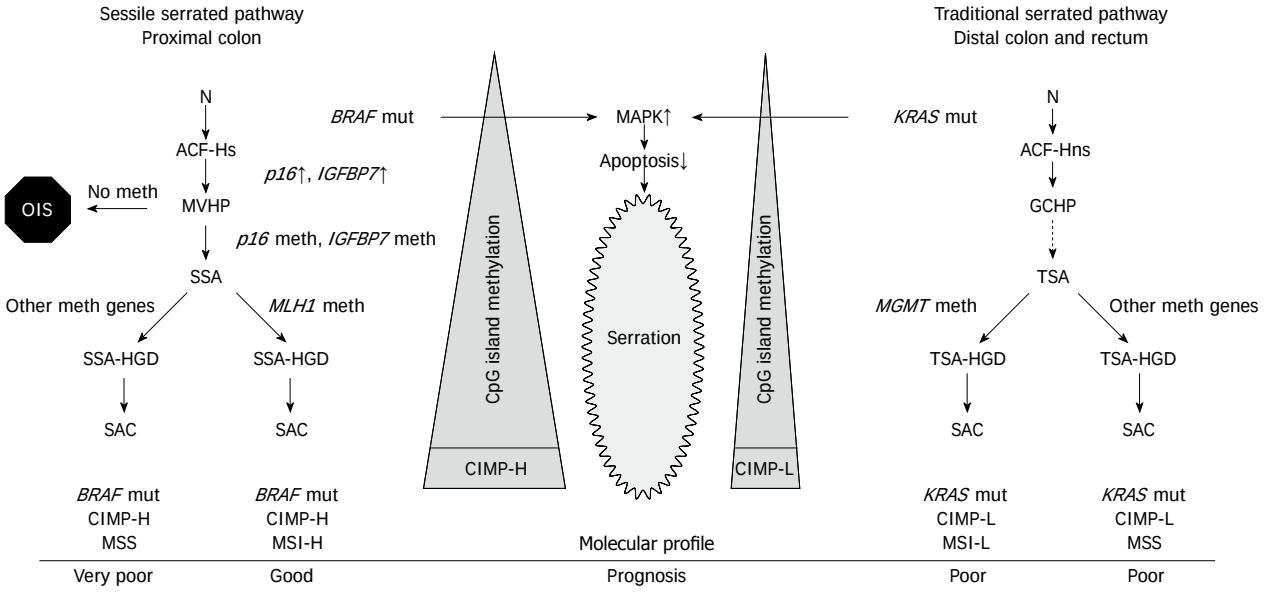


Figure 2 Schematic representation of the sessile and traditional serrated pathways. N: Normal mucosa; ACF-Hs: Serrated hyperplastic-type aberrant crypt focus; ACF-Hns: Non-serrated hyperplastic-type aberrant crypt focus; MVHP: Microvesicular hyperplastic polyp; OIS: Oncogene-induced senescence; GCHP: Goblet cell-rich hyperplastic polyp; SSA: Sessile serrated adenoma; TSA: Traditional serrated adenoma; SSA-HGD: Sessile serrated adenoma with high grade dysplasia; TSA-HGD: Traditional serrated adenoma with high grade dysplasia; SAC: Serrated adenocarcinoma; *IGFBP7*: Insulin-like growth factor-binding protein 7; MAPK: Mitogen activated protein kinase-ERK pathway; *MLH1*: MutL homolog 1; *MGMT*: O-6-methylguanine-DNA methyltransferase; CIMP-H: CpG island methylator phenotype-high; CIMP-L: CpG island methylator phenotype-low; MSI-H: High-level microsatellite instability; MSI-L: Low-level microsatellite instability; MSS: Microsatellite stable.

commonly (80%) exhibit *BRAF* V600E mutation[24], where valine is substituted for glutamic acid. As it was discussed above, this induces MAPK-ERK pathway that is followed by oncogene-induced senescence that includes overexpression of growth control genes *p16* and *IGFBP7* holding the cells in a dormant state. Aberrant CpG island methylation of the promoter region of *p16* and *IGFBP7* bypasses this dormant state[8] and drives MVHPs further to the next stage of serrated polyp progression, namely sessile serrated adenoma[9]. CpG island methylation is more pronounced in proximal MVHPs than in those located distally[25,26] (Figure 2).

Goblet cell-rich hyperplastic polyp (GCHP), also known as type 1 HP, is most commonly found in the distal colon, and is probably the most under-recognized variant. As its name implies this subtype is abundant of large, mature, distended goblet cells in the upper half of enlarged crypts and surface epithelial cells. Surface serrations are less prominent than in MVHP. Nuclear atypia is generally not present, but nuclei are slightly enlarged. *KRAS* mutations (in codon 12 and 13) were detected in almost half of these lesions[24,27], whereas *BRAF* mutations were rarely detected[27]. Successor lesions of GCHPs were rarely observed and it is open question whether they are self-limiting[9,24] or progress to advanced *KRAS*-mutated serrated polyps, presumably traditional serrated adenomas (TSA)[28] (Figure 2).

The mucin-poor hyperplastic polyp is the rarest form, almost absent of goblet cells. It has prominent nuclear atypia, hyperchromatic nuclei, lack of mucin, therefore it is considered to be a reactive version of MVHP with unknown clinical significance[29].

This distinction among these HP variants is primarily of theoretical importance, and an area of academic interest with little or no clinical importance at the moment. However, the distinction between HPs and more advanced lesions is of cardinal clinical importance[28] (Table 1).

PRECURSOR LESIONS TO SERRATED ADENOCARCINOMA

Serrated adenomas

In their landmark paper from 1990, Longacre and Fenoglio-Preiser[30] retrospectively overviewed 18 000 colorectal polyps and identified 110 (0.6%) as serrated adenomas. In 2003, Torlakovic *et al*[19] further divided serrated adenomas into two categories, TSA (those originally described by Longacre and Fenoglio-Preiser) and a new group identified as sessile serrated adenomas (SSAs), lesions with a serrated morphology without cytologic dysplasia.

Sessile serrated adenoma

SSAs are thought to be the second most common form of serrated polyps representing about 20% of all serrated polyps[18,19,31], however more recent studies have shown decreased prevalence (3%-8%)[32,33]. As mentioned above, before 2003 SSAs were labeled as "HP"[34]. It was demonstrated in a recent case series that according to the new WHO classification for serrated colonic polyps[35] a considerable proportion of HPs (especially those greater than 5 mm) were reclassified as SSAs[36].

Still today it is hard to distinguish SSAs from HPs, as

there are only subtle differences, and SSAs lack the typical features (such as cytologic dysplasia) of traditional adenomas. SSAs tend to locate in the proximal colon, but they can also be encountered in the distal colon (Table 1).

The microscopical features of SSAs were first described by Torlakovic and Snover[37] in their landmark paper in 1996, then the term was reintroduced in 2003[19,31]. Microscopically, most characteristic features include horizontal crypt extensions (inverted T- or L- shape) at the crypt bases, crypt branching, crypt invaginations and inverted crypts beneath the mucosal muscle layer (pseudoinvasion), mature goblet cells at the crypt bases, dilation in the lower crypts, serration throughout the crypt length, extending into the lower third of the crypt as well[18,38]. The proliferation zone can extend to the basis of the crypt. SSAs can exhibit mild nuclear atypia, but always lack cytologic dysplasia.

Endoscopically, SSAs are flat or slightly elevated, malleable lesions with irregular borders and may be covered with a thin layer of yellowish mucus giving them a pale appearance[28,38]. They are usually larger than 5 mm in diameter. Their surface is smooth or granular[28], sometimes resembling a prominent mucosal fold. These features altogether make it difficult to detect and remove completely with conventional white-light endoscope, therefore advanced, image-enhanced endoscopy techniques including traditional or virtual magnifying chromoendoscopy are needed. Chromoendoscopy is an image-enhanced endoscopic technique that highlights differences in colonic mucosa based on structural patterns, so-called "pit patterns". In a recent magnifying endoscopy study a new Type Ⅱ open-shape pit pattern (Type Ⅱ-O) was described and shown highly predictive of SSAs (with a sensitivity of 65.5% and a specificity of 97.3%). Progression of SSAs to more advanced lesions was associated with additional morphological changes, including the Type Ⅲ, Ⅳ and Ⅴ pit patterns[39] (Table 1).

On a molecular level, SSAs exhibit *BRAF* mutation and high level of CpG island methylation, supporting the hypothesis that they represent an intermediate stage between MVHPs and sporadic CIMP-H cancers. It was shown that SSAs can progress to dysplasia (SSA with high grade dysplasia, SSA-HGD) and then to CIMP-H cancers. Methylation and consequential loss of expression of *MLH1* (a major DNA mismatch repair gene) is thought to drive this transformation. Impaired mismatch repair leads to high level of microsatellite instability (MSI-H) (Figure 2). It was hypothesized that this malignant progression can occur at faster rate than that observed in the lesions emerging *via* the traditional pathway[40]. This is based on the observation that SACs are more prevalent than SSA-HGD[31,32,38]. The exact time of progression from SSA to SAC is unknown. In a recent case report an untreated SSA was described to transform into an early submucosal invasive cancer in a period of 8 months[41]. These data further underline the need for improved detection of these lesions.

Traditional serrated adenoma

TSAs were first described by Longacre and Fenoglio-Presier[30] in 1990. As mentioned above, until 2003 they were termed serrated adenomas when Torlakovic *et al*[19] divided serrated adenomas to SSA and TSA. In 2008 the same group further characterized these lesions[42]. TSAs represent the rarest subtype of serrated lesions, with a frequency of 1%-6%[18]. Similar to the majority of serrated lesions (unlike SSAs), TSAs have a predilection for the distal colon and the rectum. Macroscopically they resemble traditional adenomas, as having a pedunculated, polypoid appearance. Cytologic dysplasia (90% low-grade and 10% high-grade[18]), as their name implies, is a major feature of TSAs (Table 1).

In 2008, Torlakovic *et al*[42] proposed ectopic crypt formation as decisive morphological criterion for diagnosing TSAs. Ectopic crypts are newly formed aberrant crypts that lost their anchoring to the underlying muscular layer of mucosa[18]. Other characteristics include diffuse eosinophilic cytoplasm, mucosal bridges and protrusions resembling tennis-racquets[42]. On a molecular level, TSAs are frequently *KRAS* mutants; however they can exhibit *BRAF* mutation, and also lack both mutations.

Filiform serrated adenoma is an unusual, less aggressive variant of traditional serrated adenoma, with morphological features similar to TSA[43]. Unlike TSA, filiform SA is composed predominantly of prominent, thin, elongated filiform projections lined by neoplastic epithelium with a serrated contour[44].

Mixed polyps

Mixed polyps are combinations of traditional adenomas and serrated lesions. They are postulated to be the result of collision tumors[45] and successors of SSAs. It is not encouraged to use this term as it does not disclose the preinvasive nature of these lesions[18]. Still, "mixed polyp" is a widely used term and when used it is recommended to describe the components of these lesions (*e.g.*, TSA and traditional adenoma *etc.*)[18].

SERRATED ADENOCARCINOMA

Morphologic features

SAC, a special subtype of colorectal adenocarcinomas morphologically and histochemically resembling serrated polyps, was first described by Jass and Smith in 1992[46]. The relationship between serrated adenomas and SAC were further confirmed by Mäkinen *et al*[47], then histological characteristics of SAC were described[48] and reviewed by the same group[38]. Based on these seminal reports, most important diagnostic criteria of SAC include epithelial serrations, eosinophilic and abundant cytoplasm with vesicular nuclei, chromatin condensation and lack of necrosis. SAC was further classified into three major growth patterns. The most common (70%) serrated pattern contains mature, abundant, mucus-producing epithelium with well-preserved polarity, very similar to serrated

polyps[38]. The mucinous pattern (43%) strongly overlaps with the first group, and is characterized by eosinophilic papillary rods (93%) and eosinophilic cell balls floating in the mucus[38]. The least common (7%) trabecular pattern is a feature of poorly differentiated SACs, where serrated structures are absent and cancer cells grow in a trabecular pattern, but still these cases show eosinophilic epithelium with vesicular nuclei, uncharacteristic of poorly differentiated traditional CRCs[38].

Molecular features

Gene expression profiling study by Laiho *et al*[49] provided molecular evidence that SAC is a biologically distinct subclass of CRC. Comparison of SAC and conventional CRCs revealed 201 differentially expressed genes. Three potential candidates were identified that can be involved in the oncogenesis of SAC: Ephrin receptor B2 (*EPHB2*), hypoxia-inducible factor 1-alpha (*HIF1a*) and patched (*PTCH*) appeared as genes important for the oncogenesis of serrated CRC. *EPHB2* and *PTCH* expression are decreased in SAC compared to conventional CRC. On the other hand, constitutive overexpression of *HIF1a*, a major proangiogenic factor, can be the cause of infrequent necrosis seen in SAC[50]. Activating mutations of oncogenic *BRAF* and *KRAS* are common findings in SAC[26]. As mentioned above, these mutually exclusive mutations induce MAPK-ERK pathway that leads to the inhibition of apoptosis resulting in serrated appearance. Accumulation of CpG island methylation in the promoter regions and consequential silencing in key proapoptotic and tumor suppressor genes, such as *p16* or *IGFBP7* sets up a vicious circle. It is well established that methylation of *MLH1* leading to MSI-H phenotype (closely linked with the sessile serrated pathway) and methylation of *MGMT* leading to MSI-L are the main inducing factors in the malignant progression of serrated adenomas (Figure 2).

It is generally thought that CpG island hypermethylation is confined to the serrated pathway (CIMP)[51,52], however a recent study showed that sporadic CRCs and precursors arising *via* the traditional adenoma-carcinoma pathway also have a characteristic DNA methylation pattern different from those evolving through the serrated pathway[53].

Clinical characteristics

SAC predominantly locates to cecum (52%) and rectum (33%)[47]. It is estimated that 16% of proximal CRCs are SACs, whereas this proportion in the distal colon is only 6%[48]. It is hypothesized that proximal SACs (mostly MSI-H) arise from SSAs and distal SACs (MSI-L and MSS) originate from TSAs[38] (Figure 2). While serrated adenomas are more common in males, SACs are almost twice (1.9 : 1) as common in females, than in males[48]. The higher risk of malignant progression of serrated adenomas to SAC in (elderly) women was explained with postmenopausal estrogen deficiency and decreased folate level, however this needs to be further investigated[38].

The prognosis of SAC seems to be defined by its molecular profile (Figure 2). *BRAF*-mutated, microsatellite-stable cancers in the proximal colon confer a very poor survival[54] with adverse histological features such as lymphatic and perineural invasion and high tumor budding[55]. On the other hand, *BRAF*-mutated cancers with MSI-H phenotype (sporadic MSI-H CRCs) have a favorable prognosis[56].

SERRATED POLYPOSIS: A GENETIC PREDISPOSITION SYNDROME

Serrated polyposis, formerly called hyperplastic polyposis, is a rare form of intestinal polyposis, initially described in 1970 by Goldman *et al*[2]. Current diagnostic criteria, manifested in 2010[35], include (1) at least five serrated polyps proximal to the sigmoid colon with two or more of these being > 10 mm; (2) any number of serrated polyps proximal to the sigmoid colon in an individual who has a first-degree relative with serrated polyposis; and (3) > 20 serrated polyps of any size distributed throughout the colon (not all in the rectum).

Although serrated polyposis provided the first evidences for the malignant potential of serrated polyps, it is still one of the most under-recognized and poorly understood intestinal polyposis syndrome. This is probably due to its rarity (1 in 3000)[57], but also to the phenotypic plasticity[58] and overlapping clinical phenotypes within this disorder[59]. However, based on clinical observations including earlier onset of CRC, multiple cancers, increased individual and familial risk, accumulating evidence indicates that serrated polyposis is a genetic predisposition syndrome to CRC and probably confers also an increased risk for some extracolonic cancers[60].

IMPLICATIONS FOR MANAGEMENT OF SERRATED POLYPS

It is imperative to detect and completely remove serrated lesions, as majority of these lesions tend to progress, and contribute to the development of interval cancers. Data on the natural history of serrated polyps is limited, only retrospective studies with small sample size[61] are currently available. High risk serrated polyps are frequently flat and associated with synchronous advanced colorectal neoplasms[62]. Magnifying chromoendoscopy can facilitate to differentiate between serrated polyps, but it is still difficult to distinguish between SSA and typical HP[63]. Both endoscopists and pathologists should know the most important features in order to detect and diagnose these lesions (Table 1).

To date, no consensus guidelines exist on the management of serrated polyps, but new guidelines including recommendations for management and follow-up of serrated polyps are expected to be available in the near future. With the exception of small and diminutive HPs in the rectosigmoid, that confer no malignant potential,

Table 1 Most important features of serrated polyps[18,19,38,39,62]

	Sessile serrated adenoma	Traditional serrated adenoma	Hyperplastic polyps
Location	Proximal	Distal	Distal
Macroscopic characteristics	Sessile, flat, covered with mucus, poorly defined borders	Protruding, pedunculated	Flat
Color	Normochromatic, pale	Reddish	Pale
Size	> 5 mm	> 5 mm	< 5 mm
Molecular features	*BRAF* mt	*KRAS* mt	
Histological characteristics	Dilated, branched serrated crypts at the bottom	Prominent crypt serration, ectopic crypt formation	Serrations at the top
Pit pattern	Open-shape (type II-O)	Fern or pinecone-like	Starlike (type II)
Precursor	MVHP	GCHP	ACF
Malignant potential	+++	++	-
CIMP status	CIMP-H	CIMP-L	
MSI status	MSI-H or MSS	MSI-L or MSS	MSS
Gender predominance	Female	Male	Male
Dysplasia	Absent	Present	Absent
Ectopic crypt formation	Absent	Present	Absent

MVHP: Microvesicular hyperplastic polyp; GCHP: Goblet cell-rich hyperplastic polyp; ACF: Aberrant crypt focus; CIMP-H: CpG island methylator phenotype-high; CIMP-L: CpG island methylator phenotype-low; MSI-H: High-level microsatellite instability; MSI-L: Low-level microsatellite instability; MSS: Microsatellite stable.

Table 2 Colonoscopic management and surveillance strategies for serrated polyps based on experts' opinion and current literature[28,34,51,63]

Serrated polyp	Intervention	Surveillance interval
Hyperplastic polyp in the rectosigmoid	No surveillance recommended	Screening colonoscopy at 10 yr
SSA without dysplasia	Endoscopic resection (EMR)	< 3 lesions, < 1 cm: 5 yr ⩾ 3 lesions, ⩾ 1 cm: 3 yr
SSA with dysplasia	Endoscopic resection (EMR)	Complete: 2-6 mo Incomplete: Segmental colectomy
TSA	Endoscopic resection (cold-snare)	Complete: 3 yr Incomplete: Segmental colectomy
Serrated polyposis	Endoscopic resection	Follow-up colonoscopy at 6-12 mo Children: screening at an age 10 yr younger than index case
Serrated polyposis in first-degree relative	Screening at an age 10 yr younger	Follow-up colonoscopy at 12 mo

SSA: Sessile serrated adenoma; TSA: Traditional serrated adenoma; EMR: Endoscopic mucosal resection.

all other serrated polyps should be endoscopically removed. If endoscopic resection cannot be implemented, then segmental colectomy is advised[51]. There is no consensus among experts on the optimal post-polypectomy surveillance intervals, however because of lack of data and presumed faster progression rate a more intensive surveillance is recommended (Table 2).

Due to their sessile nature, SSAs are difficult to detect and remove endoscopically. For the removal of flat SSAs endoscopic mucosal resection (EMR) is the method of choice[34]. It is recommended to use a chromoendoscopy contrast dye (either onto the surface or injected submucosally) to define the border of the lesion, lifting it, then snare removing *in toto* or in multiple sessions[28,34]. It is important to note that because of the thin wall of proximal colon (where SSAs typically locate[65]), this difficult technique is even more challenging, as one has take the complications of EMR (bleeding, perforation, incomplete resection) into account and it is advised to use argon plasma coagulation to reduce the risk of complications and recurrence[34,66].

CONCLUSION

It is getting generally accepted that CRC is a heterogeneous disease. Serrated pathway is serrated pathway is a rapidly evolving concept in colorectal carcinogenesis and it is postulated that 10%-20% of CRCs arise *via* this alternative pathway. In the past two decades since its original description our knowledge of morphologic and molecular alterations of serrated lesions has greatly expanded and these lesions are getting increasingly recognized. A major challenge is how to translate these new findings into clinical practice and how to determine appropriate surveillance intervals in order to avoid interval cancers. Further investigation is needed to better characterize natural history, optimize management and improve clinical outcomes.

ACKNOWLEDGMENTS

We are deeply indebted to Orsolya Bakonyi for her assistance in the graphic design and Zsuzsa Kovács for her help in the preparation of the schematic figure.

REFERENCES

1 **Vogelstein B**, Fearon ER, Hamilton SR, Kern SE, Preisinger AC, Leppert M, Nakamura Y, White R, Smits AM, Bos JL. Genetic alterations during colorectal-tumor development. *N Engl J Med* 1988; **319**: 525-532 [PMID: 2841597 DOI: 10.1056/NEJM198809013190901]

2 **Goldman H**, Ming S, Hickock DF. Nature and significance of hyperplastic polyps of the human colon. *Arch Pathol* 1970; **89**: 349-354 [PMID: 5435674]

3 **Jass JR**. Relation between metaplastic polyp and carcinoma of the colorectum. *Lancet* 1983; **1**: 28-30 [PMID: 6129371 DOI: 10.1016/S0140-6736(83)91564-7]

4 **Young J**, Jenkins M, Parry S, Young B, Nancarrow D, English D, Giles G, Jass J. Serrated pathway colorectal cancer in the population: genetic consideration. *Gut* 2007; **56**: 1453-1459 [PMID: 17566021 DOI: 10.1136/gut.2007.126870]

5 **Snover DC**. Update on the serrated pathway to colorectal carcinoma. *Hum Pathol* 2011; **42**: 1-10 [PMID: 20869746 DOI: 10.1016/j.humpath.2010.06.002]

6 **Tateyama H**, Li W, Takahashi E, Miura Y, Sugiura H, Eimoto T. Apoptosis index and apoptosis-related antigen expression in serrated adenoma of the colorectum: the saw-toothed structure may be related to inhibition of apoptosis. *Am J Surg Pathol* 2002; **26**: 249-256 [PMID: 11812948 DOI: 10.1097/00000478-200202000-00013]

7 **Higuchi T**, Jass JR. My approach to serrated polyps of the colorectum. *J Clin Pathol* 2004; **57**: 682-686 [PMID: 15220357 DOI: 10.1136/jcp.2003.015230]

8 **Carragher LA**, Snell KR, Giblett SM, Aldridge VS, Patel B, Cook SJ, Winton DJ, Marais R, Pritchard CA. V600EBraf induces gastrointestinal crypt senescence and promotes tumour progression through enhanced CpG methylation of p16INK4a. *EMBO Mol Med* 2010; **2**: 458-471 [PMID: 20941790 DOI: 10.1002/emmm.201000099]

9 **O'Brien MJ**. Hyperplastic and serrated polyps of the colorectum. *Gastroenterol Clin North Am* 2007; **36**: 947-968, viii [PMID: 17996799 DOI: 10.1016/j.gtc.2007.08.007]

10 **Chan AO**, Broaddus RR, Houlihan PS, Issa JP, Hamilton SR, Rashid A. CpG island methylation in aberrant crypt foci of the colorectum. *Am J Pathol* 2002; **160**: 1823-1830 [PMID: 12000733 DOI: 10.1016/S0002-9440(10)61128-5]

11 **Suehiro Y**, Hinoda Y. Genetic and epigenetic changes in aberrant crypt foci and serrated polyps. *Cancer Sci* 2008; **99**: 1071-1076 [PMID: 18384435]

12 **Nucci MR**, Robinson CR, Longo P, Campbell P, Hamilton SR. Phenotypic and genotypic characteristics of aberrant crypt foci in human colorectal mucosa. *Hum Pathol* 1997; **28**: 1396-1407 [PMID: 9416697 DOI: 10.1016/S0046-8177(97)90230-6]

13 **Takayama T**, Ohi M, Hayashi T, Miyanishi K, Nobuoka A, Nakajima T, Satoh T, Takimoto R, Kato J, Sakamaki S, Niitsu Y. Analysis of K-ras, APC, and beta-catenin in aberrant crypt foci in sporadic adenoma, cancer, and familial adenomatous polyposis. *Gastroenterology* 2001; **121**: 599-611 [PMID: 11522744 DOI: 10.1053/gast.2001.27203]

14 **Stevens RG**, Swede H, Rosenberg DW. Epidemiology of colonic aberrant crypt foci: review and analysis of existing studies. *Cancer Lett* 2007; **252**: 171-183 [PMID: 17182176 DOI: 10.1016/j.canlet.2006.11.009]

15 **Rosenberg DW**, Yang S, Pleau DC, Greenspan EJ, Stevens RG, Rajan TV, Heinen CD, Levine J, Zhou Y, O'Brien MJ. Mutations in BRAF and KRAS differentially distinguish serrated versus non-serrated hyperplastic aberrant crypt foci in humans. *Cancer Res* 2007; **67**: 3551-3554 [PMID: 17440063 DOI: 10.1158/0008-5472.CAN-07-0343]

16 **Michaloglou C**, Vredeveld LC, Soengas MS, Denoyelle C, Kuilman T, van der Horst CM, Majoor DM, Shay JW, Mooi WJ, Peeper DS. BRAFE600-associated senescence-like cell cycle arrest of human naevi. *Nature* 2005; **436**: 720-724 [PMID: 16079850 DOI: 10.1038/nature03890]

17 **Wajapeyee N**, Serra RW, Zhu X, Mahalingam M, Green MR. Role for IGFBP7 in senescence induction by BRAF. *Cell* 2010; **141**: 746-747 [PMID: 20510919 DOI: 10.1016/j.cell.2010.05.014]

18 **Aust DE**, Baretton GB. Serrated polyps of the colon and rectum (hyperplastic polyps, sessile serrated adenomas, traditional serrated adenomas, and mixed polyps)-proposal for diagnostic criteria. *Virchows Arch* 2010; **457**: 291-297 [PMID: 20617338 DOI: 10.1007/s00428-010-0945-1]

19 **Torlakovic E**, Skovlund E, Snover DC, Torlakovic G, Nesland JM. Morphologic reappraisal of serrated colorectal polyps. *Am J Surg Pathol* 2003; **27**: 65-81 [PMID: 12502929 DOI: 10.1097/00000478-200301000-00008]

20 **Morimoto LM**, Newcomb PA, Ulrich CM, Bostick RM, Lais CJ, Potter JD. Risk factors for hyperplastic and adenomatous polyps: evidence for malignant potential? *Cancer Epidemiol Biomarkers Prev* 2002; **11**: 1012-1018 [PMID: 12376501]

21 **Kearney J**, Giovannucci E, Rimm EB, Stampfer MJ, Colditz GA, Ascherio A, Bleday R, Willett WC. Diet, alcohol, and smoking and the occurrence of hyperplastic polyps of the colon and rectum (United States). *Cancer Causes Control* 1995; **6**: 45-56 [PMID: 7718735 DOI: 10.1007/BF00051680]

22 **Samowitz WS**, Albertsen H, Sweeney C, Herrick J, Caan BJ, Anderson KE, Wolff RK, Slattery ML. Association of smoking, CpG island methylator phenotype, and V600E BRAF mutations in colon cancer. *J Natl Cancer Inst* 2006; **98**: 1731-1738 [PMID: 17148775 DOI: 10.1093/jnci/djj468]

23 **Limsui D**, Vierkant RA, Tillmans LS, Wang AH, Weisenberger DJ, Laird PW, Lynch CF, Anderson KE, French AJ, Haile RW, Harnack LJ, Potter JD, Slager SL, Smyrk TC, Thibodeau SN, Cerhan JR, Limburg PJ. Cigarette smoking and colorectal cancer risk by molecularly defined subtypes. *J Natl Cancer Inst* 2010; **102**: 1012-1022 [PMID: 20587792 DOI: 10.1093/jnci/djq201]

24 **O'Brien MJ**, Yang S, Mack C, Xu H, Huang CS, Mulcahy E, Amorosino M, Farraye FA. Comparison of microsatellite instability, CpG island methylation phenotype, BRAF and KRAS status in serrated polyps and traditional adenomas indicates separate pathways to distinct colorectal carcinoma end points. *Am J Surg Pathol* 2006; **30**: 1491-1501 [PMID: 17122504 DOI: 10.1097/01.pas.0000213313.36306.85]

25 **O'Brien MJ**, Yang S, Clebanoff JL, Mulcahy E, Farraye FA, Amorosino M, Swan N. Hyperplastic (serrated) polyps of the colorectum: relationship of CpG island methylator phenotype and K-ras mutation to location and histologic subtype. *Am J Surg Pathol* 2004; **28**: 423-434 [PMID: 15087661]

26 **Yang S**, Farraye FA, Mack C, Posnik O, O'Brien MJ. BRAF and KRAS Mutations in hyperplastic polyps and serrated adenomas of the colorectum: relationship to histology and CpG island methylation status. *Am J Surg Pathol* 2004; **28**: 1452-1459 [PMID: 15489648 DOI: 10.1097/01.pas.0000141404.56839.6a]

27 **Spring KJ**, Zhao ZZ, Karamatic R, Walsh MD, Whitehall VL, Pike T, Simms LA, Young J, James M, Montgomery GW, Appleyard M, Hewett D, Togashi K, Jass JR, Leggett BA. High prevalence of sessile serrated adenomas with BRAF mutations: a prospective study of patients undergoing colonoscopy. *Gastroenterology* 2006; **131**: 1400-1407 [PMID: 17101316 DOI: 10.1053/j.gastro.2006.08.038]

28 **Huang CS**, Farraye FA, Yang S, O'Brien MJ. The clinical significance of serrated polyps. *Am J Gastroenterol* 2011; **106**: 229-240; quiz 241 [PMID: 21045813 DOI: 10.1038/ajg.2010.429]

29 **Arnold CA**, Montgomery E, Iacobuzio-Donahue M. The serrated pathway of neoplasia: new insights into an evolving concept. *Diagn Histopathol* 2011; **17**: 367-375 [DOI: 10.1016/j.mpdhp.2011.06.006]

30 **Longacre TA**, Fenoglio-Preiser CM. Mixed hyperplastic adenomatous polyps/serrated adenomas. A distinct form of colorectal neoplasia. *Am J Surg Pathol* 1990; **14**: 524-537 [PMID: 2186644 DOI: 10.1097/00000478-199006000-00003]

31 **Goldstein NS**, Bhanot P, Odish E, Hunter S. Hyperplastic-like colon polyps that preceded microsatellite-unstable adenocarcinomas. *Am J Clin Pathol* 2003; **119**: 778-796 [PMID: 12817424 DOI: 10.1309/DRFQ0WFUF1G13CTK]

32 **Higuchi T**, Sugihara K, Jass JR. Demographic and pathological characteristics of serrated polyps of colorectum. *Histopathology* 2005; **47**: 32-40 [PMID: 15982321 DOI: 10.1111/j.1365-2559.2005.02180.x]

33 **Tannapfel A**, Neid M, Aust D, Baretton G. The origins of colorectal carcinoma: specific nomenclature for different pathways and precursor lesions. *Dtsch Arztebl Int* 2010; **107**: 760-766 [PMID: 21085545]

34 **East JE**, Saunders BP, Jass JR. Sporadic and syndromic hyperplastic polyps and serrated adenomas of the colon: classification, molecular genetics, natural history, and clinical management. *Gastroenterol Clin North Am* 2008; **37**: 25-46, v [PMID: 18313538 DOI: 10.1016/j.gtc.2007.12.014]

35 **Snover D**, Ahnen D, Burt R, Odze RD. Serrated Polyps of the Colon and Rectum and Serrated Polyposis. WHO Classification of Tumours of the Digestive System. 4th ed. Lyon: IARC, 2010

36 **Fidalgo CA**, Santos L, Rosa I, Fonseca R, Lage P, Claro I, Chaves P, Dias Pereira A. Hyperplastic polyp? The impact of the new classification for serrated polyps. *Gut* 2011; **60** Suppl 3: A306

37 **Torlakovic E**, Snover DC. Serrated adenomatous polyposis in humans. *Gastroenterology* 1996; **110**: 748-755 [PMID: 8608884 DOI: 10.1053/gast.1996.v110.pm8608884]

38 **Mäkinen MJ**. Colorectal serrated adenocarcinoma. *Histopathology* 2007; **50**: 131-150 [PMID: 17204027 DOI: 10.1111/j.1365-2559.2006.02548.x]

39 **Kimura T**, Yamamoto E, Yamano HO, Suzuki H, Kamimae S, Nojima M, Sawada T, Ashida M, Yoshikawa K, Takagi R, Kato R, Harada T, Suzuki R, Maruyama R, Kai M, Imai K, Shinomura Y, Sugai T, Toyota M. A novel pit pattern identifies the precursor of colorectal cancer derived from sessile serrated adenoma. *Am J Gastroenterol* 2012; **107**: 460-469 [PMID: 22233696 DOI: 10.1038/ajg.2011.457]

40 **Sheridan TB**, Fenton H, Lewin MR, Burkart AL, Iacobuzio-Donahue CA, Frankel WL, Montgomery E. Sessile serrated adenomas with low- and high-grade dysplasia and early carcinomas: an immunohistochemical study of serrated lesions "caught in the act". *Am J Clin Pathol* 2006; **126**: 564-571 [PMID: 16938659 DOI: 10.1309/C7JE8BVL8420V5VT]

41 **Oono Y**, Fu K, Nakamura H, Iriguchi Y, Yamamura A, Tomino Y, Oda J, Mizutani M, Takayanagi S, Kishi D, Shinohara T, Yamada K, Matumoto J, Imamura K. Progression of a sessile serrated adenoma to an early invasive cancer within 8 months. *Dig Dis Sci* 2009; **54**: 906-909 [PMID: 18688718 DOI: 10.1007/s10620-008-0407-7]

42 **Torlakovic EE**, Gomez JD, Driman DK, Parfitt JR, Wang C, Benerjee T, Snover DC. Sessile serrated adenoma (SSA) vs. traditional serrated adenoma (TSA). *Am J Surg Pathol* 2008; **32**: 21-29 [PMID: 18162766 DOI: 10.1097/PAS.0b013e318157f002]

43 **Yantiss RK**, Oh KY, Chen YT, Redston M, Odze RD. Filiform serrated adenomas: a clinicopathologic and immunophenotypic study of 18 cases. *Am J Surg Pathol* 2007; **31**: 1238-1245 [PMID: 17667549 DOI: 10.1097/PAS.0b013e31802d74c0]

44 **Ha SY**, Lee SM, Lee EJ, Kang SY, Jang KT, Park CK, Kim JY, Kim YH, Chang DK, Kim KM. Filiform serrated adenoma is an unusual, less aggressive variant of traditional serrated adenoma. *Pathology* 2012; **44**: 18-23 [PMID: 22157687 DOI: 10.1097/PAT.0b013e32834d7bbf]

45 **Jass JR**, Whitehall VL, Young J, Leggett BA. Emerging concepts in colorectal neoplasia. *Gastroenterology* 2002; **123**: 862-876 [PMID: 12198712 DOI: 10.1053/gast.2002.35392]

46 **Jass JR**, Smith M. Sialic acid and epithelial differentiation in colorectal polyps and cancer--a morphological, mucin and lectin histochemical study. *Pathology* 1992; **24**: 233-242 [PMID: 1289763 DOI: 10.3109/00313029209068874]

47 **Mäkinen MJ**, George SM, Jernvall P, Mäkelä J, Vihko P, Karttunen TJ. Colorectal carcinoma associated with serrated adenoma--prevalence, histological features, and prognosis. *J Pathol* 2001; **193**: 286-294 [PMID: 11241406 DOI: 10.1002/1096-9896(2000)9999:]

48 **Tuppurainen K**, Mäkinen JM, Junttila O, Liakka A, Kyllönen AP, Tuominen H, Karttunen TJ, Mäkinen MJ. Morphology and microsatellite instability in sporadic serrated and non-serrated colorectal cancer. *J Pathol* 2005; **207**: 285-294 [PMID: 16177963 DOI: 10.1002/path.1850]

49 **Laiho P**, Kokko A, Vanharanta S, Salovaara R, Sammalkorpi H, Järvinen H, Mecklin JP, Karttunen TJ, Tuppurainen K, Davalos V, Schwartz S, Arango D, Mäkinen MJ, Aaltonen LA. Serrated carcinomas form a subclass of colorectal cancer with distinct molecular basis. *Oncogene* 2007; **26**: 312-320 [PMID: 16819509 DOI: 10.1038/sj.onc.1209778]

50 **García-Solano J**, Pérez-Guillermo M, Conesa-Zamora P, Acosta-Ortega J, Trujillo-Santos J, Cerezuela-Fuentes P, Mäkinen MJ. Clinicopathologic study of 85 colorectal serrated adenocarcinomas: further insights into the full recognition of a new subset of colorectal carcinoma. *Hum Pathol* 2010; **41**: 1359-1368 [PMID: 20594582 DOI: 10.1016/j.humpath.2010.04.002]

51 **Leonard DF**, Dozois EJ, Smyrk TC, Suwanthanma W, Baron TH, Cima RR, Larson DW. Endoscopic and surgical management of serrated colonic polyps. *Br J Surg* 2011; **98**: 1685-1694 [PMID: 22034178 DOI: 10.1002/bjs.7654]

52 **Patai AV**, Molnár B, Kalmár A, Schöller A, Tóth K, Tulassay Z. Role of DNA methylation in colorectal carcinogenesis. *Dig Dis* 2012; **30**: 310-315 [PMID: 22722557 DOI: 10.1159/000337004.]

53 **Patai AV**, Galamb O, Kalmár A, Wichmann B, Valcz G, Patai A, Leiszter K, Tóth K, Tulassay Z, Molnár B. Non-sequential DNA methylation alterations in the adenoma-dysplasia-carcinoma sequence in the distal colon. *Gut* 2011; **60** Suppl 3: A76

54 **Samowitz WS**, Sweeney C, Herrick J, Albertsen H, Levin TR, Murtaugh MA, Wolff RK, Slattery ML. Poor survival associated with the BRAF V600E mutation in microsatellite-stable colon cancers. *Cancer Res* 2005; **65**: 6063-6069 [PMID: 16024606 DOI: 10.1158/0008-5472.CAN-05-0404]

55 **Pai RK**, Jayachandran P, Koong AC, Chang DT, Kwok S, Ma L, Arber DA, Balise RR, Tubbs RR, Shadrach B, Pai RK. BRAF-mutated, microsatellite-stable adenocarcinoma of the proximal colon: an aggressive adenocarcinoma with poor survival, mucinous differentiation, and adverse morphologic features. *Am J Surg Pathol* 2012; **36**: 744-752 [PMID: 22314188 DOI: 10.1097/PAS.0b013e31824430d7]

56 **Samowitz WS**, Curtin K, Ma KN, Schaffer D, Coleman LW, Leppert M, Slattery ML. Microsatellite instability in sporadic colon cancer is associated with an improved prognosis at the population level. *Cancer Epidemiol Biomarkers Prev* 2001; **10**: 917-923 [PMID: 11535541]

57 **Lockett MJ**, Atkin WS. Hyperplastic polyposis: prevalence and cancer risk. *Gut* 2001; **48** Suppl 1: A4

58 **Roberts A**, Nancarrow D, Clendenning M, Buchanan DD, Jenkins MA, Duggan D, Taverna D, McKeone D, Walters R, Walsh MD, Young BW, Jass JR, Rosty C, Gattas M, Pelzer E, Hopper JL, Goldblatt J, George J, Suthers GK, Phillips K, Parry S, Woodall S, Arnold J, Tucker K, Muir A, Drini M, Macrae F, Newcomb P, Potter JD, Pavluk E, Lindblom A, Young JP. Linkage to chromosome 2q32.2-q33.3 in familial serrated neoplasia (Jass syndrome). *Fam Cancer* 2011; **10**: 245-254 [PMID: 21165777]

59 **Kalady MF**, Jarrar A, Leach B, LaGuardia L, O'Malley M, Eng C, Church JM. Defining phenotypes and cancer risk in hyperplastic polyposis syndrome. *Dis Colon Rectum* 2011; **54**: 164-170 [PMID: 21228663 DOI: 10.1007/DCR.0b013e3181fd4c15]

60 **Jarrar AM**, Church JM, Fay S, Kalady MF. Is the phenotype

mixed or mistaken? Hereditary nonpolyposis colorectal cancer and hyperplastic polyposis syndrome. *Dis Colon Rectum* 2009; **52**: 1949-1955 [PMID: 19934914 DOI: 10.1007/DCR.0b013e3181b5450c]

61 **Teriaky A**, Driman DK, Chande N. Outcomes of a 5-year follow-up of patients with sessile serrated adenomas. *Scand J Gastroenterol* 2012; **47**: 178-183 [PMID: 22229626 DOI: 10.3109/00365521.2011.645499]

62 **Rondagh EJ**, Masclee AA, Bouwens MW, Winkens B, Riedl RG, de Bruïne AP, de Ridder R, Kaltenbach T, Soetikno RM, Sanduleanu S. Endoscopic red flags for the detection of high-risk serrated polyps: an observational study. *Endoscopy* 2011; **43**: 1052-1058 [PMID: 21971921 DOI: 10.1055/s-0030-1256770]

63 **Kashida H**, Ikehara N, Hamatani S, Kudo SE, Kudo M. Endoscopic characteristics of colorectal serrated lesions. *Hepatogastroenterology* 2011; **58**: 1163-1167 [PMID: 21937375 DOI: 10.5754/hge10093]

64 **Terdiman JP**, McQuaid KR. Surveillance guidelines should be updated to recognize the importance of serrated polyps. *Gastroenterology* 2010; **139**: 1444-1447 [PMID: 20875785 DOI: 10.1053/j.gastro.2010.09.024]

65 **Gurudu SR**, Heigh RI, De Petris G, Heigh EG, Leighton JA, Pasha SF, Malagon IB, Das A. Sessile serrated adenomas: demographic, endoscopic and pathological characteristics. *World J Gastroenterol* 2010; **16**: 3402-3405 [PMID: 20632442 DOI: 10.3748/wjg.v16.i27.3402]

66 **Brooker JC**, Saunders BP, Shah SG, Thapar CJ, Suzuki N, Williams CB. Treatment with argon plasma coagulation reduces recurrence after piecemeal resection of large sessile colonic polyps: a randomized trial and recommendations. *Gastrointest Endosc* 2002; **55**: 371-375 [PMID: 11868011 DOI: 10.1067/mge.2002.121597]

P- Reviewers Hiyama T, Ho YH **S- Editor** Gou SX
L- Editor A **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i5.616

World J Gastroenterol 2013 February 7; 19(5): 616-630
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

REVIEW

Controversial role of toll-like receptors in acute pancreatitis

Juan Vaz, Hamid Akbarshahi, Roland Andersson

Juan Vaz, Hamid Akbarshahi, Roland Andersson, Department of Surgery, Clinical Sciences Lund, Skåne University Hospital Lund, Lund University, SE-221 85 Lund, Sweden
Author contributions: Vaz J and Akbarshahi H collected the underlying material, wrote the initial draft of the manuscript; Andersson R, Vaz J and Akbarshahi H designed the outline of the paper; Andersson R finalized and revised the manuscript; all authors have read and approved the final version of the manuscript.
Correspondence to: Roland Andersson, MD, PhD, Department of Surgery, Clinical Sciences Lund, Skåne University Hospital, Lund University, SE-221 85 Lund, Sweden. roland.andersson@med.lu.se
Telephone: +46-222-46172359 Fax: +46-222-46172335
Received: November 1, 2012 Revised: December 19, 2012
Accepted: January 11, 2013
Published online: February 7, 2013

Abstract

Acute pancreatitis (AP) is a common clinical condition with an incidence of about 300 or more patients per million annually. About 10%-15% of patients will develop severe acute pancreatitis (SAP) and of those, 10%-30% may die due to SAP-associated complications. Despite the improvements done in the diagnosis and management of AP, the mortality rate has not significantly declined during the last decades. Toll-like receptors (TLRs) are pattern-recognition receptors that seem to play a major role in the development of numerous diseases, which make these molecules attractive as potential therapeutic targets. TLRs are involved in the development of the systemic inflammatory response syndrome, a potentially lethal complication in SAP. In the present review, we explore the current knowledge about the role of different TLRs that have been described associated with AP. The main candidate for targeting seems to be TLR4, which recognizes numerous damage-associated molecular patterns related to AP. TLR2 has also been linked with AP, but there are only limited studies that exclusively studied its role in AP. There is also data suggesting that TLR9 may play a role in AP.

© 2013 Baishideng. All rights reserved.

Key words: Acute pancreatitis; Severe acute pancreatitis; Pathophysiological mechanism; Toll-like receptors; Intervention

Vaz J, Akbarshahi H, Andersson R. Controversial role of toll-like receptors in acute pancreatitis. *World J Gastroenterol* 2013; 19(5): 616-630 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i5/616.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i5.616

INTRODUCTION

A central characteristic of the innate immune system is its capability to identify constitutive and conserved products of microbial metabolism. Several metabolic pathways are unique for invading microorganisms and absent in host cells. Being essential for survival, they are even highly conserved among a given class of microorganisms. A classical example is lipopolysaccharide (LPS), a molecule made by *Gram-negative bacteria*, but not by eukaryotic cells.

Molecules found in microorganisms, but not in host cells, can serve as molecular signatures that can be recognised by the innate immune system, starting an immunological response against the invasion and if necessary, assisting in the activation of the adaptive immune system[1]. As these molecules are conserved molecular patterns, they are called pathogen-associated molecular patterns (PAMPs) and interact with pattern-recognition receptors (PRRs), which are receptors of the innate immune system.

PAMPs are marvellous targets for innate immune recognition, as they are only produced by microbes, are invariant between microorganisms of a given class and are essential for microbial survival. Therefore, PRRs represents a vital component of the immune system, which probably developed early during evolution, as PRRs are found in all mammals, invertebrates and even in plants. PRRs are expressed on the cell surface or in intracellular

compartments, but they can be secreted into the blood stream and tissue fluids as well[2]. However, the role of PRRs in immune recognition is not solely associated to PAMPs, since PRRs in addition can recognize alarmins[3]. Alarmins are endogenous molecules released into the extracellular compartment by activated or necrotic cells in response to stress or tissue damage[4]. Even extracellular matrix molecules are alarmins when up-regulated upon injury or degraded following tissue damage[5]. Alarmins and PAMPs constitute damage-associated molecular patterns (DAMPs), which are the main targets of PRRs[3,4].

The relative recent discovery of a main PRR family, toll-like receptors (TLRs), has vastly increased our comprehension of the physiological and pathophysiological role of the innate immune system. While mice have twelve TLRs (TLR1 to TLR9 and TLR11 to TLR13), humans express only ten functional TLRs (TLR1 to TLR10) and ligands have been identified for all human TLRs, except for TLR10[3]. A selection of PAMPs and alarmins recognized by human TLRs are shown in Tables 1 and 2.

TLRs are type I transmembrane glycoproteins composed of an extracellular N-terminal that contains leucine-rich repeats, a single transmembrane domain and an intracellular C-terminal tail known as the Toll/IL-1 receptor (TIR)[6]. When TLRs form heterodimers or homodimers, an activating signal is started and TIR-TIR dimers are involved in the recruitment of five known signalling adaptor molecules: Myeloid differentiation primary response protein 88 (MyD88), TIR domain-containing adaptor protein (TIRAP), TIRAP inducing interferon β (TRIF), TRIF-related adaptor molecule (TRAM) and sterile-alpha and Armadillo containing motif protein[7-9].

Once the adaptor molecules are recruited, TLRs can activate two major intracellular signalling pathways. All TLRs except TLR3 can activate a MyD88-dependent pathway, in which IL-1R-associated kinases (IRAK), TNF receptor-associated factor 6 (TRAF-6) and mitogen-activated kinases are involved[5]. This pathway results in the transcription of pro-inflammatory genes through the activation of nuclear factor κβ (NFκB) and/or the activation of activating protein 1[9]. An alternative, non MyD88-dependent pathway can be activated by TLR3 and TLR4. In the TRIF pathway, the activation of interferon-regulated factors (IRF) *via* TRIF leads to the synthesis of interferon (IFN)[5].

As TLRs recognize several PAMPs and DAMPs, their involvement in the pathophysiology of several diseases has become a major research field[8,10]. Moreover, recent studies have reported the importance of NFκB pathways in the development of the systemic inflammatory response syndrome (SIRS) and the multiple organ dysfunction syndrome (MODS)[11,12]. However, NFκB pathways are not restricted to TLRs, which converts the elucidation of the role of TLRs in SIRS and MODS into a tremendous challenging task.

SIRS is a nonspecific condition that can be caused by infection, ischemia, trauma, and inflammation, or by the combination of several insults. Considered by many as a self-defense mechanism, SIRS results in a complex inflammatory cascade that involve humoral and cellular responses, complement, and cytokines cascades. Severe complications depend on the underlying etiology and the magnitude of the inflammatory response, and may include *e.g.*, single or multiple organ failure[13].

Table 1 Human Toll-like receptors: Localization, known pathogen-associated molecular patterns and their producing microorganisms

	Localization	PAMP	Origin of PAMP
TLR1	Plasma membrane	Soluble factors	*N. meningitidis*
		Triacyl lipopeptides	*Bacteria, mycobacteria*
TLR2	Plasma membrane	Glycoinositolphospholipids	*Trypanosoma cruzi*
		Glycolipids	*Treponema maltophilum*
		Haemagglutinin	*Virus*
		Lipoarabinomannan	*Mycobacteria*
		Lipoprotein/lipopeptides	*Various pathogens*
		Lipoteichoic acid	*Gram-positive bacteria*
		Peptidoglycan	*Gram-positive bacteria*
		Phenol-soluble modulin	*S. epidermidis*
		Porins	*Neisseria*
		Zymosan	*Fungi*
TLR3	Endosome	Double-stranded RNA	*Virus*
TLR4	Plasma membrane	Envelope protein	*Mouse-mammary tumour virus*
		Fusion protein	*Respiratory syncytial virus*
		Heat-shock protein 60	*Chlamydia pneumoniae*
		Lipopolysaccharide	*Gram-negative bacteria*
		Taxol	*Plants*
TLR5	Plasma membrane	Flagellin	*Bacteria*
TLR6	Plasma membrane	Diacyl lipopeptides	*Mycoplasma*
		Lipoteichoic acid	*Gram-positive bacteria*
		Zymosan	*Fungi*
TLR7	Endosome	Single-stranded RNA	*Virus*
TLR8	Endosome	Single-stranded RNA	*Virus*
TLR9	Endosome	DNA (CpG)	*Bacteria, virus*
		Haemozoin	*Plasmodium spp.*
			Rhodnius spp.
			Schistosoma spp.
TLR10	Endosome	Not determined	Not determined

Modified after Akira *et al*[7]. TLR: Toll-like receptor; PAMP: Pathogen-associated molecular patterns.

Acute pancreatitis (AP) is one common cause of SIRS and MODS. AP occurs with an incidence of about 300 or more patients per million annually[14,15], reported to have increased during the last decades[16,17]. Since the principal risk factors for AP are gallstones and excessive alcohol intake[18,19], plausible the augmented incidence reflecting the overweight epidemic observed in the Western World[20-22].

Most cases of AP are mild and self-limiting, with only brief need of clinical support[14,15]. However, about 10%-15% of patients will develop severe acute pancreatitis (SAP) and of those, 10%-30% may die due to SAP-associated complications (Table 3)[23]. Complications may be local or extrapancreatic and in up to one third, the pancreatic injury leads to pancreatic necrosis, acute fluid collections and pseudocyst formation. SIRS may develop early during the course of SAP and cause *e.g.*, adult respiratory dysfunction syndrome,

Table 2 Human toll-like receptors and a selection of known alarmins

	Proteins, peptides	Fatty acids, lipoproteins	Proteoglycans, glycosaminoglycans
TLR1	β-defensin-3		
TLR2	Antiphospholipid antibodies β-defensin-3	Serum amyloid A	Biglycan
	Eosinophil-derived neurotoxin		Hyaluronic acid fragments
	Heat-shock protein 60, 70, Gp96		Versiglycan
	High-mobility group protein B1 (HMGB1)		
	HMGB1-nucleosome complexes		
	Surfactant protein A, D		
TLR4	Antiphospholipid antibodies	Oxidised low-density protein	Biglycan
	β-defensin-2	Saturated fatty acids	Heparan sulphate fragment
	Fibrinogen	Serum amyloid A	Hyaluronic acid fragment
	Fibronectin (extra domain-A)		
	High-mobility group protein B1		
	Heat-shock protein 60, 70, 72, 22, Gp96		
	Lactoferrin MRP8, MRP14		
	Neutrophil elastase		
	Surfactant protein A, D		
	Tenascin-C (fibrinogen-like globe)		

Modified after Paccinini *et al*[5]. TLR: Toll-like receptor.

Table 3 Complications of severe acute pancreatitis

Pancreatic	Systemic
Abscess	Acute kidney failure
Fat necrosis	Acute liver failure
Hemorrhages	Adult respiratory distress syndrome
Infected necrosis	Disseminated intravascular coagulation
Pseudocyst formation	Encephalopathy
Sterile necrosis	Gut ischemia
	Hypocalcemia
	Paralytic ileus
	Shock

Modified after Baddeley *et al*[24].

acute renal and liver failure[24]. SIRS in association with SAP may also result in MODS, which carries a 40% mortality rate[15]. A model for the course of AP is shown (Figure 1).

Despite the improvements done in the diagnosis and management of SAP, the rate of mortality has only marginally declined during the last decades[24]. This is of particular concern, considering that AP patient numbers has been persistently increasing. In order to decrease the mortality rates, it is imperative to find new therapeutic strategies that target the underlying pathophysiological mechanism of SAP.

TLRs seem to play a major role in the development of numerous diseases[5,10], which make these molecules attractive as potential therapeutic targets. Since SIRS is a potentially lethal complication in AP, and TLRs are involved in the development of SIRS[25]; it is plausible that TLRs play a major role in severe acute pancreatitis.

In the present paper, we aim to explore the current knowledge about the role of different TLRs that have been reported associated with AP. In addition, future therapeutic strategies will be discussed.

TLR2-FAR AWAY FROM THE ANSWER

TLR2 is expressed on the plasma membrane of a large diversity of cells, including monocytes and macrophages, dendritic cells, polymorphonuclear leukocytes, B cells, T cells and microglia. This PRR recognises a wide range of DAMPs (Tables 1 and 2) and forms heterodimers with TLR1, TLR6 or TLR10[10]. Usually associated to the innate immune response against *Gram-positive bacteria* (several of its ligands origin in these microorganisms), TLR2 signals through a MyD88-dependent pathway (Figure 2A). CD14 is a protein found either in soluble form or anchored into the plasma membrane by a glycosylphosphatidylinositol tail. It has been reported that CD14 is required for the activation of TLR2-TLR6 dimers, since it facilitates the transport of specific ligands, such as peptidoglycan or lipoteichoic acid. Additionally, CD36, a membrane protein found in lipids rafts, seems to be important in the activation of TLR2-TLR6 pathways. However, it is unknown how CD36 enhances the formation of TL2-TLR6 dimers. CD44 is another membrane protein that has been associated to TLR2 and appears to enhance TLR2-mediated pro-inflammatory response[26]. However, the mechanisms are still unknown. There are also reports suggesting that CD44 may interact with TLR2, down-regulating TLR2-mediated inflammation[27,28].

Upon dimerization with TLR1, TLR6 or TLR10, TIR-TIR dimers recruit TIRAP, which is needed for the further recruitment of MyD88. MyD88 interacts with IRAK-4, which phosphorylates IRAK-1 and IRAK-2. IRAK-1 and IRAK-2 then activate TRAF-6, which in turn activates TGF-β activated kinase-1 (TAK-1). TAK-1 phosphorylates I-κ-β kinase β (IKKβ), which together with IKKα phosphorylate I-κ-β kinase α (IKKα). Upon phosphorylation IκBα becomes inactive, allowing the nuclear translocation of NFκB, with subsequent production of several cytokines and molecules involved in immune responses (Table 4)[29].

Besides its role in infectious diseases, TLR2 has also been reported involved in several non-infectious disorders, including atherosclerosis, asthma, renal disease, systemic lupus erythematosus and even sporadic colorectal cancer[30-34]. Though, the role of TLR2 in AP has only been evaluated in a limited number of studies.

Pancreatic damage

The TLR2mRNA expression was found to be increased

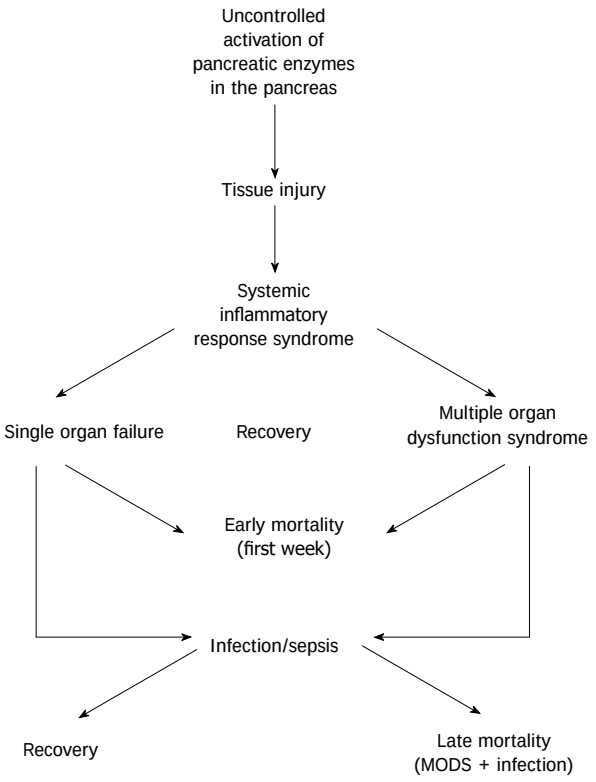


Figure 1 Systemic inflammatory response syndrome in acute pancreatitis.

in the pancreas in cerulein-induced AP in rats[35]. Furthermore, TLR2 levels in the gland were also increased, as well as TNF-α, intracellular adhesion molecule 1 and IL-6. TLR2mRNA overexpression seemed to be caused by peroxisome proliferator-activated receptor-α (PPAR-α), since WY14643, a synthetic PPAR-α antagonist, decreased TLR2 levels in treated animals. Even if the observed TLR2mRNA overexpression was associated with PPAR-α, it is uncertain how important TLR2 was in the development of inflammation and tissue damage. Possibly, hyaluronic acid fragments found in the injured pancreas could be a major source of TLR2 upregulation[36]. Small molecular weight hyaluronic acid fragments seem to need TLR2 in order to stimulate the pro-inflammatory state in mouse macrophages[37]. Heat shock proteins may leak into the extracellular compartment after necrotic cell death in AP, interact with CD14/TLR2 and induce the production of inflammatory cytokines (especially TNF-α)[38].

Lung injury

Acute lung injury (ALI) is possibly the most serious complication associated to SAP, since it accounts for most deaths in untreated patients and in hospitalised patients that die during the first week after the onset of AP[39].

In 2005, Wu *et al*[40] described that TLR2mRNA overexpression in the lungs in a sodium taurocholate-based AP rat model was coupled to elevated TNF-α levels and lower nitric oxide (NO) levels, when compared to controls. Moreover, L-Arg administration decreased TLR2mRNA expression and pulmonary TNF-α levels. Since L-Arg stimulates the production of NO, it is difficult to evaluate if the anti-inflammatory response was caused by NO itself or by a L-Arg-mediated TLR2 down-regulation. Chloroquine showed similar effects (both TLR2mRNA and TNF-α were decreased) but the lung injury was not significantly ameliorated. Chloroquine is a well-known anti-malaria drug that prevents against endosomal acidification[41] and is used to study the role of intracellular TLRs[42].

The authors concluded that SAP-associated ALI was coupled to higher TLR2 levels in the lungs and that reduced NO levels were in part responsible for the grade of inflammation, since NO has known anti-inflammatory properties. However, how NO levels are associated with TLR2 and its potential therapeutic use in AP, is still unknown.

Matsumura *et al*[43] reported that the expression of TLR2mRNA decreased in pulmonary macrophages in deoxycholate-induced AP in rats. Macrophages were obtained through bronchoalveolar lavage fluid (6 h after induction) and the cells were exposed to lipoteichoic acid. No change in TLR2mRNA was initially observed, but after six hours TLR2mRNA expression significantly decreased in macrophages. Additionally, the production of TNF-α after lipoteichoic acid stimulation was reduced. An important observation made was the increment of bacterial translocation (cultured from mesenteric lymph nodes) 18 h after AP-induction, indicating that impaired TLR2mRNA expression in pulmonary macrophages could partially be responsible for the development of ALI in SAP complicated by sepsis. It is widely accepted that $TLR2^{-/-}$ mice are vulnerable to infections[44,45]. Pulmonary macrophages have been pointed out as major players in ALI in SAP, as monocyte chemoattractant protein-1 and TNF-α are released by these cells[46]. Hence, diminished TLR2mRNA expression in pulmonary macrophages under AP is doubtfully important for the development of ALI when TLR2-related PAMPs are absent.

Liver involvement

Liver failure is a major complication in AP. Besides, there is increasing data suggesting that activated Kupffer cells mediates the inflammatory response and pulmonary damage seen in SAP[47].

Xiong *et al*[48] showed that hepatic TLR2mRNA expression was increased in AP-induced mice. The incremented TL2mRNA expression was observed 3 h after the induction, peaking at 12 h. Likewise, severe damage in the liver and SIRS ensued in the animals. However, in order to strengthen the immunological reaction, LPS was injected as well, leading to a TLR4mRNA expression increment in the liver. Thus, it is difficult to establish if SAP-related SIRS is associated with an increase in TLR2 or TLR4. Possibly, a combined increment of both TLRs account for the observed severity. Zhang *et al*[49] obtained similar results in a taurocholate-based rat model. Besides, decreased hepatic TLR2mRNA expression, chloroquine

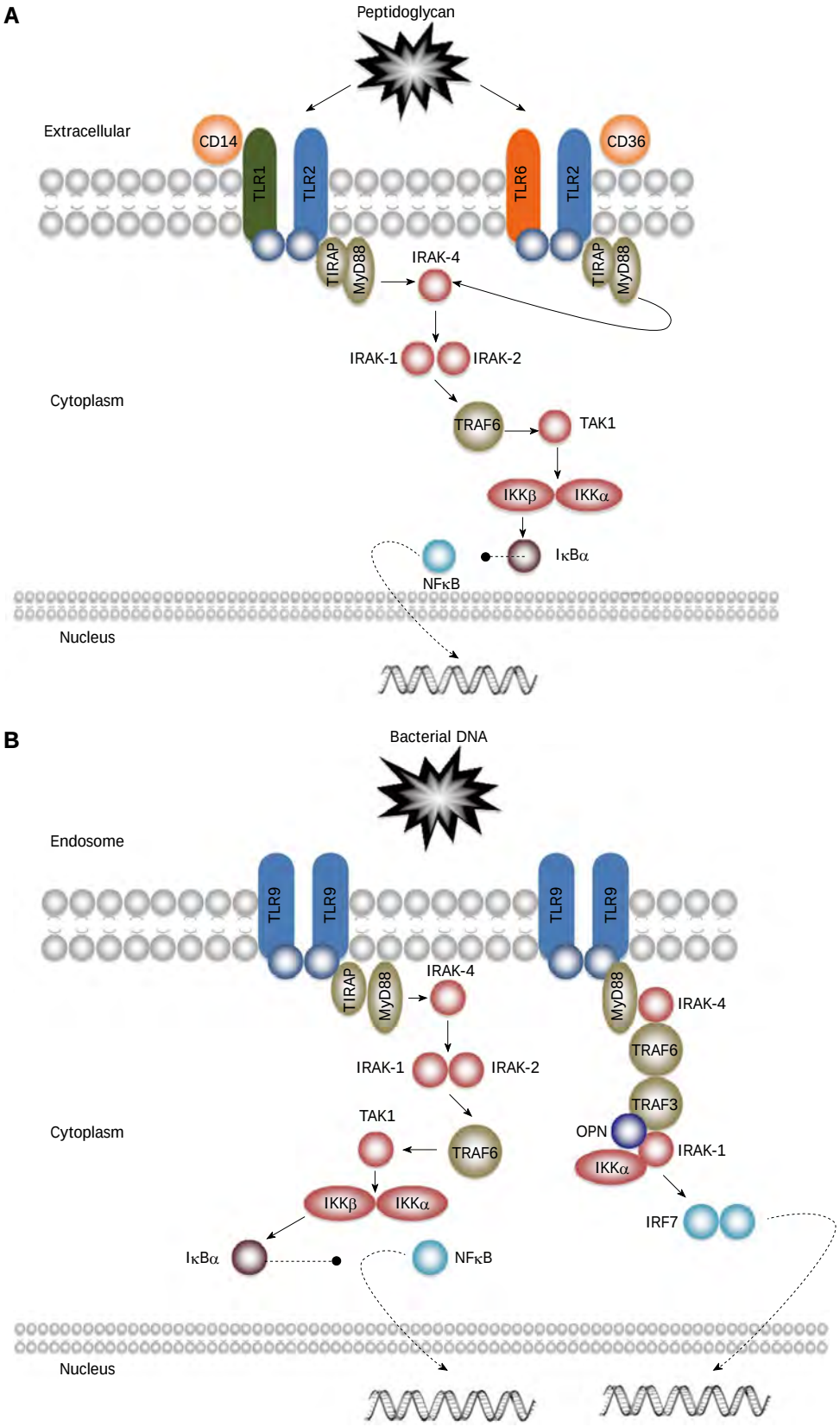


Figure 2 Toll-like receptor 2 and receptor 9 signalling pathways. A: Receptor 2; B: Receptor 9.

or L-Arg administration attenuated the liver injury and decreased TNF-α levels. Increased pro-inflammatory cytokines and chemokines are released following over-expression of TLR2mRNA in the liver of untreated animals. As previously shown in the lungs[40], NO levels also decreased in the liver. This is of special interest since reduced NO levels correlated with diminished secretion of anti-inflammatory mediators.

Table 4 A selection of genes regulated by nuclear factor κB

Acute phase proteins	C-reactive protein
	Complement factor Bf
	Complement factor C3
	TNFβ
Adhesion molecules	E-selectin
	Intracellular cell adhesion molecule 1
	Vascular cell adhesion molecule 1
Chemokines	IL8
	Monocyte chemoattractant protein-1
	Chemokine C-C motif ligand 5 (also known as RANTES)
Cytokines	IL2, 6, 12
	ILβ
	IL1β
	TNFβ
	TNFα
Growth factors	Granulocyte colony stimulating factor
	Granulocyte-macrophage colony stimulating factor
	Macrophage colony stimulating factor

Modified after Christman *et al*[11]. TLR: Toll-like receptor; TNF: Tumour necrosis factor; IL: Interleukin.

Even if several studies associate TLR2 to AP, it is still debated if TLR2 plays a pathophysiological role. Awla *et al*[50] reported that the progression of SAP was not significantly different in TLR2$^{-/-}$ mice when compared with wild-type mice. No significant differences were observed concerning pancreatic tissue damage, chemokine formation and neutrophil recruitment in taurocholate-induced AP.

TLR2 and AP in humans

In humans, microsatellite polymorphism in intron 2 in the human *TLR2* gene was associated with an increased risk for AP in Japan[51]. For this experiment, DNA was harvested from 202 patients of which 80 were diagnosed with SAP. When compared to healthy Japanese controls, AP patients showed significantly increased polymorphism rates in *TLR2* genes. Since the same polymorphism has been associated with susceptibility to colorectal cancer, tuberculosis, rheumatoid arthritis and sarcoidosis[34,52-54]; it is conceivable that TLR2 signalling is altered if intron 2 is changed. However, it remains to be elucidated if the relationship between changed *TLR2* genes and the risk for AP is just limited to Japanese subjects, or if the results can be extrapolated to other populations. Other studies have shown that gene-related vulnerability for AP in a country could not be demonstrated in other populations[55,56].

A very interesting observation was made by Szabo *et al*[57]. Monocytes were harvested from healthy volunteers before alcohol consumption and 24 h thereafter. The cells showed a decreased pro-inflammatory profile. Furthermore, *in vitro* acute alcohol stimulation of monocytes activated the pro-inflammatory state and decreased IL-production when TLR2 and TLR4 ligands were added. However, if only TLR2 ligands were added, no significant changes were observed. Therefore, acute alcohol stimulation appears to inhibit TLR2 expression in monocytes, worsening by this way the innate immune response against microorganisms that produce TLR2 specific ligands. Acute alcohol stimulation seems to be a double edged sword, since it could induce both anti and pro-inflammatory responses depending on which TLRs are involved.

In summary, TLR2 seems to be up regulated in the pancreas, lungs and liver in experimental AP animals. Moreover, TLR2-deficiency or inhibition (chloroquine or L-Arg) seems to ameliorate ALI in SAP.

How the increased TLR2 levels in vital organs are associated with SAP is still unknown. It is plausible that the overexpression of TLR2 in the pancreas, lungs and liver, amplifies the inflammatory response observed in SIRS when DAMPs released in SAP are recognised.

Despite an association found in Japan, the relationship between modified human *TLR2* genes and AP has not been reported in other populations. Even if TLR2 seems to be relevant for the development of ALI in SAP, further research has to be done before this important PRR can be considered as a potential therapeutic target in AP.

TLR4-THE MAIN CANDIDATE?

TLR4 was the first TLR identified and is widely expressed on the plasma membrane of various immune cells, including macrophages and dendritic cells[58]. TLR4 recognises several DAMPs, including LPS, fibrinogen, and various heat shock proteins (Tables 1 and 2). Upon activation, TLR4 forms homodimers or heterodimers with TLR6. The TLR4 recognition of many DAMPs demands several accessory molecules. As for TLR2, CD14 is needed for binding LPS to TLR4 dimers. Another vital protein for LPS recognition is myeloid differentiation protein-2[3].

In the same matter as other TLRs, except TLR3, TLR4 signals through a MyD88-dependent pathway, leading to the activation of NFκB (see previous section). Besides, TLR4 can signal *via* a TRIF-dependent pathway. Once activated, TIR-TIR dimers recruit TRIF and TRAM. TRIF then activates I-κ-β kinase epsilon (IKKε), which binds to TANK-binding kinase 1 (TBK1). Finally, IKKε-TBK1 phosphorylates and activates IRF3, culminating in the transcription of IFN-α and IFN-β (Figure 3)[26].

TLR4 was initially mainly studied because of its involvement in Gram-negative bacterial infection, but has in recent years also been associated with an increasing number of diseases. Many reports suggest its involvement in atherosclerosis, liver disease, obesity, cardiac disease, and renal disease, among others[59-63].

Bacteria are important microorganisms in AP. In acute haemorrhagic-necrotizing pancreatitis, intestinal bacterial translocation into the injured/necrotic pancreas or the systemic circulation, may lead to SIRS, ALI and MODS[16-19]. As TLR4 is related to LPS and a wide range of alarmins released during inflammation and tissue damage, its role in AP appears to be highly possible.

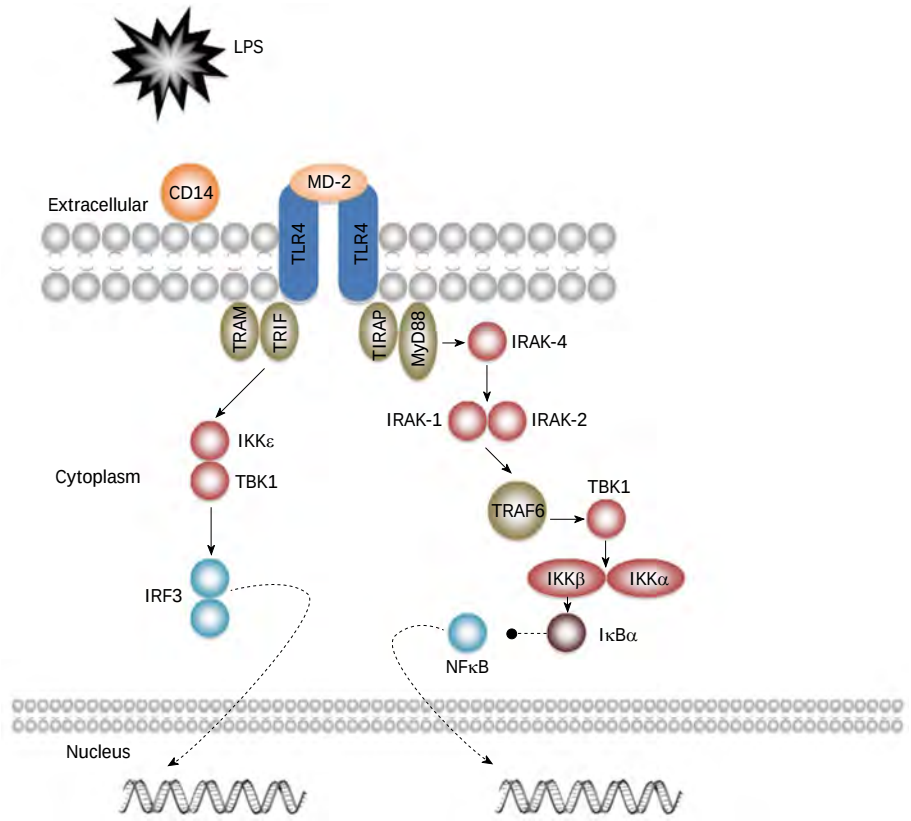


Figure 3 Toll-like receptor 4 signalling pathways.

Pancreatic damage

As for TLR2, overexpressed TLR4mRNA was also found in the pancreas in rats with cerulein-induced AP[35]. Li *et al*[64,65] reported (in the same animal model) that in both healthy and AP-induced animals, TLR4 was expressed mainly in the epithelium of the pancreatic duct and the pancreatic microcirculation. Even if some staining was observed in endocrine islets, no TLR4 protein was detected in the acinar cells. Likewise, TLR4 mRNA overexpression was observed, peaking after 1 h and returning to base levels after 4 h. In taurocholate-induced AP in TLR4$^{-/-}$ mice[50], serum amylase and myeloperoxidase levels in the pancreas decreased as compared to wild-type induced mice. Additionally, acinar cell necrosis, oedema, and haemorrhage significantly reduced. Interestingly, pancreas and serum levels of CXCL2, a chemokine, released by monocytes and macrophages, recruiting neutrophils, were strongly diminished.

Opposite results were presented by Ding *et al*[66] using the same AP model. No significant changes in serum amylase levels and pancreatic histological scores were observed between TLR4$^{-/-}$ and the controls. Curiously, TLR4$^{-/-}$ mice showed decreased levels of pro-apoptotic proteins in the pancreas 2 h after induction, but not after 4 h. TLR4 can increase the apoptotic rate in different cells[67]. As some studies suggest that apoptosis is favourable in AP[68,69], it is feasible that TLR4 is involved both in the beginning and in the resolution of AP.

Wang *et al*[70] showed that the administration of anti-CD14 antibody prior to cerulein-induction and LPS challenge in mice, reduced the severity of pancreatic injury, decreased pancreatic myeloperoxidase activity and down-regulated the secretion of pro-inflammatory cytokines.

TLR4 signalling pathways have been investigated in AP. Ding *et al*[71] showed in TLR4$^{-/-}$ mice that IRAK-4 could play a role in AP, independently of TLR4. In this experiment, lacking TLR4-mediated response did not result in increased IRAK-4 levels as was expected. This suggest that TLRs other than TLR4 may also play a role in AP, as the activation of TLR2 and TLR9 also results in decreased IRAK-4 in macrophages[72]. The hypothesis is supported by a report in which TRAF6 mediated inflammation in AP in TLR4$^{-/-}$ mice[73]. The author concluded that TLR4 may not be exclusively required for initiating AP, but its signal pathway may be of importance.

However, the association between alarmins released and TLR4-mediated inflammation in AP appears to be robust. In mononuclear inflammatory cells, the enzyme pancreatic elastase seems to activate NFκB, inducing TNF-α secretion[74,75]. Hietaranta *et al*[76] found that when human myeloid cells were exposed to porcine elastase, increased expression of NFκB and AP1 was achieved. The effects of elastase seem to be mediated by TLR4, since the blocking of TLR4 with a specific neutralizing antibody strongly prevented the expression of pro-inflammatory factors in elastase-exposed cells. Premature elastase activation has been reported in AP, implying its role in the disease[77]. Hence, TLR4-mediated inflammation may be important in AP. Blocking elastase might attenuate the severity of AP.

The degradation of matrix components appears to be connected with the pathophysiology of AP. Reports indicate that low molecular weight polysaccharides from degraded hyaluronan activates dendritic cells through TLR4[78]. Besides, dendritic cells might protect the pancreas against cell stress in AP[79]. Blycans and oligosaccharides of hyaluronan seem to signal *via* TLR4 and induce the production and secretion of great quantities of TNF-α and macrophage inflammatory protein-2 in macrophages[80]. Elastase and trypsin, both involved in the initiation of AP, cleaves heparin sulphate (HS) from cell surfaces and extra cellular matrix *in vitro*[81]. This, probably may in turn lead to free endogenous HS in damaged tissues in AP. Johnson *et al*[25] demonstrated that the rapid degradation of HS in mice causes a TLR4-mediated SIRS-like reaction in mice. When soluble HS was injected intraperitoneally, almost all wild type, but no TLR4-deficient mice, perish. This reaction was HS specific since molecules structurally similar to HS did not stimulate TLR4 and HS was not contaminated with LPS. Moreover, wild type, but not TLR4-deficient, mice had increased TNF-α serum levels 1 h after administration. Continuing in this line, Axelsson *et al*[82] confirmed that HS is involved in the initiation of AP in the rat.

Akbarshahi *et al*[83] later showed that the HS-induced TLR4-dependent immune response in the murine pancreas is IRF3-mediated. When TLR4$^{-/-}$ or MyD88$^{-/-}$ mice were challenged with HS or LPS, myeloperoxidase activity was annulled in the pancreas. The same pattern with HS was observed in IRF3$^{-/-}$ mice. However, LPS administration initiated a strong immune response in these animals. This outcome could explain preceding reports about discrepancies between TLR4-deficiency and decreased TRAF6 and IRAK-4 levels in AP[71,73]. Additionally, in this experiment, pre-treatment with the TLR4 antagonist eritoran inhibited the otherwise occurring increase in myeloperoxidase production in HS-treated wild type mouse pancreas. Eritoran is a synthetic Lipid A analogue that bind to TLR4/myeloid differentiation protein-2, thereby competing with Lipid A, resulting in inhibited LPS-mediated immune response[84].

Possibly, the inhibition of TLR4, elastase or HS, alone or in combination, could ameliorate pancreatic damage in AP, thus considerably reducing the risk for developing SAP.

Lung injury

As the role of TLR4 in AP has been investigated previously together with TLR2, similar results have been reported (see TLR2 section). Briefly, TLR4mRNA expression was increased in the lungs of AP-induced rats; leading to increased TNF-α levels combined with decreased NO levels. Chloroquine and L-Arg decreased TLR4mRNA expression and attenuated the lung injury[40]. Moreover, pulmonary macrophages decreased the TLR4mRNA expression, which caused decreased LPS-induced TNF-α levels and predisposition for intestinal bacterial translocation[41].

Pastor *et al*[85] concluded that TLR4 may not play a role in AP-associated ALI, but it may participate in pulmonary injury mediated by endotoxemia. In a cerulein-induced pancreatitis model in TLR4$^{-/-}$ mice there were no significant changes in serum amylase levels, pancreatic myeloperoxidase activity, and pancreatic oedema and acinar necrosis when compared to wild type mice. Additionally, cerulein-related pulmonary damage did not decrease in TLR4$^{-/-}$. Although, when cerulein was combined with LPS, a significant decrease in pulmonary damage was reported. Thus, TLR4 would only be of importance in AP-related ALI when the disease is worsened by sepsis.

Sharif *et al*[86] obtained pole opposed results. TLR4$^{-/-}$ mice showed decreased serum amylase activity and pancreatic damage (oedema, myeloperoxidase activity, necrosis). Importantly, myeloperoxidase activity in the lungs also decreased, indicating reduced neutrophil sequestration. Furthermore, when AP was induced in CD14$^{-/-}$ mice, pancreatic and pulmonary damage were reduced as previously observed in TLR4$^{-/-}$ animals. Since TLR4/CD14 are very important for LPS-mediated TLR4 activation, TLR4 appears to be involved in the development of ALI in AP, even when endotoxemia is absent. Still, the subject is controversial, since ALI can ensure LPS-mediated TLR4/CD11b activation in CD14$^{-/-}$ mice[87].

Matsuda *et al*[88] proposed that the TLR4-associated inflammatory response in AP complicated by endotoxemia is mediated by macrophage migration inhibitory protein (MIF). Increased MIF expression in the lungs was observed after cerulein/LPS administration in mice. MIF levels were coupled to ALI and increased TLR4 expression in the lungs. Moreover, AP-induced MIF$^{-/-}$ mice showed lower TLR4 expression than in wild type mice; and anti-MIF antibody administration greatly suppressed the pulmonary expression of TLR4. It is important to stress that MIF can mediate inflammation independently of TLR4. For instance, the induction of cytosolic phospholipase A_2, an enzyme that has been linked to ALI, may be MIF-mediated[89,90].

The role of TLR4 in AP-associated ALI is debated. While it is generally accepted that TLR4 plays a role in the course of ALI when endotoxemia ensues in AP; most reports are divided between those that suggests TLR4 as an important player (even in the absence of endotoxemia) and those that minimize its role. Fortunately, this discrepancy may lead to new experiments that focuses not only in LPS but also in the wide range of alarmins related to AP, ALI and TLR4. For instance, neuropeptide substance P, which has pro-inflammatory properties that increase vascular permeability and is correlated to AP and ALI[91]; has also been associated to the up regulation of TLR4 in AP[92]. Moreover, extracellular heat shock protein 70 can induce SIRS-like immune responses in cerulein-challenged mice[93]. The action of this protein appears to be mediated by TLR4, as TLR4$^{-/-}$ mice did not showed the same outcome.

Liver, kidneys and intestine

Similarly to TLR2, TLR4mRNA expression is increased

in the liver during AP-associated SIRS[48,49]. Briefly, after AP-induction, higher levels of liver enzymes and TNF-α were observed. Hepatic NO levels decreased. All these changes appeared to be related to the TLR4mRNA overexpression in the liver. The administration of chloroquine and L-Arg decreased TLR4mRNA expression, thus reducing the liver damage.

Peng *et al*[94] showed that the deletion of TLR4 attenuated liver injury in AP. AP was induced in mice by choline-deficient ethionine diet. Besides the expected augmentation of TLR4 mRNA in mice liver, an increment in protein kinase C-zeta (PKCζ) was detected. However, induced TLR4$^{-/-}$ mice showed less apoptosis in hepatic cells and the hepatic PKCζmRNA expression was clearly reduced. PKCζ activates NFκB, which is essential for the production of cytokines in Kupffer cells[95]. Consequently, TLR4-deficiency protects the liver in AP through down-regulation of PKCζ, resulting in less inflammation and hepatic cell apoptosis.

Sawa *et al*[96] showed that in SAP, TLR4 expression is not only increased in the liver, but also in the kidneys and small intestine. Closed duodenal loop operation was performed in mice. The increased expression of TLR4 occurred 4 h after the ligation and returned to baseline after 12 h. Curiously, TLR4-deficient mice showed the same tendency and histological analyses of the liver and kidney did not show any differences between the different groups. However, apoptosis was seen in the liver and kidney of ligated TLR4-deficient, but not in wild type, mice. Additionally, Gram-negative bacterial translocation into the pancreas occurred in higher rates in TLR4-deficient animals. The translocation ensued 12 h after induction, but not before. Even Gram-positive bacterial translocation was registered, but the difference between the groups was not significant. Bacterial translocation highly correlated to fluctuations in TLR4 expression. According to the authors, as MODS develops in the early or the late phase of SAP, the early inhibition and late stimulation of TLR4 could result in a milder clinical course, since it could prevent MODS and bacterial translocation. Yet, results from another experiment in mice challenges this hypothesis[97]. van Westerloo *et al* showed that the immune response against Escherichia coli in AP-induced TLR4$^{-/-}$ mice was not different from that observed in controls. Nevertheless, there is a chance that mice developed the compensatory anti-inflammatory response syndrome (CARS), which is generally (but not exclusively) related to SIRS and sepsis[98]. CARS is a systemic deactivation of the immune system, that can ensue after SIRS and sepsis in order to restore homeostasis[99]. Thus, CARS could explain why LPS-mediated TLR4-activation was not observed in TLR4$^{+/+}$ mice after bacterial challenge in AP.

TLR4 and AP in humans

Despite controversy, there is an increasing amount of data suggesting that TLR4 plays a role in experimental AP in rodents. However, in humans, the role of TLR4 in AP patients has been investigated mainly indirectly *via* DNA analysis or immune cells taken from blood.

In a Chinese study 310 patients were diagnosed with AP according to the Atlanta severity classification[100,101]. Pancreatic necrosis was recognized in 115 patients and in 37 of those, TLR4 mutation (*896G allele*) was identified. When compared to AP patients without TLR4 mutation (*896A allele*), patients with mutated TLR4 had an increased morbidity following Gram-negative infection. When compared to healthy volunteers (n = 80), TLR4 mutation frequency was significantly higher in patients with pancreatic necrotic infection. Thus, according to this study, TLR4 *Asp299Gly* polymorphism appears to be associated with an increased risk for pancreatic necrotic infection in AP. However, this study may have limited value as all patients were recruited from one hospital located in southwestern China, thus questioning its representative value for the whole Chinese population. In a later study, Zhang *et al*[102] could not find any significant difference in the occurrence of *Asp299Gly* polymorphism between 238 Chinese AP patients and 121 healthy volunteers.

DNA samples were harvested from 92 AP patients in a Hungarian medical centre[103]. Not only TLR4 *Asp299Gly* polymorphism was considered, but also as *Thr399Ile* polymorphism was taken into account. No significant differences were detected between AP (n = 42) and SAP patients (n = 50) in any polymorphism analysed. Likewise, no differences were found between the 92 patients and healthy controls (n = 200). Perhaps, the strongest data against the involvement of TLR4 mutations in AP comes from an intercontinental study reported by Guenther *et al*[104]. No significant differences could be found between 521 AP patients (343 from Germany and 178 from United States) and healthy controls (128 Germans and. 265 Americans, respectively) in the incidence of *Asp299Gly* and/or *Thr399Ile* polymorphism. Thus, current knowledge indicates that the investigated TLR4 polymorphisms are not important for the development and clinical course of AP, even if these mutation have been linked to impairment of LPS-induced TLR4-mediated immune response in humans[105].

In vitro acute alcohol stimulation seems to inhibit TLR4 expression in human monocytes[57]. When LPS was added, a decreased secretion of pro-inflammatory cytokines was observed. Intriguingly, the effect was abolished if LPS and TLR2 ligands were added at the same time to the cells. Li *et al*[106] showed that the expression of TLR4 in human peripheral blood mononuclear cells in mild AP, raises in the beginning of the disease to return to baseline levels after a week, when the patients recovered. As TNF-α and IL-6 showed the same pattern, the authors concluded that TLR4 might play an important role in the pathogenesis of AP.

In summary, there is strong data indicating that TLR4 plays an important role in experimental AP in rodents. Nevertheless, contrary results have been published. While most studies emphasize the role of TLR4 in the pancreas in AP, its role in AP-associated ALI is controversial. There is some evidence linking TLR4 levels and tissue

damage in the liver in AP. Likewise, the general opinion is that TLR4 is very important in the development of endotoxemia in AP.

In humans, DNA mutations in *TLR4* genes do not seem to be of importance for the development or the clinical course of AP, but TLR4 expression changes in monocytes and macrophages may be of significance. Unfortunately, the latter is only based on a very limited number of studies. There are some promising reports about the use of herbs from traditional Chinese medicine in AP that may act through TLR4 inhibition[107-109]. *Salvia miltiorrhizae* (also known as danshen) appears to inhibit the binding of LPS to TLR4 in the rat liver in SAP, which may reduce bacterial translocation and liver injury. Moreover, treatment with *emodin* and/or *baicalin* reduced serum levels of amylase, IL-6 and TNF-α in AP-induced rats. Pancreatic damage and ALI were also ameliorated. The effect of *emodin* and *baicalin* appears to be mediated by TLR4, since decreased TLR4mRNA expression and protein levels were found in the pancreas and lungs in treated animals. Its implication in experimental AP in rodents makes TLR4 a potentially very promising therapeutic target in AP, its utility though to be demonstrated in humans in future studies.

TLR9-UNEXPLORED IMPLICATIONS

As for TLR2 and TLR4, TLR9 is expressed intracellularly (endosomes) in several immune cells, including B cells and dendritic cells[110]. TLR9 recognises unmethylated *CpG* dinucleotides found in *bacteria* and *virus*[111,112]. *CpG* motifs are rare in vertebrate DNA (less 1% in human genome), but they are common in bacterial DNA. Besides, if present in vertebrate DNA, *CpG* motifs use to be methylated. *CpG* DNA directly stimulates B cells, macrophages and dendritic cells to secrete cytokines[113]. TLR9 can also recognize haemozoin, which is a disposal product formed from the digestion of erythrocytes by some parasites (*e.g.*, *Plasmodium spp.*)[114].

Upon activation, TLR9 signals through a MyD88-dependent pathway, leading to the production of pro-inflammatory cytokines (see previous sections). This pathway is mainly observed in macrophages, B cells, conventional dendritic cells and plasmacytoid dendritic cells. However, in plasmacytoid dendritic cells, TLR9 can induce the production of type I IFN through a different pathway. After the recruitment of Myd88 and IRAK-4, these interact with TRAF6, TRAF3, IRAK-1, IKKα and osteopontin. IRAK-1 and IKKα phosphorylate and activate IRF7, culminating in the production of type I IFN (Figure 2B)[26]. Apart from its role in bacterial, viral or malaria infection; TLR9 has been associated with SLE and cancer[115,116].

TLR9 and AP

To date, there are only two publications in which the role of TLR9 in AP has been investigated. Zeng *et al*[117] reported that TLR9 was expressed in rat pancreas in cerulein-induced AP. TLR9 staining was detected both in the pancreas from AP-induced rats, as in controls. The epithelium of the pancreatic duct and pancreatic microcirculation were the main sites for TLR9 staining in AP-induced animals. Controls showed staining in the vasculature, but not in the pancreatic duct. Moreover, no staining was detected in pancreatic acinar cells in either group. Interestingly, a similar pattern of staining has been reported for TLR4 in AP[64]. TLR9mRNA expression was increased after 30 min, peaked at 1 h, and remained high for the first eight hours after cerulein challenge.

In a more extensive study, Hoque *et al*[118] found that TLR9 is involved in pancreatic acinar cell death in AP-induced mice. After cerulein-administration, $TLR9^{-/-}$ mice had less oedema, leukocyte infiltration and IL-1βmRNA expression in the pancreas. Even if several cell types express TLR9 in the pancreas, bone marrow-derived $CD45^{+}$ cells (mainly macrophages) had the highest expression level. Furthermore, the action of a TLR9 antagonist (IRS954) was evaluated. $TLR9^{+/+}$ animals had IRS954 administered 1 h before inducing AP by cerulein. Pancreatic oedema, leukocyte infiltration and pancreatic cell apoptosis were ameliorated. Moreover, pancreatic pro-IL-1β elevation was also reduced, as serum amylase levels. These results were observed at 1 h after AP-induction.

The authors speculated if IRS954 might have a therapeutic value. Interestingly, the administration of IRS954 after cerulein challenge reduced pancreatic oedema, leukocyte infiltration and pancreatic cell apoptosis. Similar results were observed when another model for AP was used. Pre-treatment with IRS954 at 1 h after AP induction through taurolithocholic acid 3-sulphate challenge in $TLR9^{+/+}$, reduced serum amylase elevation, pancreatic necrosis and inflammatory cell infiltration in mice lungs. Additionally, *in vitro* experiments were performed. When isolated peritoneal macrophages were exposed to pancreatic homogenate and DNA, significant NFκB activation was observed. Interestingly, pre-treatment with IRS954 reduced NFκB activation to baseline levels.

IRS954 appears to be a good candidate for further investigation. Like TLR2 and TLR4, TLR9 may be important when AP is aggravated by sepsis. Reports indicate that during sepsis, TLR9 is expressed both in murine and human adrenal glands and its stimulation leads to corticosterone release and inflammatory response[119]. Macrophages and NK-cells in the liver mediates liver toxicity and express high levels of TLR9 in murine peritonitis[120]. Chloroquine inhibits TLR9 and may prevent sepsis-induced acute kidney injury in mice[121]. Additionally, $TLR9^{-/-}$ mice have reduced mortality in polymicrobial sepsis[122].

Still, the number of studies is very limited. Hence, it is impossible to conclude if TLR9 plays an important role in AP with a clinical course complicated with our without sepsis.

Closing remarks

An increasing number of publications suggest that TLRs

Table 5 Toll-like receptors and acute pancreatitis

	Observed change	Tissue/cell	Ref.
TLR2	Increased mRNA expression	Pancreas	[35]
		Lungs	[40]
		Liver	[48,49]
	Increased protein levels	Pancreas	[35]
		Liver	[48]
	Decreased mRNA expression	Pulmonary macrophages	[43]
TLR4	Increased mRNA expression	Pancreas	[35,64,73,92]
		Lungs	[40,88]
		Liver	[48,94,96]
		Kidney	[96]
	Increased protein levels	Small intestine	[96]
		Pancreas	[35,92]
		Lungs	[88]
		Liver	[48,49]
		Intestine	[65]
		Blood monocytes	[106]
	Decreased mRNA expression	Pulmonary macrophages	[43]
TLR9	Increased mRNA expression	Pancreas	[117,118]
	Increased protein levels	Pancreas	[118]

TLR: Toll-like receptor.

Table 6 Toll-like receptor intervention in acute pancreatitis

	Substance	Ref.
TLR2	Chloroquine	[40,49]
	L-Arg	[40,49]
	WY14643	[35]
TLR4	Anti-MIF antibody	[88]
	Baicalin	[107]
	Chloroquine	[40,49]
	Emodin	[107]
	Eritoran	[83]
	L-Arg	[40,49]
	Salvia miltiorrhizae	[108,109]
TLR9	IRS954	[118]

TLR: Toll-like receptor; MIF: Migration inhibitory protein.

are involved in the pathophysiology of several important medical conditions. In acute pancreatitis, TLRs appear to play a role, but a general consensus has not been achieved, since contradictory results have been presented.

The main candidate for targeting seems to be TLR4, which recognizes numerous DAMPs associated to AP[123,124]. TLR2 has also been linked to AP, but there are few studies that exclusively has studied its role in AP. There is data suggesting that TLR9 also can play a role in AP. The associations found between TLRs and AP, as possible novel types of therapy are presented (Tables 5 and 6). There is also some evidence indicating that TLR4 and TLR3 may be involved in chronic pancreatitis[125,126]. This is of particular importance since chronic pancreatitis may develop following repeated AP episodes, as chronic pancreatitis is to some extent a risk factor for pancreatic cancer[127].

CONCLUSION

To our knowledge, this is the first time the current understanding of the role of TLRs in acute pancreatitis is summarized. Of course, further research and elucidation of involved mechanisms is warranted, hopefully stimulated by the present review giving an update and state-of-the-art concerning the role of TLRs in acute pancreatitis and its potential future clinical implications.

REFERENCES

1 **Janeway CA**. Approaching the asymptote? Evolution and revolution in immunology. *Cold Spring Harb Symp Quant Biol* 1989; **54** Pt 1: 1-13 [PMID: 2700931 DOI: 10.1101/SQB.1989.054.01.077]

2 **Medzhitov R**. Toll-like receptors and innate immunity. *Nat Rev Immunol* 2001; **1**: 135-145 [PMID: 11905821 DOI: 10.1038/35100529]

3 **Lee CC**, Avalos AM, Ploegh HL. Accessory molecules for Toll-like receptors and their function. *Nat Rev Immunol* 2012; **12**: 168-179 [PMID: 22301850 DOI: 10.1038/nri3151]

4 **Rittirsch D**, Flierl MA, Ward PA. Harmful molecular mechanisms in sepsis. *Nat Rev Immunol* 2008; **8**: 776-787 [PMID: 18802444 DOI: 10.1038/nri2402]

5 **Piccinini AM**, Midwood KS. DAMPening inflammation by modulating TLR signalling. *Mediators Inflamm* 2010; **2010**: 672395 [PMID: 20706656 DOI: 10.1155/2010/672395]

6 **Hedayat M**, Netea MG, Rezaei N. Targeting of Toll-like receptors: a decade of progress in combating infectious diseases. *Lancet Infect Dis* 2011; **11**: 702-712 [PMID: 21719349 DOI: 10.1016/S1473-3099(11)70099-8]

7 **Akira S**, Takeda K. Toll-like receptor signalling. *Nat Rev Immunol* 2004; **4**: 499-511 [PMID: 15229469 DOI: 10.1038/nri1391]

8 **Kumar H**, Kawai T, Akira S. Toll-like receptors and innate immunity. *Biochem Biophys Res Commun* 2009; **388**: 621-625 [PMID: 19686699 DOI: 10.1016/j.bbrc.2009.08.062]

9 **O'Neill LA**, Bowie AG. The family of five: TIR-domain-containing adaptors in Toll-like receptor signalling. *Nat Rev Immunol* 2007; **7**: 353-364 [PMID: 17457343 DOI: 10.1038/nri2079]

10 **O'Neill LA**, Bryant CE, Doyle SL. Therapeutic targeting of Toll-like receptors for infectious and inflammatory diseases and cancer. *Pharmacol Rev* 2009; **61**: 177-197 [PMID: 19474110 DOI: 10.1124/pr.109.001073]

11 **Christman JW**, Lancaster LH, Blackwell TS. Nuclear factor kappa B: a pivotal role in the systemic inflammatory response syndrome and new target for therapy. *Intensive Care Med* 1998; **24**: 1131-1138 [PMID: 9876974 DOI: 10.1007/s001340050735]

12 **Abraham E**. Nuclear factor-kappaB and its role in sepsis-associated organ failure. *J Infect Dis* 2003; **187** Suppl 2: S364-S369 [PMID: 12792853 DOI: 10.1086/374750]

13 **Bone RC**, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, Schein RM, Sibbald WJ. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. *Chest* 1992; **101**: 1644-1655 [PMID: 1303622 DOI: 10.1378/chest.101.6.1644]

14 **Appelros S**, Borgström A. Incidence, aetiology and mortality rate of acute pancreatitis over 10 years in a defined urban population in Sweden. *Br J Surg* 1999; **86**: 465-470 [PMID: 10215815 DOI: 10.1046/j.1365-2168.1999.01049.x]

15 **Andersson R**, Andersson B, Haraldsen P, Drewsen G, Eckerwall G. Incidence, management and recurrence rate of acute pancreatitis. *Scand J Gastroenterol* 2004; **39**: 891-894 [PMID: 15513389 DOI: 10.1080/00365520410007061]

16 **Kingsnorth A**, O'Reilly D. Acute pancreatitis. *BMJ* 2006; **332**:

1072-1076 [PMID: 16675814 DOI: 10.1136/bmj.332.7549.1072]
17 **Goldacre MJ**, Roberts SE. Hospital admission for acute pancreatitis in an English population, 1963-98: database study of incidence and mortality. *BMJ* 2004; **328**: 1466-1469 [PMID: 15205290 DOI: 10.1136/bmj.328.7454.1466]
18 **Whitcomb DC**. Clinical practice. Acute pancreatitis. *N Engl J Med* 2006; **354**: 2142-2150 [PMID: 16707751 DOI: 10.1056/NEJMcp054958]
19 **Frossard JL**, Steer ML, Pastor CM. Acute pancreatitis. *Lancet* 2008; **371**: 143-152 [PMID: 18191686 DOI: 10.1016/S0140-6736(08)60107-5]
20 **Finucane MM**, Stevens GA, Cowan MJ, Danaei G, Lin JK, Paciorek CJ, Singh GM, Gutierrez HR, Lu Y, Bahalim AN, Farzadfar F, Riley LM, Ezzati M. National, regional, and global trends in body-mass index since 1980: systematic analysis of health examination surveys and epidemiological studies with 960 country-years and 9·1 million participants. *Lancet* 2011; **377**: 557-567 [PMID: 21295846 DOI: 10.1016/S0140-6736(10)62037-5]
21 **Flegal KM**, Carroll MD, Ogden CL, Curtin LR. Prevalence and trends in obesity among US adults, 1999-2008. *JAMA* 2010; **303**: 235-241 [PMID: 20071471 DOI: 10.1001/jama.2009.2014]
22 **Margetts B**. WHO global strategy on diet, physical activity and health.Editorial. *Public Health Nutr* 2004; **7**: 361-363 [PMID: 15153266 DOI: 10.1079/PHN2004622]
23 **McKay CJ**, Imrie CW. The continuing challenge of early mortality in acute pancreatitis. *Br J Surg* 2004; **91**: 1243-1244 [PMID: 15382103 DOI: 10.1002/bjs.4750]
24 **Baddeley R**, Skipworth J, Pereira S. Acute Pancreatitis. *Med* 2011; **39**(2): 108-115 [DOI: 10.1016/j.mpmed.2010.11.009]
25 **Johnson GB**, Brunn GJ, Platt JL. Cutting edge: an endogenous pathway to systemic inflammatory response syndrome (SIRS)-like reactions through Toll-like receptor 4. *J Immunol* 2004; **172**: 20-24 [PMID: 14688304]
26 **Abe T**, Fukuhara T, Wen X, Ninomiya A, Moriishi K, Maehara Y, Takeuchi O, Kawai T, Akira S, Matsuura Y. CD44 participates in IP-10 induction in cells in which hepatitis C virus RNA is replicating, through an interaction with Toll-like receptor 2 and hyaluronan. *J Virol* 2012; **86**: 6159-6170 [PMID: 22491449 DOI: 10.1128/JVI.06872-11]
27 **Kawana H**, Karaki H, Higashi M, Miyazaki M, Hilberg F, Kitagawa M, Harigaya K. CD44 suppresses TLR-mediated inflammation. *J Immunol* 2008; **180**: 4235-4245 [PMID: 18322236]
28 **van der Windt GJ**, van 't Veer C, Florquin S, van der Poll T. CD44 deficiency is associated with enhanced Escherichia coli-induced proinflammatory cytokine and chemokine release by peritoneal macrophages. *Infect Immun* 2010; **78**: 115-124 [PMID: 19901064 DOI: 10.1128/IAI.00949-09]
29 **Blasius AL**, Beutler B. Intracellular toll-like receptors. *Immunity* 2010; **32**: 305-315 [PMID: 20346772 DOI: 10.1016/j.immuni.2010.03.012]
30 **Satoh M**, Ishikawa Y, Minami Y, Takahashi Y, Nakamura M. Role of Toll like receptor signaling pathway in ischemic coronary artery disease. *Front Biosci* 2008; **13**: 6708-6715 [PMID: 18508689 DOI: 10.2741/3183]
31 **Eder W**, Klimecki W, Yu L, von Mutius E, Riedler J, Braun-Fahrländer C, Nowak D, Martinez FD. Toll-like receptor 2 as a major gene for asthma in children of European farmers. *J Allergy Clin Immunol* 2004; **113**: 482-488 [PMID: 15007351 DOI: 10.1016/j.jaci.2003.12.374]
32 **Anders HJ**, Banas B, Schlöndorff D. Signaling danger: toll-like receptors and their potential roles in kidney disease. *J Am Soc Nephrol* 2004; **15**: 854-867 [PMID: 15034087 DOI: 10.1097/01.ASN.0000121781.89599.16]
33 **Urbonaviciute V**, Fürnrohr BG, Meister S, Munoz L, Heyder P, De Marchis F, Bianchi ME, Kirschning C, Wagner H, Manfredi AA, Kalden JR, Schett G, Rovere-Querini P, Herrmann M, Voll RE. Induction of inflammatory and immune responses by HMGB1-nucleosome complexes: implications for the pathogenesis of SLE. *J Exp Med* 2008; **205**: 3007-3018 [PMID: 19064698 DOI: 10.1084/jem.20081165]
34 **Boraska Jelavić T**, Barisić M, Drmic Hofman I, Boraska V, Vrdoljak E, Peruzović M, Hozo I, Puljiz Z, Terzić J. Microsatelite GT polymorphism in the toll-like receptor 2 is associated with colorectal cancer. *Clin Genet* 2006; **70**: 156-160 [PMID: 16879199 DOI: 10.1111/j.1399-0004.2006.00651.x]
35 **Ding JL**, Zhou ZG, Zhou XY, Zhou B, Wang L, Wang R, Zhan L, Sun XF, Li Y. Attenuation of Acute Pancreatitis by Peroxisome Proliferator-Activated Receptor-α in Rats: The Effect on Toll-Like Receptor Signaling Pathways. *Pancreas* 2013; **42**: 114-122 [PMID: 22722259]
36 **Lorne E**, Dupont H, Abraham E. Toll-like receptors 2 and 4: initiators of non-septic inflammation in critical care medicine? *Intensive Care Med* 2010; **36**: 1826-1835 [PMID: 20689929 DOI: 10.1007/s00134-010-1983-5]
37 **Jiang D**, Liang J, Fan J, Yu S, Chen S, Luo Y, Prestwich GD, Mascarenhas MM, Garg HG, Quinn DA, Homer RJ, Goldstein DR, Bucala R, Lee PJ, Medzhitov R, Noble PW. Regulation of lung injury and repair by Toll-like receptors and hyaluronan. *Nat Med* 2005; **11**: 1173-1179 [PMID: 16244651 DOI: 10.1038/nm1315]
38 **Asea A**, Rehli M, Kabingu E, Boch JA, Bare O, Auron PE, Stevenson MA, Calderwood SK. Novel signal transduction pathway utilized by extracellular HSP70: role of toll-like receptor (TLR) 2 and TLR4. *J Biol Chem* 2002; **277**: 15028-15034 [PMID: 11836257 DOI: 10.1074/jbc.M200497200]
39 **Zhou MT**, Chen CS, Chen BC, Zhang QY, Andersson R. Acute lung injury and ARDS in acute pancreatitis: mechanisms and potential intervention. *World J Gastroenterol* 2010; **16**: 2094-2099 [PMID: 20440849 DOI: 10.3748/wjg.v16.i17.2094]
40 **Wu HS**, Zhang L, Chen Y, Guo XJ, Wang L, Xu JB, Wang CY, Zhang JH. Effect of nitric oxide on toll-like receptor 2 and 4 gene expression in rats with acute lung injury complicated by acute hemorrhage necrotizing pancreatitis. *Hepatobiliary Pancreat Dis Int* 2005; **4**: 609-613 [PMID: 16286274]
41 **Steinman RM**, Mellman IS, Muller WA, Cohn ZA. Endocytosis and the recycling of plasma membrane. *J Cell Biol* 1983; **96**: 1-27 [PMID: 6298247 DOI: 10.1083/jcb.96.1.1]
42 **Hart OM**, Athie-Morales V, O'Connor GM, Gardiner CM. TLR7/8-mediated activation of human NK cells results in accessory cell-dependent IFN-gamma production. *J Immunol* 2005; **175**: 1636-1642 [PMID: 16034103]
43 **Matsumura N**, Takeyama Y, Ueda T, Yasuda T, Shinzeki M, Sawa H, Nakajima T, Kuroda Y. Decreased expression of Toll-like receptor 2 and 4 on macrophages in experimental severe acute pancreatitis. *Kobe J Med Sci* 2007; **53**: 219-227 [PMID: 18204298]
44 **Echchannaoui H**, Frei K, Schnell C, Leib SL, Zimmerli W, Landmann R. Toll-like receptor 2-deficient mice are highly susceptible to Streptococcus pneumoniae meningitis because of reduced bacterial clearing and enhanced inflammation. *J Infect Dis* 2002; **186**: 798-806 [PMID: 12198614 DOI: 10.1086/342845]
45 **Koedel U**, Angele B, Rupprecht T, Wagner H, Roggenkamp A, Pfister HW, Kirschning CJ. Toll-like receptor 2 participates in mediation of immune response in experimental pneumococcal meningitis. *J Immunol* 2003; **170**: 438-444 [PMID: 12496429]
46 **Gea-Sorlí S**, Guillamat R, Serrano-Mollar A, Closa D. Activation of lung macrophage subpopulations in experimental acute pancreatitis. *J Pathol* 2011; **223**: 417-424 [PMID: 21171087 DOI: 10.1002/path.2814]
47 **Folch-Puy E**. Importance of the liver in systemic complications associated with acute pancreatitis: the role of Kupffer cells. *J Pathol* 2007; **211**: 383-388 [PMID: 17212343 DOI: 10.1002/path.2123]
48 **Xiong J**, Zhu ZH, Liu JS, Wang Y, Wu HS. The expression of toll-like receptor 2, 4 of livers in mice with systemic inflam-

matory response syndrome. *Hepatobiliary Pancreat Dis Int* 2006; **5**: 143-146 [PMID: 16481302]
49 **Zhang L**, Wu HS, Chen Y, Guo XJ, Wang L, Wang CY, Zhang JH, Tian Y. Role of nitric oxide in Toll-like receptor 2 and 4 mRNA expression in liver of acute hemorrhagic necrotizing pancreatitis rats. *World J Gastroenterol* 2006; **12**: 485-488 [PMID: 16489656]
50 **Awla D**, Abdulla A, Regnér S, Thorlacius H. TLR4 but not TLR2 regulates inflammation and tissue damage in acute pancreatitis induced by retrograde infusion of taurocholate. *Inflamm Res* 2011; **60**: 1093-1098 [PMID: 21863370 DOI: 10.1007/s00011-011-0370-1]
51 **Takagi Y**, Masamune A, Kume K, Satoh A, Kikuta K, Watanabe T, Satoh K, Hirota M, Shimosegawa T. Microsatellite polymorphism in intron 2 of human Toll-like receptor 2 gene is associated with susceptibility to acute pancreatitis in Japan. *Hum Immunol* 2009; **70**: 200-204 [PMID: 19280717 DOI: 10.1016/j.humimm.2009.01.006]
52 **Yim JJ**, Lee HW, Lee HS, Kim YW, Han SK, Shim YS, Holland SM. The association between microsatellite polymorphisms in intron II of the human Toll-like receptor 2 gene and tuberculosis among Koreans. *Genes Immun* 2006; **7**: 150-155 [PMID: 16437124 DOI: 10.1038/sj.gene.6364274]
53 **Lee EY**, Yim JJ, Lee HS, Lee YJ, Lee EB, Song YW. Dinucleotide repeat polymorphism in intron II of human Toll-like receptor 2 gene and susceptibility to rheumatoid arthritis. *Int J Immunogenet* 2006; **33**: 211-215 [PMID: 16712654 DOI: 10.1111/j.1744-313X.2006.00599.x]
54 **Veltkamp M**, Wijnen PA, van Moorsel CH, Rijkers GT, Ruven HJ, Heron M, Bekers O, Claessen AM, Drent M, van den Bosch JM, Grutters JC. Linkage between Toll-like receptor (TLR) 2 promotor and intron polymorphisms: functional effects and relevance to sarcoidosis. *Clin Exp Immunol* 2007; **149**: 453-462 [PMID: 17565608 DOI: 10.1111/j.1365-2249.2007.03428.x]
55 **Rahman SH**, Ibrahim K, Larvin M, Kingsnorth A, McMahon MJ. Association of antioxidant enzyme gene polymorphisms and glutathione status with severe acute pancreatitis. *Gastroenterology* 2004; **126**: 1312-1322 [PMID: 15131792 DOI: 10.1053/j.gastro.2004.02.002]
56 **Bhat YM**, Papachristou GI, Park JS, Lamb J, Slivka A, Whitcomb DC. Functional polymorphisms of the GSTT-1 gene do not predict the severity of acute pancreatitis in the United States. *Pancreatology* 2007; **7**: 180-186 [PMID: 17592232 DOI: 10.1159/000104243]
57 **Szabo G**, Mandrekar P, Oak S, Mayerle J. Effect of ethanol on inflammatory responses. Implications for pancreatitis. *Pancreatology* 2007; **7**: 115-123 [PMID: 17592223 DOI: 10.1159/000104236]
58 **Medzhitov R**, Preston-Hurlburt P, Janeway CA. A human homologue of the Drosophila Toll protein signals activation of adaptive immunity. *Nature* 1997; **388**: 394-397 [PMID: 9237759]
59 **Ding Y**, Subramanian S, Montes VN, Goodspeed L, Wang S, Han C, Teresa AS, Kim J, O'Brien KD, Chait A. Toll-like receptor 4 deficiency decreases atherosclerosis but does not protect against inflammation in obese low-density lipoprotein receptor-deficient mice. *Arterioscler Thromb Vasc Biol* 2012; **32**: 1596-1604 [PMID: 22580897 DOI: 10.1161/ATVBAHA.112.249847]
60 **Sahin H**, Trautwein C, Wasmuth HE. TLR4 stresses the liver. *Gut* 2012; **61**: 1241-1242 [PMID: 22387524 DOI: 10.1136/gutjnl-2012-302188]
61 **Kleinridders A**, Schenten D, Könner AC, Belgardt BF, Mauer J, Okamura T, Wunderlich FT, Medzhitov R, Brüning JC. MyD88 signaling in the CNS is required for development of fatty acid-induced leptin resistance and diet-induced obesity. *Cell Metab* 2009; **10**: 249-259 [PMID: 19808018 DOI: 10.1016/j.cmet.2009.08.013]
62 **Kashiwagi M**, Imanishi T, Ozaki Y, Satogami K, Masuno T, Wada T, Nakatani Y, Ishibashi K, Komukai K, Tanimoto T, Ino Y, Kitabata H, Akasaka T. Differential expression of Toll-like receptor 4 and human monocyte subsets in acute myocardial infarction. *Atherosclerosis* 2012; **221**: 249-253 [PMID: 22244044 DOI: 10.1016/j.atherosclerosis.2011.12.030]
63 **Lepenies J**, Eardley KS, Kienitz T, Hewison M, Ihl T, Stewart PM, Cockwell P, Quinkler M. Renal TLR4 mRNA expression correlates with inflammatory marker MCP-1 and profibrotic molecule TGF-β1 in patients with chronic kidney disease. *Nephron Clin Pract* 2011; **119**: c97-c104 [PMID: 21677444 DOI: 10.1159/000324765]
64 **Li Y**, Zhou ZG, Xia QJ, Zhang J, Li HG, Cao GQ, Wang R, Lu YL, Hu TZ. Toll-like receptor 4 detected in exocrine pancreas and the change of expression in cerulein-induced pancreatitis. *Pancreas* 2005; **30**: 375-381 [PMID: 15841051 DOI: 10.1097/01.mpa.0000160959.21580.41]
65 **Li Y**, Zhou ZG, Zhang J, Chen YD, Li HG, Gao HK, Wang R, Hu TZ. Microcirculatory detection of Toll-like receptor 4 in rat pancreas and intestine. *Clin Hemorheol Microcirc* 2006; **34**: 213-219 [PMID: 16543639]
66 **Ding SQ**, Li Y, Zhou ZG, Wang C, Zhan L, Zhou B. Toll-like receptor 4-mediated apoptosis of pancreatic cells in cerulein-induced acute pancreatitis in mice. *Hepatobiliary Pancreat Dis Int* 2010; **9**: 645-650 [PMID: 21134836]
67 **Salaun B**, Romero P, Lebecque S. Toll-like receptors' two-edged sword: when immunity meets apoptosis. *Eur J Immunol* 2007; **37**: 3311-3318 [PMID: 18034428 DOI: 10.1002/eji.200737744]
68 **Bhatia M**. Apoptosis of pancreatic acinar cells in acute pancreatitis: is it good or bad? *J Cell Mol Med* 2004; **8**: 402-409 [PMID: 15491516 DOI: 10.1111/j.1582-4934.2004.tb00330.x]
69 **Cao Y**, Adhikari S, Clément MV, Wallig M, Bhatia M. Induction of apoptosis by crambene protects mice against acute pancreatitis via anti-inflammatory pathways. *Am J Pathol* 2007; **170**: 1521-1534 [PMID: 17456759 DOI: 10.2353/ajpath.2007.061149]
70 **Wang X**, Wu L, Wu K, Zhang R, Dong Y. Roles of endotoxin-related signaling molecules in the progression of acute necrotizing pancreatitis in mice. *Pancreas* 2005; **31**: 251-257 [PMID: 16163057 DOI: 10.1097/01.mpa.0000175179.62916.17]
71 **Ding JL**, Li Y, Zhou XY, Wang L, Zhou B, Wang R, Liu HX, Zhou ZG. Potential role of the TLR4/IRAK-4 signaling pathway in the pathophysiology of acute pancreatitis in mice. *Inflamm Res* 2009; **58**: 783-790 [PMID: 19434478 DOI: 10.1007/s00011-009-0048-0]
72 **Hatao F**, Muroi M, Hiki N, Ogawa T, Mimura Y, Kaminishi M, Tanamoto K. Prolonged Toll-like receptor stimulation leads to down-regulation of IRAK-4 protein. *J Leukoc Biol* 2004; **76**: 904-908 [PMID: 15258191 DOI: 10.1189/jlb.0504277]
73 **Zhou XY**, Zhou ZG, Ding JL, Wang L, Wang R, Zhou B, Gu J, Sun XF, Li Y. TRAF6 as the key adaptor of TLR4 signaling pathway is involved in acute pancreatitis. *Pancreas* 2010; **39**: 359-366 [PMID: 19823099 DOI: 10.1097/MPA.0b013e3181bb9073]
74 **Jaffray C**, Yang J, Carter G, Mendez C, Norman J. Pancreatic elastase activates pulmonary nuclear factor kappa B and inhibitory kappa B, mimicking pancreatitis-associated adult respiratory distress syndrome. *Surgery* 2000; **128**: 225-231 [PMID: 10922996 DOI: 10.1067/msy.2000.107419]
75 **Jaffray C**, Mendez C, Denham W, Carter G, Norman J. Specific pancreatic enzymes activate macrophages to produce tumor necrosis factor-alpha: role of nuclear factor kappa B and inhibitory kappa B proteins. *J Gastrointest Surg* 2000; **4**: 370-377 [PMID: 11058855 DOI: 10.1016/S1091-255X(00)80015-3]
76 **Hietaranta A**, Mustonen H, Puolakkainen P, Haapiainen R, Kemppainen E. Proinflammatory effects of pancreatic elastase are mediated through TLR4 and NF-kappaB. *Biochem Biophys Res Commun* 2004; **323**: 192-196 [PMID: 15351720 DOI: 10.1016/j.bbrc.2004.08.077]

77 **Lüthen R**, Niederau C, Grendell JH. Intrapancreatic zymogen activation and levels of ATP and glutathione during caerulein pancreatitis in rats. *Am J Physiol* 1995; **268**: G592-G604 [PMID: 7537455]

78 **Termeer C**, Benedix F, Sleeman J, Fieber C, Voith U, Ahrens T, Miyake K, Freudenberg M, Galanos C, Simon JC. Oligosaccharides of Hyaluronan activate dendritic cells via toll-like receptor 4. *J Exp Med* 2002; **195**: 99-111 [PMID: 11781369 DOI: 10.1084/jem.20001858]

79 **Bedrosian AS**, Nguyen AH, Hackman M, Connolly MK, Malhotra A, Ibrahim J, Cieza-Rubio NE, Henning JR, Barilla R, Rehman A, Pachter HL, Medina-Zea MV, Cohen SM, Frey AB, Acehan D, Miller G. Dendritic cells promote pancreatic viability in mice with acute pancreatitis. *Gastroenterology* 2011; **141**: 1915-26.e1-14 [PMID: 21801698 DOI: 10.1053/j.gastro.2011.07.033]

80 **Schaefer L**, Babelova A, Kiss E, Hausser HJ, Baliova M, Krzyzankova M, Marsche G, Young MF, Mihalik D, Götte M, Malle E, Schaefer RM, Gröne HJ. The matrix component biglycan is proinflammatory and signals through Toll-like receptors 4 and 2 in macrophages. *J Clin Invest* 2005; **115**: 2223-2233 [PMID: 16025156 DOI: 10.1172/JCI23755]

81 **Klebanoff SJ**, Kinsella MG, Wight TN. Degradation of endothelial cell matrix heparan sulfate proteoglycan by elastase and the myeloperoxidase-H2O2-chloride system. *Am J Pathol* 1993; **143**: 907-917 [PMID: 8395774]

82 **Axelsson J**, Norrman G, Malmström A, Weström B, Andersson R. Initiation of acute pancreatitis by heparan sulphate in the rat. *Scand J Gastroenterol* 2008; **43**: 480-489 [PMID: 18365914 DOI: 10.1080/00365520701733814]

83 **Akbarshahi H**, Axelsson JB, Said K, Malmström A, Fischer H, Andersson R. TLR4 dependent heparan sulphate-induced pancreatic inflammatory response is IRF3-mediated. *J Transl Med* 2011; **9**: 219 [PMID: 22188870 DOI: 10.1186/1479-5876-9-219]

84 **Yeh YC**, Ko WJ, Chan KC, Fan SZ, Tsai JC, Cheng YJ, Sun WZ. Effects of eritoran tetrasodium, a toll-like receptor 4 antagonist, on intestinal microcirculation in endotoxemic rats. *Shock* 2012; **37**: 556-561 [PMID: 22392144 DOI: 10.1097/SHK.0b013e31824e20ef]

85 **Pastor CM**, Pugin J, Kwak B, Chanson M, Mach F, Hadengue A, Frossard JL. Role of Toll-like receptor 4 on pancreatic and pulmonary injury in a mice model of acute pancreatitis associated with endotoxemia. *Crit Care Med* 2004; **32**: 1759-1763 [PMID: 15286555 DOI: 10.1097/01.CCM.0000133020.47243.8E]

86 **Sharif R**, Dawra R, Wasiluk K, Phillips P, Dudeja V, Kurt-Jones E, Finberg R, Saluja A. Impact of toll-like receptor 4 on the severity of acute pancreatitis and pancreatitis-associated lung injury in mice. *Gut* 2009; **58**: 813-819 [PMID: 19201771 DOI: 10.1136/gut.2008.170423]

87 **Jeyaseelan S**, Chu HW, Young SK, Freeman MW, Worthen GS. Distinct roles of pattern recognition receptors CD14 and Toll-like receptor 4 in acute lung injury. *Infect Immun* 2005; **73**: 1754-1763 [PMID: 15731076 DOI: 10.1128/IAI.73.3.1754-1763.2005]

88 **Matsuda N**, Nishihira J, Takahashi Y, Kemmotsu O, Hattori Y. Role of macrophage migration inhibitory factor in acute lung injury in mice with acute pancreatitis complicated by endotoxemia. *Am J Respir Cell Mol Biol* 2006; **35**: 198-205 [PMID: 16574946 DOI: 10.1165/rcmb.2005-0272OC]

89 **Mitchell RA**, Metz CN, Peng T, Bucala R. Sustained mitogen-activated protein kinase (MAPK) and cytoplasmic phospholipase A2 activation by macrophage migration inhibitory factor (MIF). Regulatory role in cell proliferation and glucocorticoid action. *J Biol Chem* 1999; **274**: 18100-18106 [PMID: 10364264]

90 **Nagase T**, Uozumi N, Ishii S, Kume K, Izumi T, Ouchi Y, Shimizu T. Acute lung injury by sepsis and acid aspiration: a key role for cytosolic phospholipase A2. *Nat Immunol* 2000; **1**: 42-46 [PMID: 10881173]

91 **Bhatia M**, Moochhala S. Role of inflammatory mediators in the pathophysiology of acute respiratory distress syndrome. *J Pathol* 2004; **202**: 145-156 [PMID: 14743496 DOI: 10.1002/path.1491]

92 **Tamizhselvi R**, Shrivastava P, Koh YH, Zhang H, Bhatia M. Preprotachykinin-A gene deletion regulates hydrogen sulfide-induced toll-like receptor 4 signaling pathway in cerulein-treated pancreatic acinar cells. *Pancreas* 2011; **40**: 444-452 [PMID: 21289528]

93 **Song JM**, Liu HX, Li Y, Zeng YJ, Zhou ZG, Liu HY, Xu B, Wang L, Zhou B, Wang R. Extracellular heat-shock protein 70 aggravates cerulein-induced pancreatitis through toll-like receptor-4 in mice. *Chin Med J (Engl)* 2008; **121**: 1420-1425 [PMID: 18959120]

94 **Peng Y**, Sigua CA, Rideout D, Murr MM. Deletion of toll-like receptor-4 downregulates protein kinase C-zeta and attenuates liver injury in experimental pancreatitis. *Surgery* 2008; **143**: 679-685 [PMID: 18436016 DOI: 10.1016/j.surg.2008.01.005]

95 **Peng Y**, Sigua CA, Gallagher SF, Murr MM. Protein kinase C-zeta is critical in pancreatitis-induced apoptosis of Kupffer cells. *J Gastrointest Surg* 2007; **11**: 1253-1261 [PMID: 17653813 DOI: 10.1007/s11605-007-0193-0]

96 **Sawa H**, Ueda T, Takeyama Y, Yasuda T, Shinzeki M, Nakajima T, Kuroda Y. Role of toll-like receptor 4 in the pathophysiology of severe acute pancreatitis in mice. *Surg Today* 2007; **37**: 867-873 [PMID: 17879036 DOI: 10.1007/s00595-007-3520-x]

97 **van Westerloo DJ**, Weijer S, Bruno MJ, de Vos AF, Van't Veer C, van der Poll T. Toll-like receptor 4 deficiency and acute pancreatitis act similarly in reducing host defense during murine Escherichia coli peritonitis. *Crit Care Med* 2005; **33**: 1036-1043 [PMID: 15891333 DOI: 10.1097/01.CCM.0000162684.11375.85]

98 **Bone RC**. Sir Isaac Newton, sepsis, SIRS, and CARS. *Crit Care Med* 1996; **24**: 1125-1128 [PMID: 8674323 DOI: 10.1097/00003246-199607000-00010]

99 **Ward NS**, Casserly B, Ayala A. The compensatory anti-inflammatory response syndrome (CARS) in critically ill patients. *Clin Chest Med* 2008; **29**: 617-625, viii [PMID: 18954697 DOI: 10.1016/j.ccm.2008.06.010]

100 **Gao HK**, Zhou ZG, Li Y, Chen YQ. Toll-like receptor 4 Asp-299Gly polymorphism is associated with an increased risk of pancreatic necrotic infection in acute pancreatitis: a study in the Chinese population. *Pancreas* 2007; **34**: 295-298 [PMID: 17414051 DOI: 10.1097/mpa.0b013e3180332674a]

101 **Bradley EL**. A clinically based classification system for acute pancreatitis. Summary of the International Symposium on Acute Pancreatitis, Atlanta, Ga, September 11 through 13, 1992. *Arch Surg* 1993; **128**: 586-590 [PMID: 8489394 DOI: 10.1001/archsurg.1993.01420170122019]

102 **Zhang D**, Zheng H, Zhou Y, Yu B, Li J. TLR and MBL gene polymorphisms in severe acute pancreatitis. *Mol Diagn Ther* 2008; **12**: 45-50 [PMID: 18288881 DOI: 10.1007/BF03256267]

103 **Hofner P**, Balog A, Gyulai Z, Farkas G, Rakonczay Z, Takács T, Mándi Y. Polymorphism in the IL-8 gene, but not in the TLR4 gene, increases the severity of acute pancreatitis. *Pancreatology* 2006; **6**: 542-548 [PMID: 17124436 DOI: 10.1159/000097363]

104 **Guenther A**, Aghdassi A, Muddana V, Rau B, Schulz HU, Mayerle J, Kraft M, Whitcomb DC, Lerch MM, Weiss FU. Toll-like receptor 4 polymorphisms in German and US patients are not associated with occurrence or severity of acute pancreatitis. *Gut* 2010; **59**: 1154-1155 [PMID: 20587548 DOI: 10.1136/gut.2009.192492]

105 **Arbour NC**, Lorenz E, Schutte BC, Zabner J, Kline JN, Jones M, Frees K, Watt JL, Schwartz DA. TLR4 mutations are associated with endotoxin hyporesponsiveness in humans. *Nat Genet* 2000; **25**: 187-191 [PMID: 10835634 DOI: 10.1038/76048]

106 **Li HG**, Zhou ZG, Li Y, Zheng XL, Lei S, Zhu L, Wang Y. Alterations of Toll-like receptor 4 expression on peripheral

blood monocytes during the early stage of human acute pancreatitis. *Dig Dis Sci* 2007; **52**: 1973-1978 [PMID: 17415654 DOI: 10.1007/s10620-006-9211-4]
107 **Li Z**, Xia X, Zhang S, Zhang A, Bo W, Zhou R. Up-regulation of Toll-like receptor 4 was suppressed by emodin and baicalin in the setting of acute pancreatitis. *Biomed Pharmacother* 2009; **63**: 120-128 [PMID: 18343629 DOI: 10.1016/j.biopha.2008.01.003]
108 **Zhang X**, Liu D, Wu D, Zhu C, Ye J, Wang K, Peng L, Zhuo G. Effect of salvia miltiorrhizae on the expressions of TLR4 protein in the liver of rats with SAP or OJ. *Inflammation* 2009; **32**: 151-162 [PMID: 19370406]
109 **Xiping Z**, Dijiong W, Jianfeng L, Qihui C, Jing Y, Penghui J, Meijuan Y, Ninni Z. Effects of Salvia miltiorrhizae on ICAM-1, TLR4, NF-kappaB and Bax proteins expression in multiple organs of rats with severe acute pancreatitis or obstructive jaundice. *Inflammation* 2009; **32**: 218-232 [PMID: 19434485 DOI: 10.1007/s10753-009-9124-4]
110 **Wagner H**. The immunobiology of the TLR9 subfamily. *Trends Immunol* 2004; **25**: 381-386 [PMID: 15207506 DOI: 10.1016/j.it.2004.04.011]
111 **Hemmi H**, Takeuchi O, Kawai T, Kaisho T, Sato S, Sanjo H, Matsumoto M, Hoshino K, Wagner H, Takeda K, Akira S. A Toll-like receptor recognizes bacterial DNA. *Nature* 2000; **408**: 740-745 [PMID: 11130078 DOI: 10.1385/1592593054]
112 **Zauner L**, Nadal D. Understanding TLR9 action in Epstein-Barr virus infection. *Front Biosci* 2012; **17**: 1219-1231 [PMID: 22201799 DOI: 10.2741/3982]
113 **Akira S**, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. *Nat Immunol* 2001; **2**: 675-680 [PMID: 11477402 DOI: 10.1038/90609]
114 **Gowda NM**, Wu X, Gowda DC. TLR9 and MyD88 are crucial for the development of protective immunity to malaria. *J Immunol* 2012; **188**: 5073-5085 [PMID: 22516959 DOI: 10.4049/jimmunol.1102143]
115 **Ghaly NR**, Kotb NA, Nagy HM, Rageh ES. Toll-like receptor 9 in systemic lupus erythematosus, impact on glucocorticoid treatment. *J Dermatolog Treat* 2012; **2012**: Epub ahead of print [PMID: 22620859 DOI: 10.3109/09546634.2012.697110]
116 **Noack J**, Jordi M, Zauner L, Alessi D, Burch A, Tinguely M, Hersberger M, Bernasconi M, Nadal D. TLR9 agonists induced cell death in Burkitt's lymphoma cells is variable and influenced by TLR9 polymorphism. *Cell Death Dis* 2012; **3**: e323 [PMID: 22717578 DOI: 10.1038/cddis.2012.60]
117 **Zeng YJ**, Song JM, Li Y, Wang R, Zhou B, Zhou ZG, Liu HY, Xu B. Toll-like receptor 9 is expressed in rat pancreas and is involved in cerulein-induced pancreatitis. *Pancreas* 2008; **36**: 212-214 [PMID: 18376319 DOI: 10.1097/01.MPA.0000311840.58054.35]
118 **Hoque R**, Sohail M, Malik A, Sarwar S, Luo Y, Shah A, Barrat F, Flavell R, Gorelick F, Husain S, Mehal W. TLR9 and the NLRP3 inflammasome link acinar cell death with inflammation in acute pancreatitis. *Gastroenterology* 2011; **141**: 358-369 [PMID: 21439959 DOI: 10.1053/j.gastro.2011.03.041]
119 **Tran N**, Koch A, Berkels R, Boehm O, Zacharowski PA, Baumgarten G, Knuefermann P, Schott M, Kanczkowski W, Bornstein SR, Lightman SL, Zacharowski K. Toll-like receptor 9 expression in murine and human adrenal glands and possible implications during inflammation. *J Clin Endocrinol Metab* 2007; **92**: 2773-2783 [PMID: 17473064 DOI: 10.1210/jc.2006-2697]
120 **Tsujimoto H**, Ono S, Matsumoto A, Kawabata T, Kinoshita M, Majima T, Hiraki S, Seki S, Moldawer LL, Mochizuki H. A critical role of CpG motifs in a murine peritonitis model by their binding to highly expressed toll-like receptor-9 on liver NKT cells. *J Hepatol* 2006; **45**: 836-843 [PMID: 17030073 DOI: 10.1016/j.jhep.2006.07.024]
121 **Yasuda H**, Leelahavanichkul A, Tsunoda S, Dear JW, Takahashi Y, Ito S, Hu X, Zhou H, Doi K, Childs R, Klinman DM, Yuen PS, Star RA. Chloroquine and inhibition of Toll-like receptor 9 protect from sepsis-induced acute kidney injury. *Am J Physiol Renal Physiol* 2008; **294**: F1050-F1058 [PMID: 18305095 DOI: 10.1152/ajprenal.00461.2007]
122 **Plitas G**, Burt BM, Nguyen HM, Bamboat ZM, DeMatteo RP. Toll-like receptor 9 inhibition reduces mortality in polymicrobial sepsis. *J Exp Med* 2008; **205**: 1277-1283 [PMID: 18474631 DOI: 10.1084/jem.20080162]
123 **Zhang X**, Zhu C, Wu D, Jiang X. Possible role of toll-like receptor 4 in acute pancreatitis. *Pancreas* 2010; **39**: 819-824 [PMID: 20664479 DOI: 10.1097/MPA.0b013e3181ca065c]
124 **Hoque R**, Malik AF, Gorelick F, Mehal WZ. Sterile inflammatory response in acute pancreatitis. *Pancreas* 2012; **41**: 353-357 [PMID: 22415665 DOI: 10.1097/MPA.0b013e3182321500]
125 **Vonlaufen A**, Phillips PA, Xu Z, Zhang X, Yang L, Pirola RC, Wilson JS, Apte MV. Withdrawal of alcohol promotes regression while continued alcohol intake promotes persistence of LPS-induced pancreatic injury in alcohol-fed rats. *Gut* 2011; **60**: 238-246 [PMID: 20870739 DOI: 10.1136/gut.2010.211250]
126 **Soga Y**, Komori H, Miyazaki T, Arita N, Terada M, Kamada K, Tanaka Y, Fujino T, Hiasa Y, Matsuura B, Onji M, Nose M. Toll-like receptor 3 signaling induces chronic pancreatitis through the Fas/Fas ligand-mediated cytotoxicity. *Tohoku J Exp Med* 2009; **217**: 175-184 [PMID: 19282652 DOI: 10.1620/tjem.217.175]
127 **Dítě P**, Hermanová M, Trna J, Novotný I, Růžička M, Liberda M, Bártková A. The role of chronic inflammation: chronic pancreatitis as a risk factor of pancreatic cancer. *Dig Dis* 2012; **30**: 277-283 [PMID: 22722552 DOI: 10.1159/000336991]

P- Reviewers Coelho AMM, Hac SAY, Maria D, Blanco-Guillermo I
S- Editor Huang XZ **L- Editor** A **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i5.631

World J Gastroenterol 2013 February 7; 19(5): 631-637
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

Prophecy about post-endoscopic retrograde cholangiopancreatography pancreatitis: From divination to science

Sung-Hoon Moon, Myung-Hwan Kim

Sung-Hoon Moon, Department of Internal Medicine, Hallym University Sacred Heart Hospital, Hallym University College of Medicine, Anyang 431-070, South Korea
Myung-Hwan Kim, Department of Internal Medicine, University of Ulsan College of Medicine, Asan Medical Center, Seoul 138-736, South Korea
Author contributions: Moon SH and Kim MH wrote the paper; Moon SH and Kim MH advised and informed about the knowledge.
Correspondence to: Myung-Hwan Kim, MD, PhD, Department of Internal Medicine, University of Ulsan College of Medicine, Asan Medical Center, Asanbyeongwon-Gil 86, Songpa-Gu, Seoul 138-736, South Korea. mhkim@amc.seoul.kr
Telephone: +82-2-30103183 Fax: +82-41-5563256
Received: October 15, 2012 Revised: December 28, 2012
Accepted: January 11, 2013
Published online: February 7, 2013

Abstract

One unresolved issue of endoscopic retrograde cholangiopancreatography (ERCP) is post-ERCP pancreatitis (PEP), which occurs in up to 40% of patients. Identification of risk factors for PEP is especially important in the field of ERCP practice because it may assist physicians in taking protective measures in situations with high risk. A decade ago, Freeman *et al* meticulously evaluated a large number of potentially relevant risk factors for PEP, which can be divided into patient-related and procedure-related issues. In this commentary, we summarize this classic article and reevaluate the risk factors for PEP from the current point of view. This is followed by assessment of strategies for prevention of PEP that can be divided into mechanical and pharmacologic methods.

© 2013 Baishideng. All rights reserved.

Key words: Endoscopic retrograde cholangiopancreatography; Post-endoscopic retrograde cholangiopancreatography pancreatitis; Risk factor; Prevention of post-endoscopic retrograde cholangiopancreatography pancreatitis; Pancreatic stents; Nonsteroidal antiinflammatory drugs

Moon SH, Kim MH. Prophecy about post-endoscopic retrograde cholangiopancreatography pancreatitis: From divination to science. *World J Gastroenterol* 2013; 19(5): 631-637 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i5/631.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i5.631

COMMENTARY ON HOT TOPICS

One unresolved issue of endoscopic retrograde cholangiopancreatography (ERCP) is post-ERCP pancreatitis (PEP), which occurs after 1% to 40% of procedures[1-4]. This variable frequency of PEP is due to a number of factors, including the definition used, the patient population, the type of maneuvers, the type and duration of patient follow-up, and the personnel performing the ERCP[2,3,5]. Postulated mechanisms for pancreatitis after ERCP include mechanical, chemical, hydrostatic, enzymatic, microbiologic, and thermal disruptions[1,2]. A widely used consensus definition for PEP is: (1) new or worsened abdominal pain; (2) new or prolongation of hospitalization for at least 2 d; and (3) serum amylase three-fold or more above the upper limit of normal, measured more than 24 h after the procedure[6,7].

Prediction of PEP is of utmost importance in the field of ERCP practice. Although PEP is sometimes inevitable and can occur in the best of hands, identification of its risk factors may assist physicians in taking protective measures in situations with high a priori risk[8,9]. This article is intended as a commentary on the classic article "Risk factors for post-ERCP pancreatitis: a prospective, multicenter study" by Freeman *et al*[1] published in *Gastro-*

intestinal Endoscopy, a landmark study in the field of PEP, and it also provides strategies for the prevention of PEP.

In the classic article[1], comprehensive evaluation of the risk factors for PEP were performed by 26 endoscopists from 11 centers in the United States (6 private practices and 5 university-affiliated teaching hospitals), who collaborated in a painstaking prospective study of almost 2000 procedures[10]. The authors of the classic article meticulously determined eye-opening numbers (n = 32) of potentially relevant risk factors for PEP and provided vigilant analysis.

Risk factors for PEP

In the classic article[1], the authors classified risk factors into clearly demarcated patient-related and procedure-related factors. This allowed them to demonstrate that the risk of PEP is determined as much by patient characteristics as by endoscopic technique or maneuvers. Stratification of patients into low-risk or high-risk categories for PEP is important in order to ensure that gastroenterologists remove borderline indications of ERCP in high-risk patients and that adequate pre-procedure information is provided to the patient[5].

Patient-related risk factors: Of the 16 patient-related risk factors identified, in the classic article, 5 variables were statistically significant by multivariate analysis: history of PEP, suspected sphincter of Oddi dysfunction, female gender, normal serum bilirubin, and absence of chronic pancreatitis. Although a few other factors, such as young age and absence of common bile duct stones, were only significant in univariate analysis, subsequent multivariate studies by other groups or further meta-analysis showed that young age, non-dilated extrahepatic ducts, and absence of common bile duct stones are factors that may increase the risk for PEP (Table 1)[3,5,6,11,12]. A recent multivariate study, published in abstract form, suggested that obesity and hyperlipidemia were independent risk factors for PEP based on a nationwide database analysis[13]. Interestingly, some of the patient-related risk factors, such as normal serum bilirubin, non-dilated extrahepatic ducts, and absence of common bile duct stones, are poor indicators of ERCP. This may attest to the statement by Cotton[10,14] that "ERCP is most dangerous for those who need it least".

Procedure-related risk factors: Of the 16 procedure-related risk factors identified, in the classic article, 4 variables were significant by multivariate analysis: biliary balloon sphincter dilation, moderate-to-difficult cannulation, pancreatic sphincterotomy, and pancreatic contrast injections. Subsequent multivariate studies by other groups or further meta-analysis showed that precut sphincterotomy, ampullectomy, pancreatic brush cytology, failure to clear bile duct stones, and involvement of trainees are all factors that may increase the risk for PEP (Table 2)[3,5,6,11,12].

Although pancreatic stent placement was associated with risk of pancreatitis by univariate analysis, no independent contribution of pancreatic stent placement was evident in the multivariate analysis because pancreatic stents were primarily placed in patients with multiple other independent risk factors for PEP. Actually, pancreatic stent placement can reduce the risk of PEP in a number of settings, so that prophylactic pancreatic stent placement has become a standard of care for reducing PEP in high-risk cases[15-17].

The classic article also indicated the importance of the general similarity of the overall risk of pancreatitis for diagnostic and therapeutic ERCP and showed that the performance of biliary sphincterotomy did not appear to add significant independent risk of pancreatitis to ERCP[1]. The article emphasized that these observations point not to the safety of sphincterotomy, but rather to the risk of diagnostic ERCP[1]. Despite the general similarity of the overall risk of PEP for diagnostic and therapeutic ERCP, certain high risk procedures such as pancreatic sphincterotomy, pancreatic brush cytology, and ampullectomy may increase the risk for PEP. Therefore, we should pay attention to the type and complexity of maneuvers when comparing the incidence of PEP between studies.

Interactive effect of patient-related and procedure-related risk factors: The striking message of the classic article was that patient-related and procedure-related risk factors are cumulative and perhaps even synergistic[1,10]. Subsequent studies confirmed that patients with multiple factors had an extremely high chance (up to 40%) of developing PEP[5,17-20]. The typical very high risk patient is a young to middle-aged woman with recurrent abdominal pain, normal serum bilirubin, no biliary obstruction, and difficult cannulation. The combinations of patient-related and procedure-related risk factors allow reliable prediction of the possibility that an individual patient will develop PEP[17]. Furthermore, this cumulative effect may influence the severity as well as the incidence of PEP. According to the literature[1,21-23], nearly all patients who developed severe or fatal pancreatitis after ERCP had multiple risk factors. Gastroenterologists are now more able to predict PEP.

Field of vision after a decade from the classic article: Even from the current point of view, the classic article investigated most of the potentially relevant risk factors for PEP, dividing these into patient-related and procedure-related issues. Over the past decade, subsequent studies have usually confirmed or just slightly altered the significance of these risk factors. The confirmation of the risk of diagnostic ERCP has focused the role of ERCP into an exclusively therapeutic modality with the advent of other diagnostic modalities[22]. Confirmed risk factors for PEP have also enabled the conception of several strategies for the prevention of PEP in actual practice[3,5,17,22,24,25].

Strategy for prevention of PEP

The most important goal of recognizing the risk factors for PEP is the development of a strategy for prevention

Table 1 Comparison of patient-related risk factors for post-endoscopic retrograde cholangiopancreatography pancreatitis by multivariate analysis in the classic article and current knowledge by meta-analysis or multivariate studies

Risk factors in the classic article	Current knowledge[1]
Significant in multivariate analysis	High risk factors
Suspected sphincter of Oddi dysfunction	Suspected sphincter of Oddi dysfunction
Female gender	Female gender
History of post-ERCP pancreatitis	Previous pancreatitis
Normal serum bilirubin	Normal serum bilirubin
Absence of chronic pancreatitis	Young age
Significant only in univariate analysis	Possible risk factors
Pancreas divisum	Non-dilated extrahepatic ducts
Recurrent abdominal pain	Absence of chronic pancreatitis
History of acute pancreatitis of any etiology	Absence of definite common bile duct stone
Cholangiogram normal	Obesity[2]
Pancreatogram normal	
Age < 55 yr	
Prior cholecystectomy	
Absence of definite common bile duct stone	
Not significant	Not related
Previous sphincterotomy	Pancreas divisum
Distal common bile duct diameter ⩽ 5 mm	Allergy to contrast media
Prior failed ERCP	Prior failed ERCP

[1]Current knowledge is based on recent guidelines by American Society for Gastrointestinal Endoscopy[6] and European Society of Gastrointestinal Endoscopy[5], and relevant articles[3,22]; [2]Based on recent multivariate analysis in abstract form[13]. ERCP: Endoscopic retrograde cholangiopancreatography.

Table 2 Comparison of procedure-related risk factors for post-endoscopic retrograde cholangiopancreatography pancreatitis by multivariate analysis in the classic article and current knowledge by meta-analysis or multivariate studies

Risk factors in the classic article	Current knowledge[1]
Significant in multivariate analysis	High risk factors
Difficult cannulation	Difficult or failed cannulation
Balloon dilation of biliary sphincter	Balloon dilation of biliary sphincter
Pancreatic sphincterotomy	Pancreatic sphincterotomy
⩾ 1 pancreatic contrast injections	Pancreatic duct injection
	Precut sphincterotomy
	Failed attempts at placing pancreatic duct stent
Significant only in univariate analysis	Possible risk factors
Sphincter of Oddi manometry	Ampullectomy
Pancreatic stent placement	Pancreatic acinarization
Minor papilla cannulation	Pancreatic brush cytology
Precut (access) papillotomy	Failure to clear bile duct stones
⩾ 1 pancreatic deep wire pass/cannulation	Involvement of trainee during ERCP
Endoscopist performing > 2 ERCP/wk	
Not significant	Not related
Acinarization of pancreas	Sphincter of Oddi manometry (using aspirated catheter)
Biliary sphincterotomy	Biliary sphincterotomy
Intramural contrast injection	Intramural contrast injection
Pancreatic stricture dilation by any method	Prior failed ERCP
Pancreatic duct tissue sampling by any method	Therapeutic *vs* diagnostic
Training fellow involved	

[1]Current knowledge is based on recent guidelines by American Society for Gastrointestinal Endoscopy[6] and European Society of Gastrointestinal Endoscopy[5], and relevant articles[3,22]. ERCP: Endoscopic retrograde cholangiopancreatography.

of PEP. A practical strategy appears to be the combination of careful patient selection - which means avoiding inappropriate ERCP in high-risk patients - and selection of appropriate preventive measures (Table 3)[2,6].

Patient selection: Gastroenterologists should take much more care in the selection of patients for ERCP. The clinical role of ERCP has diminished substantially with the advance of relevant diagnostic and therapeutic modalities[6,9,26,27]. For diagnostic purposes, a plethora of relevant diagnostic procedures now are available, such as magnetic resonance cholangiopancreatography and endoscopic ultrasonography (EUS), which may obviate the need for ERCP or better focus its application[9]. For therapeutic purposes, minimally invasive surgeries now show considerable improvements in safety and outcomes[9]. Thus, balancing the benefit with risk is a prerequisite for determining the indication for ERCP. If the potential risk

Table 3 Clinical pearls to help avoid post-endoscopic retrograde cholangiopancreatography pancreatitis

Remember that ERCP is the most dangerous endoscopic procedure that can be associated with bad outcomes
Instead of diagnostic ERCP, use alternative imaging techniques such as magnetic resonance cholangiopancreatography or EUS, especially in high-risk patients
Rectal NSAIDs before or after ERCP procedure can be a simple measure to prevent PEP
Tailor a variety of cannulation techniques to the individual risk profile and the papillary anatomy of the patient
In cases of difficult cannulation, early precut or fistulotomy technique with a pancreatic stent (performed by an expert endoscopist) can decrease the risk of PEP
Quit the ERCP procedure earlier in high-risk patients if success is not achieved quickly. After a failed ERCP, alternative therapeutic methods such as percutaneous or EUS-guided approaches can be considered
In high risk patients, make sure that a prophylactic pancreatic stent is placed. In cases with equivocal risk at the end of the procedure, a prophylactic pancreatic stent can eliminate the fear of PEP

ERCP: Endoscopic retrograde cholangiopancreatography; PEP: Post-endoscopic retrograde cholangiopancreatography pancreatitis; EUS: Endoscopic ultrasonography; NSAIDs: Non-steroidal anti-inflammatory drugs.

is much greater than the possible benefit, gastroenterologists should seek other diagnostic and/or therapeutic modalities or refer their high-risk patients to tertiary centers that have more experience and other tools.

Once a decision for ERCP is made, the gastroenterologist should reassess the risk profile of the patient and apply several mechanical and pharmacological interventions in order to reduce the likelihood of PEP[2]. In addition, an adequate consent process is very important to ensure that patients and their relatives understand that ERCP is potentially dangerous[14].

Mechanical prevention: Careful endoscopic techniques in cannulation and therapy are naturally important, but these are not sufficient to prevent PEP in high-risk patients[17]. Several modifications in endoscopic technique have been identified that can reduce the risk of PEP. (1) Prophylactic placement of pancreatic duct stents**:** The prophylactic placement of pancreatic duct stents improves the drainage of the manipulated pancreatic duct. Otherwise, this might be impaired by mechanical injury to the pancreatic sphincter from catheter and guidewire manipulation, and from thermal injury caused by biliary and pancreatic sphincterotomy[17]. The general consensus in the literature is that prophylactic pancreatic stents can reduce the risk of PEP in high-risk populations, such as those undergoing ampullectomy, pancreatic sphincterotomy, sphincter of Oddi manometry, precut sphincterotomy, pancreatic brush cytology, difficult cannulation, and pancreatic duct injection[3,8,17,28,29]. Recent meta-analyses of high-risk populations demonstrated that prophylactic pancreatic stents reduced the incidence of PEP from 18.6% to 5.6%[28,29]. An elegant cost-effectiveness analysis also suggested that prophylactic pancreatic stents in high-risk patients were a cost-effective strategy[30]. Actually, the routine use of pancreatic stents in high-risk cases has reduced the incidence and severity of PEP to a more acceptable level in advanced centers, allaying the fear of ERCP in high risk settings[17,31]; (2) Wire-guided cannulation: Compared with conventional use of contrast injection, wire-guided cannulation may avoid inadvertent injection of contrast into the pancreatic duct and decrease the risk of PEP[3,8]. Several meta-analyses have demonstrated a greater success of biliary cannulation and a lowered risk of PEP[21,32], but recent studies have shown that guidewire manipulation of the pancreatic duct for guidewire biliary cannulation is an another independent risk factor for PEP[33-35]. Prophylactic pancreatic stents might be recommended after pancreatic-guidewire assisted biliary cannulation to reduce the incidence of PEP[33]; and (3) Early precut biliary sphincterotomy and fistulotomy in cases of difficult cannulation: Although precut biliary sphincterotomy is an independent risk factor for PEP, prolonged cannulation attempts using standard techniques may also impart a higher risk for PEP than does the precut biliary sphincterotomy itself[5,36-39]. Early precut technique can be considered in cases of difficult biliary cannulation by advanced endoscopists with expertise in various cannulation techniques[5]. Fistulotomy is a variation of the precut needle-knife techniques that creates a direct bilio-enteric fistula by making an incision at the upper end of the ampullary region of the major papilla[40,41]. This technique has a potential advantage in that it evades the pancreatic orifice, which is the probable site of initiation of the cascade of PEP. Several studies have reported a lower incidence of PEP (up to 0%) in patients who underwent fistulotomy, with or without the prophylactic pancreatic stent[40-43]. Fistulotomy, however, may be more feasible in patients with bulging papillae and clear landmarks than in patients with tiny or diminutive papillae[40, 44]. For sparing the pancreatic orifice, reported investigational techniques include suprapapillary puncture with a needle-tip catheter, blunt dissection using a cotton swab, and EUS-guided suprapapillary puncture[39,45-47].

Pharmacologic prevention: Numerous trials have been attempted with many kinds of pharmacologic agents in order to reduce the risk of PEP. Pharmacologic agents, based on various theoretical benefits, have included nitroglycerin, ceftazidime, somatostatin, octreotide, gabexate, ulinastatin, nafamostat, antioxidants, allopurinol, glucocorticoid, non-steroidal anti-inflammatory drugs (NSAIDs), and *etc.*[5,24,48-52]. Early studies suggested that protease inhibitors such as gabexate or nafamostat may decrease the incidence of PEP[53,54]. At present, the dismally universal finding is that a strategy of pharmacologic prevention that proves effective in a few trials ultimately yields largely disappointing results over the long term

Table 4 Unresolved issues with prevention of post-endoscopic retrograde cholangiopancreatography pancreatitis

The ideal design of a prophylactic pancreatic stent
Cannulation technique to lower incidence of PEP, tailored to the shape of the major papilla
The ideal pharmacologic agent
Comparison of rectal NSAIDs *vs* pancreatic stent placement *vs* combination in high risk patients
The route (rectal or intravenous) and the timing (before or after ERCP) of NSAIDs administration

ERCP: Endoscopic retrograde cholangiopancreatography; PEP: Post-ERCP pancreatitis; NSAIDs: Non-steroidal anti-inflammatory drugs.

when adopted in routine clinical practice[55,56]. Endoscopists in the ERCP field appear to believe that mechanical techniques such as pancreatic stents, much more than pharmacologic prophylaxis, play a key role in the prevention of PEP[55].

Despite a current climate of skepticism regarding the efficacy of any prophylactic medication for PEP, evidence for the efficacy of rectal NSAIDs in reducing PEP continues to accumulate[15,57-60]. Rectal NSAIDs are particularly attractive because of their low cost, easy administration, and known favorable risk profiles.[15] In addition to several meta-analyses[15,58,59], a recent well-designed randomized controlled trial beautifully showed the effect of rectal indomethacin in preventing PEP in 602 high risk patients (9.2% in the indomethacin group *vs* 16.9% in the placebo group, $P = 0.005$)[57]. However, only time will tell whether rectal NSAIDs can significantly reduce PEP.

Selection of preventive measures: According to the European Society of Gastrointestinal Endoscopy guidelines, periprocedural rectal administration of NSAIDs is recommended for low-risk ERCPs, whereas prophylactic pancreatic stent placement should be strongly considered for high-risk ERCPs[5]. However, the combined use of NSAIDs and prophylactic pancreatic stent placement might further reduce the rate of PEP in high-risk patients[61]. Therefore, rectal administration of NSAIDs for all ERCPs and prophylactic pancreatic stent placement for high-risk ERCPs might be more practical. A further practical strategy might be rectal administration of NSAIDs for patient-related high risk and prophylactic pancreatic stent placement for procedure-related high risk. However, the possibility remains that rectal NSAIDs may obviate the need for prophylactic pancreatic stent placement[61]. Studies to compare the effectiveness of rectal NSAIDs and pancreatic stent placement in high risk patients are warranted.

Future prospects for research

Although PEP has benefited from evolved understanding, there is still room for continuing research. In the future, the individual incidence of PEP should be accurately calculated according to the previously listed patient-related and procedure-related risk factors for PEP. In addition, the complexity of ERCP procedures[62,63] should be incorporated into the calculation of the individual incidence of PEP. Regarding the prevention of PEP, many issues still remain to be resolved (Table 4).

In conclusion, more than a decade has passed since the publication of the classic article that revealed the risk factors for PEP. Subsequent multivariate analyses have confirmed a number of risk factors for PEP that can be divided into patient-related and procedure-related issues. Prophylactic pancreatic stent placement has become a standard of care in high-risk patients and rectal NSAIDs have become a potential candidate as an ideal pharmacologic agent for preventing PEP. However, PEP is still the most frequent and most feared complication of ERCP. In the past decade, indications of ERCP have become more stringent owing to the development of other diagnostic and therapeutic modalities. To minimize PEP and maximize benefits[9], ERCP should be done for the best indications, while recognizing accurate risks to the individual and using meticulous endoscopic techniques with optimal preventive measures.

REFERENCES

1 **Freeman ML**, DiSario JA, Nelson DB, Fennerty MB, Lee JG, Bjorkman DJ, Overby CS, Aas J, Ryan ME, Bochna GS, Shaw MJ, Snady HW, Erickson RV, Moore JP, Roel JP. Risk factors for post-ERCP pancreatitis: a prospective, multicenter study. *Gastrointest Endosc* 2001; **54**: 425-434 [PMID: 11577302 DOI: 10.1067/mge.2001.117550]

2 **Freeman ML**, Guda NM. Prevention of post-ERCP pancreatitis: a comprehensive review. *Gastrointest Endosc* 2004; **59**: 845-864 [PMID: 15173799 DOI: 10.1016/S0016-5107(04)00353-0]

3 **Cooper ST**, Slivka A. Incidence, risk factors, and prevention of post-ERCP pancreatitis. *Gastroenterol Clin North Am* 2007; **36**: 259-276, vii-viii [PMID: 17533078 DOI: 10.1016/j.gtc.2007.03.006]

4 **Woods KE**, Willingham FF. Endoscopic retrograde cholangiopancreatography associated pancreatitis: A 15-year review. *World J Gastrointest Endosc* 2010; **2**: 165-178 [PMID: 21160744 DOI: 10.4253/wjge.v2.i5.165]

5 **Dumonceau JM**, Andriulli A, Deviere J, Mariani A, Rigaux J, Baron TH, Testoni PA. European Society of Gastrointestinal Endoscopy (ESGE) Guideline: prophylaxis of post-ERCP pancreatitis. *Endoscopy* 2010; **42**: 503-515 [PMID: 20506068 DOI: 10.1055/s-0029-1244208]

6 **Anderson MA**, Fisher L, Jain R, Evans JA, Appalaneni V, Ben-Menachem T, Cash BD, Decker GA, Early DS, Fanelli RD, Fisher DA, Fukami N, Hwang JH, Ikenberry SO, Jue TL, Khan KM, Krinsky ML, Malpas PM, Maple JT, Sharaf RN, Shergill AK, Dominitz JA. Complications of ERCP. *Gastrointest Endosc* 2012; **75**: 467-473 [PMID: 22341094 DOI: 10.1016/j.gie.2011.07.010]

7 **Cotton PB**, Lehman G, Vennes J, Geenen JE, Russell RC, Meyers WC, Liguory C, Nickl N. Endoscopic sphincterotomy complications and their management: an attempt at consensus. *Gastrointest Endosc* 1991; **37**: 383-393 [PMID: 2070995]

8 **Aabakken L**. Endoscopic retrograde cholangiopancreatography. *Gastrointest Endosc* 2012; **76**: 516-520 [PMID: 22898410 DOI: 10.1016/j.gie.2012.06.029]

9 **Cotton PB**. Endoscopic retrograde cholangiopancreatography: maximizing benefits and minimizing risks. *Gastrointest*

Endosc Clin N Am 2012; **22**: 587-599 [PMID: 22748250 DOI: 10.1016/j.giec.2012.05.002]
10 **Cotton PB**. ERCP is most dangerous for people who need it least. *Gastrointest Endosc* 2001; **54**: 535-536 [PMID: 11577330]
11 **Masci E**, Mariani A, Curioni S, Testoni PA. Risk factors for pancreatitis following endoscopic retrograde cholangiopancreatography: a meta-analysis. *Endoscopy* 2003; **35**: 830-834 [PMID: 14551860 DOI: 10.1055/s-2003-42614]
12 **Williams EJ**, Taylor S, Fairclough P, Hamlyn A, Logan RF, Martin D, Riley SA, Veitch P, Wilkinson ML, Williamson PR, Lombard M. Risk factors for complication following ERCP; results of a large-scale, prospective multicenter study. *Endoscopy* 2007; **39**: 793-801 [PMID: 17703388 DOI: 10.1055/s-2007-966723]
13 **Kumar N**, Gergi MA, Thompson CC. Obesity is an independent risk factor for post-ERCP pancreatitis: results of a nationwide database analysis. *Gastrointest Endosc* 2012; **75**(4S): AB142
14 **Cotton PB**. Analysis of 59 ERCP lawsuits; mainly about indications. *Gastrointest Endosc* 2006; **63**: 378-82; quiz 464 [PMID: 16500382 DOI: 10.1016/j.gie.2005.06.046]
15 **Elmunzer BJ**, Waljee AK, Elta GH, Taylor JR, Fehmi SM, Higgins PD. A meta-analysis of rectal NSAIDs in the prevention of post-ERCP pancreatitis. *Gut* 2008; **57**: 1262-1267 [PMID: 18375470 DOI: 10.1136/gut.2007.140756]
16 **Freeman ML**. Post-ERCP pancreatitis: patient and technique-related risk factors. *JOP* 2002; **3**: 169-176 [PMID: 12432183]
17 **Freeman ML**. Pancreatic stents for prevention of post-endoscopic retrograde cholangiopancreatography pancreatitis. *Clin Gastroenterol Hepatol* 2007; **5**: 1354-1365 [PMID: 17981248 DOI: 10.1016/j.cgh.2007.09.007]
18 **Andriulli A**, Forlano R, Napolitano G, Conoscitore P, Caruso N, Pilotto A, Di Sebastiano PL, Leandro G. Pancreatic duct stents in the prophylaxis of pancreatic damage after endoscopic retrograde cholangiopancreatography: a systematic analysis of benefits and associated risks. *Digestion* 2007; **75**: 156-163 [PMID: 17684365 DOI: 10.1159/000106774]
19 **Fazel A**, Quadri A, Catalano MF, Meyerson SM, Geenen JE. Does a pancreatic duct stent prevent post-ERCP pancreatitis? A prospective randomized study. *Gastrointest Endosc* 2003; **57**: 291-294 [PMID: 12612504 DOI: 10.1067/mge.2003.124]
20 **Harewood GC**, Pochron NL, Gostout CJ. Prospective, randomized, controlled trial of prophylactic pancreatic stent placement for endoscopic snare excision of the duodenal ampulla. *Gastrointest Endosc* 2005; **62**: 367-370 [PMID: 16111953 DOI: 10.1016/j.gie.2005.04.020]
21 **Cennamo V**, Fuccio L, Zagari RM, Eusebi LH, Ceroni L, Laterza L, Fabbri C, Bazzoli F. Can a wire-guided cannulation technique increase bile duct cannulation rate and prevent post-ERCP pancreatitis?: A meta-analysis of randomized controlled trials. *Am J Gastroenterol* 2009; **104**: 2343-2350 [PMID: 19532133 DOI: 10.1038/ajg.2009.269]
22 **Freeman ML**. Complications of endoscopic retrograde cholangiopancreatography: avoidance and management. *Gastrointest Endosc Clin N Am* 2012; **22**: 567-586 [PMID: 22748249 DOI: 10.1016/j.giec.2012.05.001]
23 **Trap R**, Adamsen S, Hart-Hansen O, Henriksen M. Severe and fatal complications after diagnostic and therapeutic ERCP: a prospective series of claims to insurance covering public hospitals. *Endoscopy* 1999; **31**: 125-130 [PMID: 10223360 DOI: 10.1055/s-1999-13659]
24 **Feurer ME**, Adler DG. Post-ERCP pancreatitis: review of current preventive strategies. *Curr Opin Gastroenterol* 2012; **28**: 280-286 [PMID: 22450899 DOI: 10.1097/MOG.0b013e3283528e68]
25 **Dumonceau JM**, Rigaux J, Kahaleh M, Gomez CM, Vandermeeren A, Devière J. Prophylaxis of post-ERCP pancreatitis: a practice survey. *Gastrointest Endosc* 2010; **71**: 934-939 [PMID: 20226455 DOI: 10.1016/j.gie.2009.10.055]
26 **Simmons DT**, Baron TH. pERCePtions on ERCP utilization in the United States. *Am J Gastroenterol* 2007; **102**: 976-977 [PMID: 17489782 DOI: 10.1111/j.1572-0241.2007.01121.x]
27 **Mazen Jamal M**, Yoon EJ, Saadi A, Sy TY, Hashemzadeh M. Trends in the utilization of endoscopic retrograde cholangiopancreatography (ERCP) in the United States. *Am J Gastroenterol* 2007; **102**: 966-975 [PMID: 17319932 DOI: 10.1111/j.1572-0241.2007.01127.x]
28 **Mazaki T**, Masuda H, Takayama T. Prophylactic pancreatic stent placement and post-ERCP pancreatitis: a systematic review and meta-analysis. *Endoscopy* 2010; **42**: 842-853 [PMID: 20886403 DOI: 10.1055/s-0030-1255781]
29 **Choudhary A**, Bechtold ML, Arif M, Szary NM, Puli SR, Othman MO, Pais WP, Antillon MR, Roy PK. Pancreatic stents for prophylaxis against post-ERCP pancreatitis: a meta-analysis and systematic review. *Gastrointest Endosc* 2011; **73**: 275-282 [PMID: 21295641 DOI: 10.1016/j.gie.2010.10.039]
30 **Das A**, Singh P, Sivak MV, Chak A. Pancreatic-stent placement for prevention of post-ERCP pancreatitis: a cost-effectiveness analysis. *Gastrointest Endosc* 2007; **65**: 960-968 [PMID: 17331513 DOI: 10.1016/j.gie.2006.07.031]
31 **Freeman ML**. Pancreatic stents for prevention of post-ERCP pancreatitis: for everyday practice or for experts only? *Gastrointest Endosc* 2010; **71**: 940-944 [PMID: 20438883 DOI: 10.1016/j.gie.2009.12.043]
32 **Shao LM**, Chen QY, Chen MY, Cai JT. Can wire-guided cannulation reduce the risk of post-endoscopic retrograde cholangiopancreatography pancreatitis? A meta-analysis of randomized controlled trials. *J Gastroenterol Hepatol* 2009; **24**: 1710-1715 [PMID: 20136957 DOI: 10.1111/j.1440-1746.2009.06010.x]
33 **Ito K**, Fujita N, Noda Y, Kobayashi G, Obana T, Horaguchi J, Takasawa O, Koshita S, Kanno Y, Ogawa T. Can pancreatic duct stenting prevent post-ERCP pancreatitis in patients who undergo pancreatic duct guidewire placement for achieving selective biliary cannulation? A prospective randomized controlled trial. *J Gastroenterol* 2010; **45**: 1183-1191 [PMID: 20607310 DOI: 10.1007/s00535-010-0268-7]
34 **Wang P**, Li ZS, Liu F, Ren X, Lu NH, Fan ZN, Huang Q, Zhang X, He LP, Sun WS, Zhao Q, Shi RH, Tian ZB, Li YQ, Li W, Zhi FC. Risk factors for ERCP-related complications: a prospective multicenter study. *Am J Gastroenterol* 2009; **104**: 31-40 [PMID: 19098846 DOI: 10.1038/ajg.2008.5]
35 **Mariani A**, Giussani A, Di Leo M, Testoni S, Testoni PA. Guidewire biliary cannulation does not reduce post-ERCP pancreatitis compared with the contrast injection technique in low-risk and high-risk patients. *Gastrointest Endosc* 2012; **75**: 339-346 [PMID: 22075192 DOI: 10.1016/j.gie.2011.09.002]
36 **Cennamo V**, Fuccio L, Zagari RM, Eusebi LH, Ceroni L, Laterza L, Fabbri C, Bazzoli F. Can early precut implementation reduce endoscopic retrograde cholangiopancreatography-related complication risk? Meta-analysis of randomized controlled trials. *Endoscopy* 2010; **42**: 381-388 [PMID: 20306386 DOI: 10.1055/s-0029-1243992]
37 **Gong B**, Hao L, Bie L, Sun B, Wang M. Does precut technique improve selective bile duct cannulation or increase post-ERCP pancreatitis rate? A meta-analysis of randomized controlled trials. *Surg Endosc* 2010; **24**: 2670-2680 [PMID: 20414680 DOI: 10.1007/s00464-010-1033-y]
38 **Testoni PA**, Giussani A, Vailati C, Testoni S, Di Leo M, Mariani A. Precut sphincterotomy, repeated cannulation and post-ERCP pancreatitis in patients with bile duct stone disease. *Dig Liver Dis* 2011; **43**: 792-796 [PMID: 21733768 DOI: 10.1016/j.dld.2011.05.010]
39 **Baillie J**. Advanced cannulation technique and precut. *Gastrointest Endosc Clin N Am* 2012; **22**: 417-434 [PMID: 22748240 DOI: 10.1016/j.giec.2012.05.010]
40 **Freeman ML**, Guda NM. ERCP cannulation: a review of reported techniques. *Gastrointest Endosc* 2005; **61**: 112-125 [PMID: 15672074]
41 **Madácsy L**, Kurucsai G, Fejes R, Székely A, Székely I. Prophylactic pancreas stenting followed by needle-knife fistulotomy in patients with sphincter of Oddi dysfunction and

difficult cannulation: new method to prevent post-ERCP pancreatitis. *Dig Endosc* 2009; **21**: 8-13 [PMID: 19691794 DOI: 10.1111/j.1443-1661.2008.00819.x]
42 **Harewood GC**, Baron TH. An assessment of the learning curve for precut biliary sphincterotomy. *Am J Gastroenterol* 2002; **97**: 1708-1712 [PMID: 12135022 DOI: 10.1111/j.1572-0241.2002.05829.x]
43 **Mavrogiannis C**, Liatsos C, Romanos A, Petoumenos C, Nakos A, Karvountzis G. Needle-knife fistulotomy versus needle-knife precut papillotomy for the treatment of common bile duct stones. *Gastrointest Endosc* 1999; **50**: 334-339 [PMID: 10462652 DOI: 10.1053/ge.1999.v50.98593]
44 **Freeman ML**. Cannulation techniques for ERCP: one size does not fit all. *Gastrointest Endosc* 2007; **65**: 132-133 [PMID: 17185092 DOI: 10.1016/j.gie.2006.07.038]
45 **Artifon EL**, Sakai P, Ishioka S, Hondo FY, Raju GS. Suprapapillary puncture of the common bile duct for selective biliary access: a novel technique (with videos). *Gastrointest Endosc* 2007; **65**: 124-131 [PMID: 17185091 DOI: 10.1016/j.gie.2006.06.032]
46 **Hashiba K**, D'Assunção MA, Armellini S, Hassegawa RT, Cappellanes CA, Moribe D. Endoscopic suprapapillary blunt dissection of the distal common bile duct in cases of difficult cannulation: a pilot series. *Endoscopy* 2004; **36**: 317-321 [PMID: 15057681 DOI: 10.1055/s-2004-814410]
47 **Perez-Miranda M**, Barclay RL, Kahaleh M. Endoscopic ultrasonography-guided endoscopic retrograde cholangiopancreatography: endosonographic cholangiopancreatography. *Gastrointest Endosc Clin N Am* 2012; **22**: 491-509 [PMID: 22748245 DOI: 10.1016/j.giec.2012.05.004]
48 **Foster E**, Leung J. Pharmacotherapy for the prevention of post-ERCP pancreatitis. *Am J Gastroenterol* 2007; **102**: 52-55 [PMID: 17266688 DOI: 10.1111/j.1572-0241.2006.00950.x]
49 **Seta T**, Noguchi Y. Protease inhibitors for preventing complications associated with ERCP: an updated meta-analysis. *Gastrointest Endosc* 2011; **73**: 700-706.e1-2 [PMID: 21145053 DOI: 10.1016/j.gie.2010.09.022]
50 **Yoo KS**, Huh KR, Kim YJ, Kim KO, Park CH, Hahn T, Park SH, Kim JH, Park CK, Kwon YJ, Lehman GA. Nafamostat mesilate for prevention of post-endoscopic retrograde cholangiopancreatography pancreatitis: a prospective, randomized, double-blind, controlled trial. *Pancreas* 2011; **40**: 181-186 [PMID: 21206331 DOI: 10.1097/MPA.0b013e3181f94d46]
51 **Mohseni Salehi Monfared SS**, Vahidi H, Abdolghaffari AH, Nikfar S, Abdollahi M. Antioxidant therapy in the management of acute, chronic and post-ERCP pancreatitis: a systematic review. *World J Gastroenterol* 2009; **15**: 4481-4490 [PMID: 19777606 DOI: 10.3748/wjg.15.4481]
52 **Martinez-Torres H**, Rodriguez-Lomeli X, Davalos-Cobian C, Garcia-Correa J, Maldonado-Martinez JM, Medrano-Muñoz F, Fuentes-Orozco C, Gonzalez-Ojeda A. Oral allopurinol to prevent hyperamylasemia and acute pancreatitis after endoscopic retrograde cholangiopancreatography. *World J Gastroenterol* 2009; **15**: 1600-1606 [PMID: 19340902]
53 **Manes G**, Ardizzone S, Lombardi G, Uomo G, Pieramico O, Porro GB. Efficacy of postprocedure administration of gabexate mesylate in the prevention of post-ERCP pancreatitis: a randomized, controlled, multicenter study. *Gastrointest Endosc* 2007; **65**: 982-987 [PMID: 17531632 DOI: 10.1016/j.gie.2007.02.055]
54 **Park KT**, Kang DH, Choi CW, Cho M, Park SB, Kim HW, Kim DU, Chung CW, Yoon KT. Is high-dose nafamostat mesilate effective for the prevention of post-ERCP pancreatitis, especially in high-risk patients? *Pancreas* 2011; **40**: 1215-1219 [PMID: 21775918 DOI: 10.1097/MPA.0b013e31822116d5]
55 **Testoni PA**. Facts and fiction in the pharmacologic prevention of post-ERCP pancreatitis: a never-ending story. *Gastrointest Endosc* 2006; **64**: 732-734 [PMID: 17055866 DOI: 10.1016/j.gie.2006.07.004]
56 **Testoni PA**. Simple measures to prevent post-ERCP pancreatitis? *Gut* 2008; **57**: 1197-1198 [PMID: 18719133 DOI: 10.1136/gut.2008.153080]
57 **Elmunzer BJ**, Scheiman JM, Lehman GA, Chak A, Mosler P, Higgins PD, Hayward RA, Romagnuolo J, Elta GH, Sherman S, Waljee AK, Repaka A, Atkinson MR, Cote GA, Kwon RS, McHenry L, Piraka CR, Wamsteker EJ, Watkins JL, Korsnes SJ, Schmidt SE, Turner SM, Nicholson S, Fogel EL. A randomized trial of rectal indomethacin to prevent post-ERCP pancreatitis. *N Engl J Med* 2012; **366**: 1414-1422 [PMID: 22494121 DOI: 10.1056/NEJMoa1111103]
58 **Dai HF**, Wang XW, Zhao K. Role of nonsteroidal anti-inflammatory drugs in the prevention of post-ERCP pancreatitis: a meta-analysis. *Hepatobiliary Pancreat Dis Int* 2009; **8**: 11-16 [PMID: 19208508]
59 **Zheng MH**, Xia HH, Chen YP. Rectal administration of NSAIDs in the prevention of post-ERCP pancreatitis: a complementary meta-analysis. *Gut* 2008; **57**: 1632-1633 [PMID: 18941015]
60 **Ding X**, Chen M, Huang S, Zhang S, Zou X. Nonsteroidal anti-inflammatory drugs for prevention of post-ERCP pancreatitis: a meta-analysis. *Gastrointest Endosc* 2012; **76**: 1152-1159 [PMID: 23164513 DOI: 10.1016/j.gie.2012.08.021]
61 **Baron TH**, Abu Dayyeh BK, Zinsmeister AR. Rectal indomethacin to prevent post-ERCP pancreatitis. *N Engl J Med* 2012; **367**: 277-28; author reply 277-28; [PMID: 22808973 DOI: 10.1056/NEJMc1205928#SA1]
62 **Cotton PB**, Eisen G, Romagnuolo J, Vargo J, Baron T, Tarnasky P, Schutz S, Jacobson B, Bott C, Petersen B. Grading the complexity of endoscopic procedures: results of an ASGE working party. *Gastrointest Endosc* 2011; **73**: 868-874 [PMID: 21377673 DOI: 10.1016/j.gie.2010.12.036]
63 **Schutz SM**, Abbott RM. Grading ERCPs by degree of difficulty: a new concept to produce more meaningful outcome data. *Gastrointest Endosc* 2000; **51**: 535-539 [PMID: 10805837]

P- Reviewers Urbain D, Gonzalez-Ojeda A **S- Editor** Gou SX
L- Editor A **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i5.638

World J Gastroenterol 2013 February 7; 19(5): 638-647
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

TOPIC HIGHLIGHT

Luis Bujanda, PhD, Professor, *Series Editor*

Alcohol consumption on pancreatic diseases

Marta Herreros-Villanueva, Elizabeth Hijona, Jesus Maria Bañales, Angel Cosme, Luis Bujanda

Marta Herreros-Villanueva, Schulze Center for Novel Therapeutics, Division of Oncology Research, Department of Medicine, Mayo Clinic, Rochester, MA 55905, United States
Marta Herreros-Villanueva, Elizabeth Hijona, Jesus Maria Bañales, Angel Cosme, Luis Bujanda, Department of Gastroenterology, Center for Biomedical Research in Digestive and Liver Diseases Network (CIBERehd), Donostia Hospital, University of the Basque UPV/EHU, 20010 San Sebastian, Spain
Author contributions: Herreros-Villanueva M, Hijona E, Bañales JM, Cosme A and Bujanda L designed and wrote the review.
Supported by CIBERehd Funded by the Health Institute of Carlos III
Correspondence to: Luis Bujanda, MD, PhD, Professor, Department of Gastroenterology, Center for Biomedical Research in Digestive and Liver Diseases Network (CIBERehd), Donostia Hospital, University of the Basque UPV/EHU, Avda Sancho El Sabio 17-2, 20010 San Sebastian, Spain. medik@telefonica.net
Telephone: +34-94-3007173 Fax: +34-94-3007065
Received: June 20, 2012 Revised: August 14, 2012
Accepted: August 16, 2012
Published online: February 7, 2013

Abstract

Although the association between alcohol and pancreatic diseases has been recognized for a long time, the impact of alcohol consumption on pancreatitis and pancreatic cancer (PC) remains poorly defined. Nowadays there is not consensus about the epidemiology and the beverage type, dose and duration of alcohol consumption causing these diseases. The objective of this study was to review the epidemiology described in the literature for pancreatic diseases as a consequence of alcoholic behavior trying to understand the association between dose, type and frequency of alcohol consumption and risk of pancreatitis and PC. The majority of the studies conclude that high alcohol intake was associated with a higher risk of pancreatitis (around 2.5%-3% between heavy drinkers and 1.3% between non drinkers). About 70% of pancreatitis are due to chronic heavy alcohol consumption. Although this incidence rate differs between countries, it is clear that the risk of developing pancreatitis increases with increasing doses of alcohol and the average of alcohol consumption vary since 80 to 150 g/d for 10-15 years. With regard to PC, the role of alcohol consumption remains less clear, and low to moderate alcohol consumption do not appear to be associated with PC risk, and only chronic heavy drinking increase the risk compared with lightly drinkers. In a population of 10%-15% of heavy drinkers, 2%-5% of all PC cases could be attributed to alcohol consumption. However, as only a minority (less than 10% for pancreatitis and 5% for PC) of heavily drinkers develops these pancreatic diseases, there are other predisposing factors besides alcohol involved. Genetic variability and environmental exposures such as smoking and diet modify the risk and should be considered for further investigations.

© 2013 Baishideng. All rights reserved.

Key words: Acute pancreatitis; Chronic pancreatitis; Alcohol consumption; Alcohol metabolism; Genetic variability; Pancreatic cancer; Risk

Herreros-Villanueva M, Hijona E, Bañales JM, Cosme A, Bujanda L. Alcohol consumption on pancreatic diseases. *World J Gastroenterol* 2013; 19(5): 638-647 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i5/638.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i5.638

INTRODUCTION

Alcohol causes different diseases and they are considered an important medical and social burden on society. Alcohol has been identified as a leading risk factor for death and disability globally, accounting for 3.8% of death and 4.6% of disability adjusted life years lost in 2004[1].

Pancreas is one of the most important organs adversely affected by alcohol consumption. An association between alcohol abuse and pancreatic injury was reported by Friedreich[2] as early as 1878. Freidreich recognized an

association of alcohol abuse with chronic pancreatic injure.

Since Friedreich's initial observation, many studies have confirmed that excessive alcohol intake is associated with pancreatic damage. Nowadays it is widely recognized that pancreatic injure due to alcohol consumption ranges from isolated episodes of acute pancreatitis (AP) to chronic manifestations that with time could move to pancreatic cancer (PC). However, there is not a consensus in the epidemiology and it is no clear how different drinks or dose of alcohol affect to the development of pancreatic diseases and finally, how drinking trigger pancreatic injury only in a minority of alcoholics.

Here, we review different studies and meta-analysis that have been published in the last years relating alcohol and pancreatic diseases as AP, chronic pancreatitis (CP) and PC. We summarize the effects of alcohol consumption in these diseases and at the end we present the possible mechanisms that have been proposed as a cause of this pancreatic pathogenesis.

Alcohol consumption measures

The incidence and prevalence of alcoholic pancreatic diseases are difficult to ascertain with precision, in part because of the variable considerations of drinking behavior and alcohol intakes measures.

Most of the studies in cohorts measures alcohol intake from wine, beer, and spirits separately. The final value of the daily alcohol intake is expressed in grams for each beverage based on the frequency of consumption, the alcohol content of the beverage, and the average quantity consumed. The United States Department of Agriculture established that alcoholic beverages are 12.8 g for 335 mL of alcohol for a bottle of beer (12-oz, 5% alcohol), 11.0 g for 118 mL (4-oz, 12% alcohol) glass of wine, and 14.0 g for 44 mL (1.5 oz, 40% alcohol) of 80-proof liquor.

The definition of a "standard drinker" differs between countries[3,4]: in United States it is 14 to 15 g, in Great Britain is 8 g and 19.75 g of alcohol in Japan. In addition to the amount to alcohol in one drink, the cut points to define "moderate" and "heavy" drinking also vary. In general, and to simplify a "standard drink" can be considered equal to 10 g of alcohol. A glass of wine, beer or half a glass of liquor would equivalente a beverage. For example in United States the standards are as following: moderate drinking is for women less than 2 drinks per day and less than 3 drinks for men; Heavy drinking is more than 7 drinks per week or 3 per occasion and more than 14 drinks per week or 4 per occasion; Bingo drinkers is 4 or more drinks in a row for women and more than 5 for men[5].

PANCREATITIS

Alcoholic pancreatitis is a potentially fatal illness that may be short term (*i.e.*, acute) or long term (*i.e.*, chronic). The relationship between acute and CP is complex. Symptoms are shared by acute and CP and include abdominal pain and interference with normal pancreatic functions. Although the prevalence of alcoholic pancreatitis in the population is unknown, clinicians usually agree that both acute and chronic alcoholic pancreatitis are responsible for a significant amount of illness and death in the world. However, the proportion of cases of pancreatitis attributed to alcohol varies widely among countries and even among different studies in the same country.

There are not universally accepted criteria to assign alcohol as an etiology of patient's pancreatitis but experts defines that varying from consumption of over 50 to 80 g (4-7 drinks/d) with or without a minimum drinking duration[6-9]. An international consensus defined alcoholic CP based on typical clinical history, threshold alcohol consumption (80 g or more of alcohol for a few years in males and less in females) and morphological evidence of CP on imaging studies or histology[10].

Besides the alcohol intake, other list of etiologies have been described as factors that could increase the risk for pancreatitis as gallstones, cystic fibrosis, hyperlipidemia, hyperparathyroidism, pancreas divisum or traumas, infections, genetic factors and autoimmune disease[11,12].

There is only a few numbers of epidemiological studies on the quantitative aspects of risk estimations for pancreatitis in relation to alcohol. In 2004, Corrao *et al*[13] conducted a meta-analysis of case-control and cohort studies published between 1966 and 1995 that assessed the association between alcohol consumption and CP. Then, Irving *et al*[14] in a review and meta-analysis published in 2009 analyzed studies published between 1980 and 2008 and assessed the association between alcohol consumption and the risk of pancreatitis. They concluded that the threshold between alcohol consumption and pancreatitis is 4 drinks daily.

However, about 70% of pancreatitis cases are believed to be attributable to chronic, heavy alcohol consumption but this percentage differs between countries[13,15,16]. Autopsies done on alcohol abusers have shown that up to 75% of them present CP[17]. On another hand, as less than 10% of consumers of alcohol in excess develop CP, other factors modify ethanol toxicity *in vivo*[16].

ACUTE PANCREATITIS

Historically it has been well-known that the risk of developing AP increases with increasing doses of alcohol[18] and with the duration of alcohol abuse[19], although epidemiological studies have shown that only a minority of heavy drinkers develop evident pancreatitis episodes[20,21]. This correlation shows again that besides of alcohol effects, additional factors are involved in AP develop.

Alcohol is the second most common cause of AC after gallstones[22]. The clinical features at the time of initial presentation are abdominal pain, jaundice, distal common bile duct obstruction and exocrine or endocrine insufficiency. Generally, the onset of alcoholic pancreatitis occurs in the 4th decade of males with an average of alcohol consumption around 150 g/d for a period of

Table 1 Alcoholic pancreatitis epidemiology in the different countries

Ref.	Country	Alcoholic etiology
Layer *et al*[7]	United States	56%
Coté *et al*[31]	United States	45%
Yadav *et al*[32]	United States	51%
Lin *et al*[36]	Japan	56%
Cavallini *et al*[6]	Italy	43%
Frulloni *et al*[37]	Italy	79%
Lankisch *et al*[9]	Germany	78%
Nøjgaard *et al*[29]	Denmark	44%
Díte *et al*[33]	Czech Republic	60%
Ammann *et al*[38]	Switzerland	71%
Robles-Díaz *et al*[39]	Mexico	68%
Dani *et al*[40]	Brazil	90%
Marks *et al*[41]	South Africa	80%
Balakrishnan *et al*[42]	India	33%

10-15 years[23]. Initially patients present acute abdominal pain, elevated serum levels of pancreatic enzymes and evidence of pancreatic damage in imaging studies.

These acute toxic effects of alcohol on the pancreas are considered AP but the progression of acute episodes (potentially reversible) leads to chronic disease with irreversible changes in the pancreas.

Traditionally, AP has been classed as fundamentally different from CP as the first one is characterized by restoration of normal pancreatic histology after full clinical recovery[24]. However, acute, recurrent acute and CP are now regarded as a disease continuum[25,26]. For this reason, the majority of the epidemiologic studies of alcoholic pancreatitis are referred to CP.

There is no data in the literature showing whether the heavy alcohol intake (> 80 g/d) in abstainers or moderately drinkers is a risk factor for AP. Also it is unknown whether this risk would be the same for all alcoholic beverages (wine, beer or spirits).

CHRONIC PANCREATITIS

CP is an inflammatory disorder of the pancreas typically associated with heavy alcohol consumption. Clinical features of CP consist of abdominal pain, recurrent attacks of clinical AP, and exocrine and/or endocrine insufficiency[27].

Alcohol is the most common cause for CP[22]. Clinical and experimental studies analyzing alcoholic CP development have concluded that alcoholic pancreatitis begins as a acute process that progresses to chronic irreversible pancreatic damage as a consequence of repeated acute attacks[23,28].

The overall survival in patients with alcoholic pancreatitis is significantly lower compared with the background population, but most of the patients die from causes unrelated to pancreatitis[29,30].

Dufour *et al*[16] concluded that CP development is proportional to the dose and duration of alcohol consumption (minimum, 6-12 years of approximately 80 g of alcohol per day).

The incidence rates of CP and the proportion of cases attributed directly to alcohol etiology differ based on geographic distribution (see Table 1).

In United States alcohol etiology accounts around 50%-55% depending on the study. Layer *et al*[7] published a study with patients obtained since 1976 to 1982 and they found that 56% were heavy drinkers (> 50 g/d) and present alcoholic etiology. Coté *et al*[31] published their results from a multicenter study (2000-2006) in 2010 concluding that alcohol contributes to CP in 45% of the patients. In 2011, Yadav *et al*[32] published a population-based study with patients from Mayo Clinic in Olmsted County, MN concluding that 51% presented alcoholic CP. In these studies patients with alcohol consumption of > 50 g/d were defined as alcoholic CP.

In general this percentage is lower than Europeans series[6,9,33,34] or other regions[35] (67%-89%) but similar to those from Japan (56%)[36].

In European series there is some variability between countries. For example in Italy the described percentage was 43%-79%[6,37], Germany 78%[9], Denmark 44%[29], Czech Republic 60%[33] and Switzerland 71%[38].

The series published in Mexico show 68%[39] of patients with alcoholic etiology, while alcohol is the responsible factor in 90%[40] of patients in Brazil, 80%[41] in South Africa and 33%[42] in India.

Taken together the data presented above, independently of the region it has been shown that 50% or more of AP or CP are associated with alcohol consumption.

Besides of the geographic distribution, CP due to alcohol consumption could be different based on the race but not on the sex. Lowenfels *et al*[43] determined that at equal levels of consumption the rates of alcoholic pancreatitis are similar for males and females although patients with alcoholic pancreatitis are more likely males. This is due to the overrepresentation of males among patients with alcoholic pancreatitis, showing a higher prevalence of alcohol consumption than sex-based differences in susceptibility.

Also, regardless to the race it was initially described by Lowenfels *et al*[30] and then confirmed by other authors[44-46] that blacks have two to three folds higher rates of alcoholic pancreatitis. Other authors have described that polymorphisms associated to specific populations and races are associated with functional differences in alcohol metabolizing enzymes leading to variation in pancreas damage[47].

However, detailed reasons for geographic or racial differences in susceptibility to alcoholic pancreatitis are still unknown and most likely are because differences in alcohol intake influenced by habits and genetic susceptibility. Further investigations are needed to confirm that or look in detail for cofactors associated to alcohol consumption.

Based on alcohol consumption, Kristiansen *et al*[48] estimated that the risk of any pancreatitis among non drinkers (abstainers) is 1.33% while it is among 2.5% (1.6% acute and 1.6% chronic) in heavy drinkers (> 35 drinks/wk or > 5 drinks/d). Lowenfels *et al*[30] concluded that the risk of pancreatitis among heavy drinkers (> 5

drinks/d) is about 2%-3%.

Also, some studies have been made to evaluate whether the type of beverage (beer, wine or spirits) or the frequency of drinking (daily, almost daily, weekly or monthly) is associate with the risk of pancreatitis[48]. This study concluded that consumption (> 14 drinks/wk) of beer but not wine or spirits increase the risk of pancreatitis. However this analysis was limited by the number of cases at high levels of consumption. These authors did not find an independent association between the frequency of consumption and risk. In addition some experimental studies carried out in animals conclude that prolonged ethanol feeding does not induce CP although causes little histological changes and variation in pancreatic enzymes[49,50].

Although it has been well established that pancreatic inflammation appears to increase the risk of PC[51] and it could be that alcoholic pancreatitis could leads also to PC, future epidemiological studies are needed to analyze this correlation in population.

Some studies have indicated that CP can result in type 2 diabetes, and with a time (up to 20 years) 2%-4% of these pancreatitis cases will develop PC[52]. Also, long-standing type 2 diabetes is a risk factor for PC[53]

Even in heavy alcoholics, CP and alcoholic cirrhosis seldom occur together in the same patient[54,55], despite a few contradictory reports. The mechanisms or factors determining this dichotomy of some alcoholics developing pancreatitis and others developing liver cirrhosis have not been adequately explained. Whether this can be explained by the quantity, duration, and pattern of drinking, or by other cofactors, tobacco smoking, genetic predispositions or dietary factors is debatable.

Veena *et al*[56] showed that a longer duration of use and bigger amounts of alcohol consumption are necessary before cirrhosis disease compared with CP.

PANCREATIC CANCER

Looking at the risk factors of PC, cigarette smoking represent one of the most important contributing lifestyle risk factor accounting around 20% of the patients. Other factors include CP, diabetes, *Helicobacter pilory* infection, obesity, family history of PC and also heavy alcohol consumption[57].

While the association between alcohol abuse and pancreatitis is well established the association between alcohol consumption and PC is less clear and remains controversial.

Historical studies in different cohorts have shown that patients with CP have an increased risk of PC; the excess risk was observed in men and women[52].

Epidemiological data on alcohol and PC are difficult to interpret due to several reasons as: the small sample size (and limited power to detect a possible weak effect of a rare exposure), variability in dose and time of alcohol exposure and other causes influencing (genetic or environmental).

Since 2009 three pooled data analysis and one meta-analysis have been performed. The first pooled study published in 2009 was adjusted for age, smoking status, diabetes, weight, food intake and time of exposure. The control group was no drinkers (0 g ethanol/d). The authors showed a moderate effect of heavy drinking (> 30 g ethanol/d or approximately more than 2-3 alcoholic beverages/d) in women but not in men although the difference in the results by gender was not statistically significant. No associations were observed for the different types of beverage (beer, wine or liquor). This finding is consistent with a modest increase in risk of PC for alcohol intakes of at least 30 g/d[58].

The second pooled analysis[59] was performed using PanScan study. Analysis was adjusted for age, study, race, smoking status, diabetes and body mass index. The control group was a very light drinkers (> 0-4.99 g ethanol/d) for the total alcohol intake and non-drinkers (0 ethanol/d) for the beverage type group. The author concluded that heavily consumption of alcohol from liquor (> 45 g ethanol/d) was associated with PC in men but not in women and not in men and women combined.

The third pooled analysis[60] included a big number of PC cases. Analysis was adjusted for age, sex, race, area, smoking status, education, body mass index and diabetes. The study concluded that heavy drinkers (> 9 drinks/d) have a moderately increased risk of PC compared with lightly drinkers (< 1 drink/d).

The meta-analysis of alcohol consumption and PC includes 21 case-control studies and 11 cohort studies published since 2009[61]. The results obtained indicate that heavy drinkers (> 3 drinks/d) but not moderate or low drinkers, have an increased risk of PC. This result is valid for both men and women.

In summary, these analysis and meta-analysis suggest that heavy drinkers (>30-40 g ethanol/day or > 3 drinks/d) can result in an increased risk of PC. The authors conclude that in a population of 10%-15% of heavy drinkers, 2%-5% of all PC cases could be attributed to alcohol consumption.

Most of the data published in the literature indicate that alcohol drinking at the levels typically consumed by the general population is probably not a risk factor for PC, however heavy alcohol drinking may be related to PC risk[62]. As an extreme case, Gupta *et al*[63] conclude that men binge drinkers (> 5 drinks/episode or > 70g ethanol/episode) leads to a 3.5-fold increased risk of PC and this risk is higher with the numbers of drinks per binge episode. This effect is greater in current smokers than in former or never smokers.

In contrast, most previous epidemiological studies could not demonstrate an excess risk for PC associated with moderate alcohol intake. Velema *et al*[64] did not find sufficient evidence for a causal relationship. Ye *et al*[65] concluded that the excess risk for PC among alcoholics was small, and may be totally attributable to a mix of causes, for example also increased by smoking.

Some of these studies demonstrated no significant

elevation in risk of PC was observed among alcohol consumers less than 55 years old; however, no similar elevation in risk was observed among those 55 or more years old[66].

In conclusion, there is several difficulties to evaluate the association between alcohol and PC and in epidemiological studies the small sample size has probably contributed to the problem of limited power to detect a possible weak effect of a rare exposure (*i.e.*, chronic heavy alcohol drinking). Also causes like exposure measurement error, limited number of cases under study and competing causes have made difficult study the potential effects of alcohol consumption on the risk of PC[67].

Compared with the general population, alcoholic drinkers without alcoholic CP or alcoholic liver cirrhosis have a modest 40% excess risk to develop PC. Even patients diagnosed with alcoholic CP or liver cirrhosis, have only two folds risk for PC. In addition, it has been shown that non-alcoholic CP patients have a markedly greater excess risk for PC, and this risk is higher than that among alcoholic CP patients[65]. These data suggest that in some circumstances, pancreatitis may be an early manifestation of an as yet undiagnosed PC. Also Kudo *et al*[68] concluded that alcohol drinking was not significant risk factor for developing PC in CP patients. So far, no studies have demonstrated that alcoholic pancreatitis leads to an increased risk of PC compared with non-alcoholic pancreatitis.

Taking these data together, although the role of alcohol consumption in PC remains unclear, it appears that low to moderate alcohol consumption is not associated with PC risk, but chronic heavy drinking (and perhaps prolonged bingering, although available data are confusing) may increase risk of PC[58,59,61,63]. Heavy alcohol consumption may increase PC risk by potentiating the effects of other risk factors such as tobacco smoking, poor nutrition, and inflammatory pathways related to CP, but also may have independent genetic and epigenetic effects.

Overall alcohol intake is only one dimension of drinking behavior. Other considerations such as spacing of drinking occasions, quantity of alcohol consumed, type of alcoholic beverages, ways in which beverages are mixed and commodities consumed in conjunction with alcoholic beverages as salt[69], coffee or tobacco or food[70] are important of pancreatic disease and were described many years ago.

POTENTIAL MECHANISMS OF ALCOHOL CONSUMPTION AND PANCREATIC DISEASES

Over the last decades there have been numerous efforts to elucidate the mechanisms by which alcohol damages the pancreas.

The injurious effects of ethanol on the pancreas are mediated through different mechanisms[71] as (1) sensitization of acinar cells to cholecystokinin (CCK) inducing premature activation of zymogens[72]; (2) potentiation of the effect of CCK on the activation of transcription factors, nuclear factor-κB and activating protein-1[73,74]; (3) generation of toxic metabolites such as acetaldehyde and fatty acid ethyl esters; (4) sensitization of the pancreas to the toxic effects of coxsackievirus B3[75]; and (5) activation of pancreatic stellate cells by acetaldehyde and oxidative stress and subsequent increased production of collagen and other matrix proteins[76].

Chronic alcohol exposure leads to impaired exocytosis mediated by acetaldehyde-induced microtubular dysfunction and apical cytoskeleton reorganization in acinar cells, with a subsequent accumulation of intracellular enzymes[77]. In addition, alcohol decreases the stability of zymogen and lysosomal membranes and enhancers acinar cell sensibility to CCK further increasing susceptibility to pathological enzyme activation[78,79]. Some theories also show that physiologically ethanol leads to the formation of protein secretory plugs that obstruct pancreatic ducts, spam of the sphincter of Oddi or decreased tone of the sphincter causing reflux[80-82].

Ethanol and its major metabolite, acetaldehyde, are classified by the International Agency for Research on Cancer as group 1 carcinogens[83].

Alcohol metabolism depends on enzymes that transform ethanol. Genes for these modifying enzymes have specific polymorphisms that differ between subjects and races leading to differences in susceptibility to alcohol effects and alcohol dependence[47].

Although the liver is the major ethanol-metabolizing organ in the body, the pancreas can metabolize alcohol both via oxidative and non-oxidative pathways.

The oxidative pathway is catalyzed by the enzyme alcohol dehydrogenase (ADH) and the cytochrome P450 and produces the metabolite acetaldehyde. Finally, the oxidative alcohol metabolism results in the generation of oxygen species (ROS)[84] and a depletion of the ROS scavenger glutathione[85]. The increased ROS production (which damage DNA and proteins) and a reduction of proteins that eliminate this ROS (glutathione and enzymes related) lead to oxidant stress and resultant damage in tissue. This stress could be responsible for induce alcoholic pancreatitis as has been demonstrated by several models[86-88].

But in pancreas, the non-oxidative pathways may be more important than oxidative metabolism, generating fatty acid ethyl esters (FAEE) by fatty acid ethyl ester synthases (FAEE synthases)[89]. It has been shown that pancreas exhibits higher FAEE synthase activity than liver[90] and FAEEs accumulation have been observed in human and rat pancreas after alcohol intake[91-93].

The products of alcohol oxidation (acetaldehyde and ROS) and of non-oxidative metabolism have been reported to cause acinar cell injury. Acetaldehyde cause morphological changes in rat and dog's pancreas[94] and it has been showed that inhibits CKK-simululated cinar cell secretion[95]. Also, several studies have demonstrated that alcohol intake causes oxidant stress within the pan-

creas[86-88] which may play a role in the alcohol-induced destabilization of zymogen granules and lysosomes. In addition, alcohol oxidation contributes to acinar damage altering the intracellular redox state (a reduced NAD/NADH ratio and increased lactate/piruvate ratio). Other results obtained in isolated mouse pancreatic acinar cells suggest that FAEEs leads to mitochondrial damage, loss of ATP and rise in cytosolic free calcium, which leads to acinar cell toxicity[96]. Other authors have shown that acute application of ethanol at clinically relevant concentrations (1-50 nmol/L) of isolated acinar cells resulted in calcium influx due to the production of oxidative metabolites of alcohol[97]. Together, these data show that the role of alcohol metabolites in acinar cell damage could be due to aberrant calcium signals[98]. FAEEs can elevate Calcium greater that ethanol alone. In addition, FAEEs and their products, fatty acids induces necrosis in acinar cells and this process could be avoided by calcium chelation[99].

These physiological changes leads to the pathobiology found in alcoholic pancreatitis including acute and chronic inflammation, elimination of parenchymal cells of the pancreas by a deregulation of apoptosis/necrosis and/or modification in cell proliferation[49]. The hypothesis called "necrosis-fibrosis sequence" shows these pathological changes where in the early episodes of pancreatitis, patients present focal necrosis and mild fibrosis while patients evaluated years later of the onset of symptoms presents fibrosis and calcifications but not necrosis[100].

But the fact that only a minority of heavily drinkers develops pancreatitis or PC indicates that other susceptibility factors as lipid tolerance, smoking or hereditary factors play an important role. In the last decades, genetic susceptibility has been considered between the factors that contribute mainly to the development to alcoholic pancreatic diseases.

One study showed an association between a polymorphism of the gene for one FAEE synthase enzymes, carboxylester lipase and risk of developing alcoholic pancreatitis[101].

In addition, the G191R variant in the anionic trypsinogen gene *PRSS2*, has been shown to result in a form of trypsin that is easily degraded, is more infrequent in alcoholic pancreatitis patients compared with healthy controls[102].

Other studies have demonstrated that mutation N34S in *SPINK1* gene is found in 5%-5.8% of patients with pancreatitis compared with 1% in healthy controls[103,104]. But still the functional consequences of this mutation are unknown.

One of the enzymes that have been also related to alcoholism and drug dependence for decades is ADH. Li *et al*[105] performed a recent meta-analyses and confirmed strong associations of the *ADH1B* and *ALDH2* genes with alcoholism and alcohol-related medical diseases[106]. Recently, Celorrio *et al*[107] demonstrated that some specific polymorphism in the genes *TH*, *ADH1B* increase the risk to develop diseases a consequence of excessive consumption of alcohol.

Although it is clear that alcohol consumption is genetically influenced, but characterized by incomplete penetrance, phenocopies, heterogeneity, and polygenic inheritance.

In conclusion, nowadays it appears clear that alcohol consumption is the first or second most common cause of pancreatitis. Based on the different epidemiology studies published in the literature the percentage of pancreatitis cases attributable to alcohol abuse vary since 30% to 90% between countries. A statistical association has been shown with a threshold of $\geqslant$ 5 drinks per day with a dose of alcohol $\geqslant$ 50 g/d.

But despite that excessive alcohol consumption is primarily responsible for most cases of pancreatitis, alcohol intake alone is not sufficient to lead to this disease, as less than 10% of heavily drinkers develop pancreatitis.

Regarding to PC, the role of alcohol consumption remains less clear, and low to moderate alcohol consumption do not appear to be associated with PC risk, but only chronic heavy drinking increase the risk compared with lightly drinkers.

Genetic variability and environmental exposures such as smoking and diet could act synergistically with regard to pancreatitis and PC and should be considered for further investigations. Probably heavy alcohol consumption may increase pancreatic disease risk most likely potentiating the effects of these other risk factors, but also may have independent genetic and epigenetic effects.

REFERENCES

1 **World Health Organization**. Global Health Risks. Mortality and burden of disease attributable to selected mayor risks. Geneva: World Health Organization, 2009

2 **Friedreich N**. Disease of the pancreas. Cyclopoedia of the Practice of Medicine. New York: William Wood, 1878

3 **National Institute of Alcohol Abuse and Alcoholism**. The physicians' guide to helping patient with alcohol problems. Wasintong DC: Goverment Printing Office, 1995

4 **Kloner RA**, Rezkalla SH. To drink or not to drink? That is the question. *Circulation* 2007; **116**: 1306-1317 [PMID: 17846344 DOI: 10.1161/CIRCULATIONAHA.106.678375]

5 **Kerr WC**, Greenfield TK, Tujague J, Brown SE. A drink is a drink? Variation in the amount of alcohol contained in beer, wine and spirits drinks in a US methodological sample. *Alcohol Clin Exp Res* 2005; **29**: 2015-2021 [PMID: 16340459 DOI: 10.1097/01.alc.0000187596.92804.bd]

6 **Cavallini G**, Frulloni L, Pederzoli P, Talamini G, Bovo P, Bassi C, Di Francesco V, Vaona B, Falconi M, Sartori N, Angelini G, Brunori MP, Filippini M. Long-term follow-up of patients with chronic pancreatitis in Italy. *Scand J Gastroenterol* 1998; **33**: 880-889 [PMID: 9754738 DOI: 10.1080/00365529850171567]

7 **Layer P**, Yamamoto H, Kalthoff L, Clain JE, Bakken LJ, DiMagno EP. The different courses of early- and late-onset idiopathic and alcoholic chronic pancreatitis. *Gastroenterology* 1994; **107**: 1481-1487 [PMID: 7926511]

8 **Ammann RW**, Muellhaupt B. Progression of alcoholic acute to chronic pancreatitis. *Gut* 1994; **35**: 552-556 [PMID: 8174996 DOI: 10.1136/gut.35.4.552]

9 **Lankisch PG**, Assmus C, Maisonneuve P, Lowenfels AB. Epidemiology of pancreatic diseases in Lüneburg County. A

study in a defined german population. *Pancreatology* 2002; **2**: 469-477 [PMID: 12378115 DOI: 10.1159/000064713]
10 **Ammann RW**. A clinically based classification system for alcoholic chronic pancreatitis: summary of an international workshop on chronic pancreatitis. *Pancreas* 1997; **14**: 215-221 [PMID: 9094150 DOI: 10.1097/00006676-199704000-00001]
11 **Whitcomb DC**, Yadav D, Adam S, Hawes RH, Brand RE, Anderson MA, Money ME, Banks PA, Bishop MD, Baillie J, Sherman S, DiSario J, Burton FR, Gardner TB, Amann ST, Gelrud A, Lo SK, DeMeo MT, Steinberg WM, Kochman ML, Etemad B, Forsmark CE, Elinoff B, Greer JB, O'Connell M, Lamb J, Barmada MM. Multicenter approach to recurrent acute and chronic pancreatitis in the United States: the North American Pancreatitis Study 2 (NAPS2). *Pancreatology* 2008; **8**: 520-531 [PMID: 18765957 DOI: 10.1159/000152001]
12 **Harper SJ**, Cheslyn-Curtis S. Acute pancreatitis. *Ann Clin Biochem* 2011; **48**: 23-37 [PMID: 20926469 DOI: 10.1258/acb.2010.010196]
13 **Corrao G**, Bagnardi V, Zambon A, La Vecchia C. A meta-analysis of alcohol consumption and the risk of 15 diseases. *Prev Med* 2004; **38**: 613-619 [PMID: 15066364 DOI: 10.1016/j.ypmed.2003.11.027]
14 **Irving HM**, Samokhvalov AV, Rehm J. Alcohol as a risk factor for pancreatitis. A systematic review and meta-analysis. *JOP* 2009; **10**: 387-392 [PMID: 19581740]
15 **Whitcomb DC**. Inflammation and Cancer V. Chronic pancreatitis and pancreatic cancer. *Am J Physiol Gastrointest Liver Physiol* 2004; **287**: G315-G319 [PMID: 15246966 DOI: 10.1152/ajpgi.00115.2004]
16 **Dufour MC**, Adamson MD. The epidemiology of alcohol-induced pancreatitis. *Pancreas* 2003; **27**: 286-290 [PMID: 14576488 DOI: 10.1097/00006676-200311000-00002]
17 **Schenker S**, Montalvo R. Alcohol and the pancreas. *Recent Dev Alcohol* 1998; **14**: 41-65 [PMID: 9751942]
18 **Sarles H**. Alcoholism and pancreatitis. *Scand J Gastroenterol* 1971; **6**: 193-198 [PMID: 4934709 DOI: 10.3109/00365527109180692]
19 **Secknus R**, Mössner J. [Changes in incidence and prevalence of acute and chronic pancreatitis in Germany]. *Chirurg* 2000; **71**: 249-252 [PMID: 10789040]
20 **Dreiling DA**, Koller M. The natural history of alcoholic pancreatitis: update 1985. *Mt Sinai J Med* 1985; **52**: 340-342 [PMID: 3874352]
21 **Steinberg W**, Tenner S. Acute pancreatitis. *N Engl J Med* 1994; **330**: 1198-1210 [PMID: 7811319]
22 **Yadav D**, Lowenfels AB. Trends in the epidemiology of the first attack of acute pancreatitis: a systematic review. *Pancreas* 2006; **33**: 323-330 [PMID: 17079934 DOI: 10.1097/01.mpa.0000236733.31617.52]
23 **Lankisch PG**, Löhr-Happe A, Otto J, Creutzfeldt W. Natural course in chronic pancreatitis. Pain, exocrine and endocrine pancreatic insufficiency and prognosis of the disease. *Digestion* 1993; **54**: 148-155 [PMID: 8359556 DOI: 10.1159/000201029]
24 **Sarles H**. Etiopathogenesis and definition of chronic pancreatitis. *Dig Dis Sci* 1986; **31**: 91S-107S [PMID: 3525051 DOI: 10.1007/BF01295992]
25 **Mitchell RM**, Byrne MF, Baillie J. Pancreatitis. *Lancet* 2003; **361**: 1447-1455 [PMID: 12727412]
26 **Whitcomb DC**. Mechanisms of disease: Advances in understanding the mechanisms leading to chronic pancreatitis. *Nat Clin Pract Gastroenterol Hepatol* 2004; **1**: 46-52 [PMID: 16265044 DOI: 10.1038/ncpgasthep0025]
27 **Etemad B**, Whitcomb DC. Chronic pancreatitis: diagnosis, classification, and new genetic developments. *Gastroenterology* 2001; **120**: 682-707 [PMID: 11179244 DOI: 10.1053/gast.2001.22586]
28 **Perides G**, Tao X, West N, Sharma A, Steer ML. A mouse model of ethanol dependent pancreatic fibrosis. *Gut* 2005; **54**: 1461-1467 [PMID: 15870229 DOI: 10.1136/gut.2004.062919]
29 **Nøjgaard C**, Bendtsen F, Becker U, Andersen JR, Holst C, Matzen P. Danish patients with chronic pancreatitis have a four-fold higher mortality rate than the Danish population. *Clin Gastroenterol Hepatol* 2010; **8**: 384-390 [PMID: 20036762 DOI: 10.1016/j.cgh.2009.12.016]
30 **Lowenfels AB**, Maisonneuve P, Cavallini G, Ammann RW, Lankisch PG, Andersen JR, DiMagno EP, Andrén-Sandberg A, Domellöf L, Di Francesco V. Prognosis of chronic pancreatitis: an international multicenter study. International Pancreatitis Study Group. *Am J Gastroenterol* 1994; **89**: 1467-1471 [PMID: 8079921]
31 **Coté GA**, Yadav D, Slivka A, Hawes RH, Anderson MA, Burton FR, Brand RE, Banks PA, Lewis MD, Disario JA, Gardner TB, Gelrud A, Amann ST, Baillie J, Money ME, O'Connell M, Whitcomb DC, Sherman S. Alcohol and smoking as risk factors in an epidemiology study of patients with chronic pancreatitis. *Clin Gastroenterol Hepatol* 2011; **9**: 266-273; quiz e27 [PMID: 21029787]
32 **Yadav D**, Timmons L, Benson JT, Dierkhising RA, Chari ST. Incidence, prevalence, and survival of chronic pancreatitis: a population-based study. *Am J Gastroenterol* 2011; **106**: 2192-2199 [PMID: 21946280 DOI: 10.1038/ajg.2011.328]
33 **Díte P**, Starý K, Novotný I, Precechtelová M, Dolina J, Lata J, Zboril V. Incidence of chronic pancreatitis in the Czech Republic. *Eur J Gastroenterol Hepatol* 2001; **13**: 749-750 [PMID: 11434607 DOI: 10.1097/00042737-200106000-00024]
34 **Andersen BN**, Pedersen NT, Scheel J, Worning H. Incidence of alcoholic chronic pancreatitis in Copenhagen. *Scand J Gastroenterol* 1982; **17**: 247-252 [PMID: 7134849 DOI: 10.3109/00365528209182047]
35 **Dani R**, Mott CB, Guarita DR, Nogueira CE. Epidemiology and etiology of chronic pancreatitis in Brazil: a tale of two cities. *Pancreas* 1990; **5**: 474-478 [PMID: 2381901 DOI: 10.1097/00006676-199007000-00016]
36 **Lin Y**, Tamakoshi A, Matsuno S, Takeda K, Hayakawa T, Kitagawa M, Naruse S, Kawamura T, Wakai K, Aoki R, Kojima M, Ohno Y. Nationwide epidemiological survey of chronic pancreatitis in Japan. *J Gastroenterol* 2000; **35**: 136-141 [PMID: 10680669 DOI: 10.1007/s005350050026]
37 **Frulloni L**, Gabbrielli A, Pezzilli R, Zerbi A, Cavestro GM, Marotta F, Falconi M, Gaia E, Uomo G, Maringhini A, Mutignani M, Maisonneuve P, Di Carlo V, Cavallini G. Chronic pancreatitis: report from a multicenter Italian survey (PanCroInfAISP) on 893 patients. *Dig Liver Dis* 2009; **41**: 311-317 [PMID: 19097829 DOI: 10.1016/j.dld.2008.07.316]
38 **Ammann RW**, Buehler H, Muench R, Freiburghaus AW, Siegenthaler W. Differences in the natural history of idiopathic (nonalcoholic) and alcoholic chronic pancreatitis. A comparative long-term study of 287 patients. *Pancreas* 1987; **2**: 368-377 [PMID: 3628234 DOI: 10.1097/00006676-198707000-00002]
39 **Robles-Díaz G**, Vargas F, Uscanga L, Fernández-del Castillo C. Chronic pancreatitis in Mexico City. *Pancreas* 1990; **5**: 479-483 [PMID: 2381902 DOI: 10.1097/00006676-199007000-00017]
40 **Dani R**, Penna FJ, Nogueira CE. Etiology of chronic calcifying pancreatitis in Brazil: a report of 329 consecutive cases. *Int J Pancreatol* 1986; **1**: 399-406 [PMID: 3681031]
41 **Marks IN**, Bank S, Louw JH. Chronic pancreatitis in the Western Cape. *Digestion* 1973; **9**: 447-453 [PMID: 4784706]
42 **Balakrishnan V**, Unnikrishnan AG, Thomas V, Choudhuri G, Veeraraju P, Singh SP, Garg P, Pai CG, Devi RN, Bhasin D, Jayanthi V, Premalatha N, Chacko A, Kar P, Rai RR, Rajan R, Subhalal N, Mehta R, Mishra SP, Dwivedi M, Vinayakumar KR, Jain AK, Biswas K, Mathai S, Varghese J, Ramesh H, Alexander T, Philip J, Raj VV, Vinodkumar A, Mukevar S, Sawant P, Nair P, Kumar H, Sudhindran S, Dhar P, Sudheer OV, Sundaram KR, Tantri BV, Singh D, Nath TR. Chronic pancreatitis. A prospective nationwide study of 1,086 subjects from India. *JOP* 2008; **9**: 593-600 [PMID: 18762690]

43 **Lowenfels AB**, Maisonneuve P, Lankisch PG. Chronic pancreatitis and other risk factors for pancreatic cancer. *Gastroenterol Clin North Am* 1999; **28**: 673-85, x [PMID: 10503143]

44 **Morton C**, Klatsky AL, Udaltsova N. Smoking, coffee, and pancreatitis. *Am J Gastroenterol* 2004; **99**: 731-738 [PMID: 15089909 DOI: 10.1111/j.1572-0241.2004.04143.x]

45 **Yadav D**, Hawes RH, Brand RE, Anderson MA, Money ME, Banks PA, Bishop MD, Baillie J, Sherman S, DiSario J, Burton FR, Gardner TB, Amann ST, Gelrud A, Lawrence C, Elinoff B, Greer JB, O'Connell M, Barmada MM, Slivka A, Whitcomb DC. Alcohol consumption, cigarette smoking, and the risk of recurrent acute and chronic pancreatitis. *Arch Intern Med* 2009; **169**: 1035-1045 [PMID: 19506173 DOI: 10.1001/archinternmed.2009.125]

46 **Yang AL**, Vadhavkar S, Singh G, Omary MB. Epidemiology of alcohol-related liver and pancreatic disease in the United States. *Arch Intern Med* 2008; **168**: 649-656 [PMID: 18362258 DOI: 10.1001/archinte.168.6.649]

47 **Celorrio D**, Bujanda L, Chbel F, Sánchez D, Martinez-Jarreta B, de Pancorbo MM. Alcohol-metabolizing enzyme gene polymorphisms in the Basque Country, Morocco, and Ecuador. *Alcohol Clin Exp Res* 2011; **35**: 879-884 [PMID: 21303386 DOI: 10.1111/j.1530-0277.2010.01418.x]

48 **Kristiansen L**, Grønbaek M, Becker U, Tolstrup JS. Risk of pancreatitis according to alcohol drinking habits: a population-based cohort study. *Am J Epidemiol* 2008; **168**: 932-937 [PMID: 18779386 DOI: 10.1093/aje/kwn222]

49 **Pandol SJ**, Raraty M. Pathobiology of alcoholic pancreatitis. *Pancreatology* 2007; **7**: 105-114 [PMID: 17592222 DOI: 10.1159/000104235]

50 **Li J**, Guo M, Hu B, Liu R, Wang R, Tang C. Does chronic ethanol intake cause chronic pancreatitis?: evidence and mechanism. *Pancreas* 2008; **37**: 189-195 [PMID: 18665082 DOI: 10.1097/MPA.0b013e31816459b7]

51 **Whitcomb DC**, Pogue-Geile K. Pancreatitis as a risk for pancreatic cancer. *Gastroenterol Clin North Am* 2002; **31**: 663-678 [PMID: 12134623 DOI: 10.1016/S0889-8553(02)00004-3]

52 **Lowenfels AB**, Maisonneuve P, Cavallini G, Ammann RW, Lankisch PG, Andersen JR, Dimagno EP, Andrén-Sandberg A, Domellöf L. Pancreatitis and the risk of pancreatic cancer. International Pancreatitis Study Group. *N Engl J Med* 1993; **328**: 1433-1437 [PMID: 8479461 DOI: 10.1056/NEJM199305203282001]

53 **Huxley R**, Ansary-Moghaddam A, Berrington de González A, Barzi F, Woodward M. Type-II diabetes and pancreatic cancer: a meta-analysis of 36 studies. *Br J Cancer* 2005; **92**: 2076-2083 [PMID: 15886696 DOI: 10.1038/sj.bjc.6602619]

54 **Aparisi L**, Sabater L, Del-Olmo J, Sastre J, Serra MA, Campello R, Bautista D, Wassel A, Rodrigo JM. Does an association exist between chronic pancreatitis and liver cirrhosis in alcoholic subjects? *World J Gastroenterol* 2008; **14**: 6171-6179 [PMID: 18985807 DOI: 10.3748/wjg.14.6171]

55 **Pace A**, de Weerth A, Berna M, Hillbricht K, Tsokos M, Bläker M, Pueschel K, Lohse AW. Pancreas and liver injury are associated in individuals with increased alcohol consumption. *Clin Gastroenterol Hepatol* 2009; **7**: 1241-1246 [PMID: 19560556 DOI: 10.1016/j.cgh.2009.06.010]

56 **Veena AB**, Rajesh G, Varghese J, Sundaram KR, Balakrishnan V. Alcoholic chronic pancreatitis and alcoholic liver cirrhosis: differences in alcohol use habits and patterns in Indian subjects. *Pancreas* 2012; **41**: 703-706 [PMID: 22481284]

57 **Klein AP**. Genetic susceptibility to pancreatic cancer. *Mol Carcinog* 2012; **51**: 14-24 [PMID: 22162228 DOI: 10.1002/mc.20855]

58 **Genkinger JM**, Spiegelman D, Anderson KE, Bergkvist L, Bernstein L, van den Brandt PA, English DR, Freudenheim JL, Fuchs CS, Giles GG, Giovannucci E, Hankinson SE, Horn-Ross PL, Leitzmann M, Männistö S, Marshall JR, McCullough ML, Miller AB, Reding DJ, Robien K, Rohan TE, Schatzkin A, Stevens VL, Stolzenberg-Solomon RZ, Verhage BA, Wolk A, Ziegler RG, Smith-Warner SA. Alcohol intake and pancreatic cancer risk: a pooled analysis of fourteen cohort studies. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 765-776 [PMID: 19258474 DOI: 10.1158/1055-9965.EPI-08-0880]

59 **Michaud DS**, Vrieling A, Jiao L, Mendelsohn JB, Steplowski E, Lynch SM, Wactawski-Wende J, Arslan AA, Bas Bueno-de-Mesquita H, Fuchs CS, Gross M, Helzlsouer K, Jacobs EJ, Lacroix A, Petersen G, Zheng W, Allen N, Ammundadottir L, Bergmann MM, Boffetta P, Buring JE, Canzian F, Chanock SJ, Clavel-Chapelon F, Clipp S, Freiberg MS, Michael Gaziano J, Giovannucci EL, Hankinson S, Hartge P, Hoover RN, Allan Hubbell F, Hunter DJ, Hutchinson A, Jacobs K, Kooperberg C, Kraft P, Manjer J, Navarro C, Peeters PH, Shu XO, Stevens V, Thomas G, Tjønneland A, Tobias GS, Trichopoulos D, Tumino R, Vineis P, Virtamo J, Wallace R, Wolpin BM, Yu K, Zeleniuch-Jacquotte A, Stolzenberg-Solomon RZ. Alcohol intake and pancreatic cancer: a pooled analysis from the pancreatic cancer cohort consortium (PanScan). *Cancer Causes Control* 2010; **21**: 1213-1225 [PMID: 20373013 DOI: 10.1007/s10552-010-9548-z]

60 **Lucenteforte E**, La Vecchia C, Silverman D, Petersen GM, Bracci PM, Ji BT, Bosetti C, Li D, Gallinger S, Miller AB, Bueno-de-Mesquita HB, Talamini R, Polesel J, Ghadirian P, Baghurst PA, Zatonski W, Fontham E, Bamlet WR, Holly EA, Gao YT, Negri E, Hassan M, Cotterchio M, Su J, Maisonneuve P, Boffetta P, Duell EJ. Alcohol consumption and pancreatic cancer: a pooled analysis in the International Pancreatic Cancer Case-Control Consortium (PanC4). *Ann Oncol* 2012; **23**: 374-382 [PMID: 21536662 DOI: 10.1093/annonc/mdr120]

61 **Tramacere I**, Scotti L, Jenab M, Bagnardi V, Bellocco R, Rota M, Corrao G, Bravi F, Boffetta P, La Vecchia C. Alcohol drinking and pancreatic cancer risk: a meta-analysis of the dose-risk relation. *Int J Cancer* 2010; **126**: 1474-1486 [PMID: 19816941]

62 **Silverman DT**, Brown LM, Hoover RN, Schiffman M, Lillemoe KD, Schoenberg JB, Swanson GM, Hayes RB, Greenberg RS, Benichou J. Alcohol and pancreatic cancer in blacks and whites in the United States. *Cancer Res* 1995; **55**: 4899-4905 [PMID: 7585527]

63 **Gupta S**, Wang F, Holly EA, Bracci PM. Risk of pancreatic cancer by alcohol dose, duration, and pattern of consumption, including binge drinking: a population-based study. *Cancer Causes Control* 2010; **21**: 1047-1059 [PMID: 20349126 DOI: 10.1007/s10552-010-9533-6]

64 **Velema JP**, Walker AM, Gold EB. Alcohol and pancreatic cancer. Insufficient epidemiologic evidence for a causal relationship. *Epidemiol Rev* 1986; **8**: 28-41 [PMID: 3533583]

65 **Ye W**, Lagergren J, Weiderpass E, Nyrén O, Adami HO, Ekbom A. Alcohol abuse and the risk of pancreatic cancer. *Gut* 2002; **51**: 236-239 [PMID: 12117886 DOI: 10.1136/gut.51.2.236]

66 **Michaud DS**, Giovannucci E, Willett WC, Colditz GA, Fuchs CS. Coffee and alcohol consumption and the risk of pancreatic cancer in two prospective United States cohorts. *Cancer Epidemiol Biomarkers Prev* 2001; **10**: 429-437 [PMID: 11352851]

67 **Duell EJ**. Epidemiology and potential mechanisms of tobacco smoking and heavy alcohol consumption in pancreatic cancer. *Mol Carcinog* 2012; **51**: 40-52 [PMID: 22162230 DOI: 10.1002/mc.20786]

68 **Kudo Y**, Kamisawa T, Anjiki H, Takuma K, Egawa N. Incidence of and risk factors for developing pancreatic cancer in patients with chronic pancreatitis. *Hepatogastroenterology* 2011; **58**: 609-611 [PMID: 21661440]

69 **Tuyns AJ**. Sodium chloride, alcohol, and cancer of the digestive tract. *Nutr Cancer* 1983; **5**: 92-95 [PMID: 6647043 DOI: 10.1080/01635588309513784]

70 **Room R**. Measurements of drinking patterns in the general population and possible applications in studies of the role

of alcohol in cancer. *Cancer Res* 1979; **39**: 2830-2833 [PMID: 376125]
71 Mechanisms of alcoholic pancreatitis. Proceedings of a conference. Chicago, Illinois, USA, November 2002. *Pancreas* 2003; **27**: 281-355 [PMID: 14576487]
72 **Lerch MM**, Gorelick FS. Early trypsinogen activation in acute pancreatitis. *Med Clin North Am* 2000; **84**: 549-563, viii [PMID: 10872413]
73 **Chen X**, Ji B, Han B, Ernst SA, Simeone D, Logsdon CD. NF-kappaB activation in pancreas induces pancreatic and systemic inflammatory response. *Gastroenterology* 2002; **122**: 448-457 [PMID: 11832459 DOI: 10.1053/gast.2002.31060]
74 **Gukovsky I**, Lugea A, Shahsahebi M, Cheng JH, Hong PP, Jung YJ, Deng QG, French BA, Lungo W, French SW, Tsukamoto H, Pandol SJ. A rat model reproducing key pathological responses of alcoholic chronic pancreatitis. *Am J Physiol Gastrointest Liver Physiol* 2008; **294**: G68-G79 [PMID: 17884979 DOI: 10.1152/ajpgi.00006.2007]
75 **Jerrells TR**, Vidlak D, Strachota JM. Alcoholic pancreatitis: mechanisms of viral infections as cofactors in the development of acute and chronic pancreatitis and fibrosis. *J Leukoc Biol* 2007; **81**: 430-439 [PMID: 17095612 DOI: 10.1189/jlb.1004622]
76 **Apte MV**, Pirola RC, Wilson JS. Battle-scarred pancreas: role of alcohol and pancreatic stellate cells in pancreatic fibrosis. *J Gastroenterol Hepatol* 2006; **21** Suppl 3: S97-S101 [PMID: 16958684 DOI: 10.1111/j.1440-1746.2006.04587.x]
77 **Vonlaufen A**, Apte MV, Imhof BA, Frossard JL. The role of inflammatory and parenchymal cells in acute pancreatitis. *J Pathol* 2007; **213**: 239-248 [PMID: 17893879 DOI: 10.1002/path.2231]
78 **Haber PS**, Wilson JS, Apte MV, Korsten MA, Pirola RC. Chronic ethanol consumption increases the fragility of rat pancreatic zymogen granules. *Gut* 1994; **35**: 1474-1478 [PMID: 7525419 DOI: 10.1136/gut.35.10.1474]
79 **Gorelick FS**. Alcohol and zymogen activation in the pancreatic acinar cell. *Pancreas* 2003; **27**: 305-310 [PMID: 14576492 DOI: 10.1097/00006676-200311000-00006]
80 **Guy O**, Robles-Diaz G, Adrich Z, Sahel J, Sarles H. Protein content of precipitates present in pancreatic juice of alcoholic subjects and patients with chronic calcifying pancreatitis. *Gastroenterology* 1983; **84**: 102-107 [PMID: 6401181]
81 **Singh M**, Simsek H. Ethanol and the pancreas. Current status. *Gastroenterology* 1990; **98**: 1051-1062 [PMID: 2179029 DOI: 10.1016/0016-5085(90)90033-W]
82 **Pfeffer RB**, Stasior O, Hinton JW. The clinical picture of the sequential development of acute hemorrhagic pancreatitis in the dog. *Surg Forum* 1957; **8**: 248-251 [PMID: 13529599]
83 **Baan R**, Grosse Y, Straif K, Secretan B, El Ghissassi F, Bouvard V, Benbrahim-Tallaa L, Guha N, Freeman C, Galichet L, Cogliano V. A review of human carcinogens--Part F: chemical agents and related occupations. *Lancet Oncol* 2009; **10**: 1143-1144 [PMID: 19998521 DOI: 10.1016/S1470-2045(09)70358-4]
84 **Ekström G**, Ingelman-Sundberg M. Rat liver microsomal NADPH-supported oxidase activity and lipid peroxidation dependent on ethanol-inducible cytochrome P-450 (P-450IIE1). *Biochem Pharmacol* 1989; **38**: 1313-1319 [PMID: 2495801 DOI: 10.1016/0006-2952(89)90338-9]
85 **Shaw S**, Jayatilleke E. The role of cellular oxidases and catalytic iron in the pathogenesis of ethanol-induced liver injury. *Life Sci* 1992; **50**: 2045-2052 [PMID: 1608288 DOI: 10.1016/0024-3205(92)90570-F]
86 **Altomare E**, Grattagliano I, Vendemiale G, Palmieri V, Palasciano G. Acute ethanol administration induces oxidative changes in rat pancreatic tissue. *Gut* 1996; **38**: 742-746 [PMID: 8707122 DOI: 10.1136/gut.38.5.742]
87 **Casini A**, Galli A, Pignalosa P, Frulloni L, Grappone C, Milani S, Pederzoli P, Cavallini G, Surrenti C. Collagen type I synthesized by pancreatic periacinar stellate cells (PSC) co-localizes with lipid peroxidation-derived aldehydes in chronic alcoholic pancreatitis. *J Pathol* 2000; **192**: 81-89 [PMID: 10951404 DOI: 3.0.CO;]
88 **Norton ID**, Apte MV, Lux O, Haber PS, Pirola RC, Wilson JS. Chronic ethanol administration causes oxidative stress in the rat pancreas. *J Lab Clin Med* 1998; **131**: 442-446 [PMID: 9605109 DOI: 10.1016/S0022-2143(98)90145-7]
89 **Wilson JS**, Apte MV. Role of alcohol metabolism in alcoholic pancreatitis. *Pancreas* 2003; **27**: 311-315 [PMID: 14576493 DOI: 10.1097/00006676-200311000-00007]
90 **Gukovskaya AS**, Mouria M, Gukovsky I, Reyes CN, Kasho VN, Faller LD, Pandol SJ. Ethanol metabolism and transcription factor activation in pancreatic acinar cells in rats. *Gastroenterology* 2002; **122**: 106-118 [PMID: 11781286 DOI: 10.1053/gast.2002.30302]
91 **Lange LG**. Nonoxidative ethanol metabolism: formation of fatty acid ethyl esters by cholesterol esterase. *Proc Natl Acad Sci USA* 1982; **79**: 3954-3957 [PMID: 6955782 DOI: 10.1073/pnas.79.13.3954]
92 **Haber PS**, Apte MV, Moran C, Applegate TL, Pirola RC, Korsten MA, McCaughan GW, Wilson JS. Non-oxidative metabolism of ethanol by rat pancreatic acini. *Pancreatology* 2004; **4**: 82-89 [PMID: 15056978 DOI: 10.1159/000077608]
93 **Werner J**, Saghir M, Fernandez-del Castillo C, Warshaw AL, Laposata M. Linkage of oxidative and nonoxidative ethanol metabolism in the pancreas and toxicity of nonoxidative ethanol metabolites for pancreatic acinar cells. *Surgery* 2001; **129**: 736-744 [PMID: 11391373 DOI: 10.1067/msy.2001.113891]
94 **Nordback IH**, MacGowan S, Potter JJ, Cameron JL. The role of acetaldehyde in the pathogenesis of acute alcoholic pancreatitis. *Ann Surg* 1991; **214**: 671-678 [PMID: 1720611 DOI: 10.1097/00000658-199112000-00006]
95 **Sankaran H**, Lewin MB, Wong A, Deveney CW, Wendland MF, Leimgruber RM, Geokas MC. Irreversible inhibition by acetaldehyde of cholecystokinin-induced amylase secretion from isolated rat pancreatic acini. *Biochem Pharmacol* 1985; **34**: 2859-2863 [PMID: 2411269 DOI: 10.1016/0006-2952(85)90007-3]
96 **Criddle DN**, Raraty MG, Neoptolemos JP, Tepikin AV, Petersen OH, Sutton R. Ethanol toxicity in pancreatic acinar cells: mediation by nonoxidative fatty acid metabolites. *Proc Natl Acad Sci USA* 2004; **101**: 10738-10743 [PMID: 15247419 DOI: 10.1073/pnas.0403431101]
97 **Del Castillo-Vaquero A**, Salido GM, González A. Increased calcium influx in the presence of ethanol in mouse pancreatic acinar cells. *Int J Exp Pathol* 2010; **91**: 114-124 [PMID: 20002836 DOI: 10.1111/j.1365-2613.2009.00691.x]
98 **Thrower EC**, Gorelick FS, Husain SZ. Molecular and cellular mechanisms of pancreatic injury. *Curr Opin Gastroenterol* 2010; **26**: 484-489 [PMID: 20651589 DOI: 10.1097/MOG.0b013e32833d119e]
99 **Criddle DN**, Murphy J, Fistetto G, Barrow S, Tepikin AV, Neoptolemos JP, Sutton R, Petersen OH. Fatty acid ethyl esters cause pancreatic calcium toxicity via inositol trisphosphate receptors and loss of ATP synthesis. *Gastroenterology* 2006; **130**: 781-793 [PMID: 16530519 DOI: 10.1053/j.gastro.2005.12.031]
100 **Oruc N**, Whitcomb DC. Theories, mechanisms, and models of alcoholic chronic pancreatitis. *Gastroenterol Clin North Am* 2004; **33**: 733-750, v-vi [PMID: 15528015]
101 **Miyasaka K**, Ohta M, Takano S, Hayashi H, Higuchi S, Maruyama K, Tando Y, Nakamura T, Takata Y, Funakoshi A. Carboxylester lipase gene polymorphism as a risk of alcohol-induced pancreatitis. *Pancreas* 2005; **30**: e87-e91 [PMID: 15841033 DOI: 10.1097/01.mpa.0000160960.21580.mL]
102 **Witt H**, Sahin-Tóth M, Landt O, Chen JM, Kähne T, Drenth JP, Kukor Z, Szepessy E, Halangk W, Dahm S, Rohde K, Schulz HU, Le Maréchal C, Akar N, Ammann RW, Truninger K, Bargetzi M, Bhatia E, Castellani C, Cavestro GM, Cerny M, Destro-Bisol G, Spedini G, Eiberg H, Jansen JB, Koudova M, Rausova E, Macek M, Malats N, Real FX,

Menzel HJ, Moral P, Galavotti R, Pignatti PF, Rickards O, Spicak J, Zarnescu NO, Böck W, Gress TM, Friess H, Ockenga J, Schmidt H, Pfützer R, Löhr M, Simon P, Weiss FU, Lerch MM, Teich N, Keim V, Berg T, Wiedenmann B, Luck W, Groneberg DA, Becker M, Keil T, Kage A, Bernardova J, Braun M, Güldner C, Halangk J, Rosendahl J, Witt U, Treiber M, Nickel R, Férec C. A degradation-sensitive anionic trypsinogen (PRSS2) variant protects against chronic pancreatitis. *Nat Genet* 2006; **38**: 668-673 [PMID: 16699518 DOI: 10.1038/ng1797]

103 **Witt H**, Luck W, Becker M, Böhmig M, Kage A, Truninger K, Ammann RW, O'Reilly D, Kingsnorth A, Schulz HU, Halangk W, Kielstein V, Knoefel WT, Teich N, Keim V. Mutation in the SPINK1 trypsin inhibitor gene, alcohol use, and chronic pancreatitis. *JAMA* 2001; **285**: 2716-2717 [PMID: 11386926 DOI: 10.1001/jama.285.21.2716-a]

104 **Diaconu BL**, Ciobanu L, Mocan T, Pfützer RH, Scafaru MP, Acalovschi M, Singer MV, Schneider A. Investigation of the SPINK1 N34S mutation in Romanian patients with alcoholic chronic pancreatitis. A clinical analysis based on the criteria of the M-ANNHEIM classification. *J Gastrointestin Liver Dis* 2009; **18**: 143-150 [PMID: 19565042]

105 **Li D**, Zhao H, Gelernter J. Further clarification of the contribution of the ADH1C gene to vulnerability of alcoholism and selected liver diseases. *Hum Genet* 2012; **131**: 1361-1374 [PMID: 22476623]

106 **Li D**, Zhao H, Gelernter J. Strong association of the alcohol dehydrogenase 1B gene (ADH1B) with alcohol dependence and alcohol-induced medical diseases. *Biol Psychiatry* 2011; **70**: 504-512 [PMID: 21497796 DOI: 10.1016/j.biopsych.2011.02.024]

107 **Celorrio D**, Bujanda L, Caso C, Landabaso M, Oria JC, Ogando J, de Pancorbo MM. A comparison of Val81Met and other polymorphisms of alcohol metabolising genes in patients and controls in Northern Spain. *Alcohol* 2012; **46**: 427-431 [PMID: 22560290]

P- Reviewer Gukovskaya AS **S- Editor** Gou SX **L- Editor** A **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i5.648

World J Gastroenterol 2013 February 7; 19(5): 648-653
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

Use of antibiotics in the treatment of Crohn's disease

Maria Lia Scribano, Cosimo Prantera

Maria Lia Scribano, Cosimo Prantera, Gastroenterology Operative Unit, Azienda Ospedaliera San Camillo-Forlanini, 00152 Rome, Italy
Author contributions: Scribano ML and Prantera C contributed equally to this work.
Correspondence to: Maria Lia Scribano, MD, Gastroenterology Operative Unit, Azienda Ospedaliera San Camillo-Forlanini, Circonvallazione Gianicolense 87, 00152 Rome, Italy. marialiascribano@virgilio.it
Telephone: +39-6-58703308 Fax: +39-6-58704505
Received: April 26, 2012 Revised: July 26, 2012
Accepted: July 29, 2012
Published online: February 7, 2013

Abstract

Many data coming from animal models and clinical observations support an involvement of intestinal microbiota in the pathogenesis of Crohn's disease (CD). It is hypothesized in fact, that the development of chronic intestinal inflammation is caused by an abnormal immune response to normal flora in genetically susceptible hosts. The involvement of bacteria in CD inflammation has provided the rationale for including antibiotics in the therapeutic armamentarium. However, randomized controlled trials have failed to demonstrate an efficacy of these drugs in patients with active uncomplicated CD, even if a subgroup of patients with colonic location seems to get benefit from antibiotics. Nitroimidazole compounds have been shown to be efficacious in decreasing CD recurrence rates in operated patients, and the use of metronidazole and ciprofloxacin is recommended in perianal disease. However, the appearance of systemic side effects limits antibiotic long-term employment necessary for treating a chronic relapsing disease. Rifaximin, characterized by an excellent safety profile, has provided promising results in inducing remission of CD.

© 2013 Baishideng. All rights reserved.

Key words: Antibiotics; Crohn's disease; Gut microbiota; Mycobacteria

Scribano ML, Prantera C. Use of antibiotics in the treatment of Crohn's disease. *World J Gastroenterol* 2013; 19(5): 648-653 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i5/648.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i5.648

ROLE OF GUT MICROBIOTA IN CROHN'S DISEASE

Crohn's disease (CD) is a chronic inflammatory bowel disease characterized by an altered composition of the intestinal commensal bacteria (dysbiosis)[1,2]. Dysbiosis is considered to have a preeminent role in CD pathogenesis by inducing an abnormal immune response in genetically susceptible individuals[3-7].

Intestinal sites commonly affected by CD lesion, in fact, are those with the highest bacterial concentration, such as colon, terminal ileum, especially after the loss of the ileocecal valve, and upstream from strictures. The luminal content is necessary for causing intestinal inflammation, and CD lesions do not appear when it is diverted from the gut, whereas restoration of bowel continuity or infusion of faecal material into the bypassed intestine rapidly results in recurrence of the disease[8-10]. In genetically engineered rodent models susceptible to spontaneous colitis, inflammation does not occur when the animals are raised in germ-free conditions[11]. Patients with CD have an altered composition of gut microbiota with increased concentrations of invasive bacteria, especially *Escherichia coli* (*E. coli*) and a decreased number of protective bifidobacteria, lactobacilli and the more recently studied *Faecalibacterium prausnitzii,* that has been shown to have anti-inflammatory properties[12-15]. Genetic susceptibly in a subgroup of patients with CD is related to polymorphisms in the *NOD2/CARD15* gene, suggesting that individuals with mutations in *NOD2* present defective intestinal immune responses to gut microbiota[16-20]. About 20% of CD patients are homozygous for *NOD2* variants and they may have an increased susceptibility to CD localized at the ileum[21].

Moreover, a loss of immunologic tolerance to the

commensal flora has been observed in patients with active IBD[22].

Experimental studies in animal models have shown that broad-spectrum antibiotics are efficacious in almost all models of acute and chronic colitis and ileitis[23]. However, they have only a transient benefit in HLA-B27 transgenic rats[24]. Broad-spectrum antibiotics can both prevent and treat experimental colitis of rodent models, while metronidazole and ciprofloxacin can only prevent the onset of colitis, but not reverse the established disease[25,26].

ANTIBIOTIC TREATMENT IN CROHN'S DISEASE

These and other data, confirming the role of the gut microbiota in the pathogenesis of CD, provide the rationale for a therapeutic manipulation of the intestinal flora through the use of antibiotics and probiotics[27-30].

Some of the suggested mechanisms of action for antibiotics in CD are the ability to reduce luminal and mucosa adherent bacteria concentrations, to eliminate in a selective way aggressive bacterial species, to decrease bacterial tissue invasion and translocation. Additionally, some antibiotics also exert a potential immunosuppressive action.

The role of antibiotics as primary or adjunctive treatment of active, uncomplicated CD has not been clearly demonstrated and their use is controversial. A recent meta-analysis suggests that antibiotics may be effective as primary therapy of CD[31], but guidelines do not recommend their use except for the treatment of septic complications of CD, symptoms attributable to bacterial overgrowth, or perianal disease[32].

ANTIBIOTICS FOR INDUCTION AND MAINTENANCE OF DISEASE REMISSION

The similarity of CD to tuberculous enteritis and Johne's disease of ruminants, caused by *Mycobacterium Avium* subspecies *Paratuberculosis* (MAP), and the isolation of atypical Mycobacteria from blood and tissue of CD subjects, have lead to evaluate the efficacy of anti-mycobacterial drugs in these patients[33-37]. However, the results of the randomized controlled trials performed with antibiotics active against atypical Mycobacteria for obtaining and maintaining CD remission have been conflicting.

A meta-analysis that considered eight trials employing different associations of anti-mycobacterial drugs showed that these drugs seem to be ineffective for inducing remission without a course of steroid therapy[38].

In the largest study 213 Australian patients were randomized to receive a combination of clarithromycin plus rifabutin and clofazimine, antibiotics active against MAP, or placebo for up 2 years, in addition to a 16-week course of corticosteroids[39]. The results showed a significant benefit only at 16 wk, when the antibiotic combination was added to steroids, confirming the data founded by the meta-analysis, and suggesting that the short-term advantage could be related to a generic antibacterial effect. Therefore, this study does not support a significant role for MAP in CD pathogenesis, although several objections to this conclusion have been raised[40-44]. At the present time the mycobacterial hypothesis cannot be completely ruled out, and it continues to be plausible that an infectious agent could start the inflammatory process[45].

Pathogenic adherent and invasive *E. coli* have been detected in Crohn's ileal and colonic tissue[46-49]. This bacterium can invade and replicate within macrophages, inducing the secretion of large quantities of tumor necrosis factor[50]. Clarithromycin is a broad spectrum macrolide antibiotic that can penetrate into macrophages, and may therefore be effective in eradicating the bacteria. However, a study comparing clarithromycin 1 gr to placebo for 3 mo in patients with active CD, was stopped because of poor efficacy[51].

Metronidazole, which is active against anaerobic bacteria and some parasites, and ciprofloxacin, particularly active against *E. coli* and *Enterobacteriacee*, are the most frequent studied and used antibiotics. Several randomized clinical trials have been performed employing metronidazole and/or ciprofloxacin for induction of CD remission.

The results of the trials have indicated that metronidazole is efficacious in active Crohn's colitis and ileo-colitis, but not in small bowel location[52-55]. Five randomized controlled studies evaluating the efficacy of ciprofloxacin, alone or in association with metronidazole, in patients with active CD, have shown uncertain results[56-60].

Patients with colonic involvement get more benefit from antibiotics, probably because of the high concentration of bacteria in the colon.

The efficacy of antibiotics in CD seems to be related to a prolonged therapy, that is frequently burdened by an elevated number of systemic adverse events (AEs). In particular, there is concern about the *Clostridium difficile* infection caused by antibiotics, especially fluoroquinolones such as ciprofloxacin[61]. *Clostridium difficile* infection can induce CD relapse and gastroenterologists in charge of CD patients must be aware of this serious complication. In 2007 two retrospective studies demonstrated a dramatically increased incidence of *Clostridium difficile* infection in patients with IBD, who appeared to be more susceptible to this infection than non IBD-patients[61,62].

The minimally absorbed antibiotic rifaximin, which is active against gram-positive and gram-negative bacteria, has been shown to be effective in active CD patients. In an exploratory study, a gastro resistant formulation of rifaximin [extended intestinal release (EIR)] (rifaximin-EIR) at a dose of 800 mg twice daily for 12 wk reported significantly higher rates of remission and response compared to placebo, in a subgroup of patients with mild to moderate CD and an elevated value of C-reactive protein[63].

In a recent, larger study 402 patients with moderately active CD were randomized to receive rifaximin-EIR 400, 800, 1200 mg or placebo twice daily for 12 wk[64]. The

results showed that rifaximin-EIR 800 mg twice daily was significantly superior to placebo in inducing remission, defined as a Crohn's Disease Activity Index (CDAI) score < 150, after 12 wk of therapy (62 % *vs* 43%). The effect was maintained throughout a subsequent 12-wk follow-up period in 65% of patients. Remission rates in patients treated with 400 and 1200 mg twice daily (54 % and 47 %, respectively) showed a trend towards but without reaching the statistical significance in comparison with placebo. The lack of a dose-response relationship was probably caused by the higher percentage of subjects who discontinued the treatment due to AEs in the 1200 mg twice daily group. Median CRP values over time showed no statistically significant differences between treatment groups. The most frequent AEs were of gastrointestinal origin, either determined by an underlying disease, with consequent CDAI score increase and treatment failure, or to rifaximin related side effects. Also in this study colonic location appeared to be associated with a higher response to the antibiotic therapy. Overall, the safety profile of rifaximin-EIR was good, indicating that rifaximin could be administered for a long period of time. However, *Clostridium difficile* infection was reported in a single patient with rifaximin 800 mg twice daily 20 d after the end of the treatment period. Rifaximin has been successfully employed for the treatment of CDI in metronidazole resistant patients[65], however it is probable that rare clones of rifaximin-unresponsive *Clostridium* can develop[66].

ANTIBIOTICS FOR PREVENTION OF POSTOPERATIVE RECURRENCE

Prevention of recurrence after intestinal resection is one of the major aims in the treatment of CD, and antibiotics have been used in this setting in 3 randomized placebo-controlled studies. The rationale for the employment of antibiotics is that bacteria are strongly suspected to be the main reason for the recurrence of lesions. In the first trial, metronidazole, at a dosage of 20 mg/kg per day for a 3-mo period, significantly decreased the incidence of early severe endoscopic recurrence, and also seemed to delay the symptomatic recurrence at 1 year, but it was associated with a high percentage of side effects[67]. The same authors have later performed a 12 mo placebo-controlled trial employing ornidazole, which has the same bacterial spectrum with a better safety profile. Ornidazole at a dose of 1 g/d proved significantly to reduce the clinical recurrence rate at 1 year, but more than 30% of patients in the antibiotic group discontinued the therapy because of side effects[68]. More recently, D' Haens *et al*[69]compared metronidazole (250 mg 3 times per day) given for 3 mo plus azathioprine for 12 mo to metronidazole alone in 81 operated CD patients at high risk of recurrence. This drug combination uses metronidazole as bridge therapy, given the slowness of azathioprine activity. At 3 mo after surgery severe endoscopic recurrence occurred in 34.3% of patients in the metronidazole/azathioprine group and in 52.6% of patients in the metronidazole/placebo group (P = 0.11). At month 12, severe endoscopic recurrence was observed in 43.7% of patients in the metronidazole/azathioprine group and in 69.0% of patients in the metronidazole/placebo group (P = 0.048). The study treatment was well tolerated and only 3 patients discontinued the therapy in the first 3 mo because of side effects, probably ascribable to metronidazole. The authors concluded that the combined treatment seems to be recommendable to CD patients with an elevated risk for postoperative recurrence. Recurrence prevention requires a long-term treatment, and this is burdened by a high number of AEs. Given its high safety profile, rifaximin, provided that its efficacy is completely demonstrated, should be employed for long term recurrence prevention.

ANTIBIOTICS FOR TREATMENT OF PERIANAL DISEASE

Antibiotics are widely employed for treatment of perianal CD, alone or as adjuvant therapy, and, despite the lack of controlled trials, European Crohn's and Colitis Organisation consensus statements recommend them in simple and complex fistulising perianal disease[70].

Most of the studies of perianal disease treated with antibiotics are, in fact, uncontrolled with a small sample size. In these studies, metronidazole and ciprofloxacin used alone or in combination have proved to induce a decrease of fistula drainage, but rarely induce closure. Moreover, symptoms tend to recur after suspending the treatment[71-73]. AEs resulting from prolonged use of antibiotics may, however, limit their use.

Recently, a randomized, placebo-controlled pilot study evaluating ciprofloxacin or metronidazole for the treatment of perianal fistulas in 25 CD patients failed to demonstrate a significant benefit of either antibiotic treatment over placebo in the cessation of drainage[74]. However the study was probably too small to permit detecting differences between treatment arms.

In conclusion, the different antibiotic regimens evaluated in the randomized controlled studies, the limited number of patient enrolled, the heterogeneity between the trials, and the uncertain results have led to the conclusion that antibiotics cannot be recommended for treatment of active CD, except for septic complications, symptoms attributable to bacterial overgrowth, or perianal disease. In addition, their efficacy could be limited by the prolonged therapy usually required for treating CD. However, there seems to be a subgroup of patients with colonic disease who can respond to these medications, likely due to the differences in gut microbiota between ileal and colonic location. Nitroimidazole antibiotics seem to be effective in decreasing both endoscopic and clinical recurrence rates after surgery, but their long-term use is complicated by an elevated number of AEs.

Treatment of patients with mild and moderate CD with rifaximin seems promising, but further larger studies are needed.

REFERENCES

1 **Chassaing B**, Darfeuille-Michaud A. The commensal microbiota and enteropathogens in the pathogenesis of inflammatory bowel diseases. *Gastroenterology* 2011; **140**: 1720-1728 [PMID: 21530738 DOI: 10.1053/j.gastro.2011.01.054]

2 **Tamboli CP**, Neut C, Desreumaux P, Colombel JF. Dysbiosis in inflammatory bowel disease. *Gut* 2004; **53**: 1-4 [PMID: 14684564 DOI: 10.1136/gut.53.1.1]

3 **Podolsky DK**. Inflammatory bowel disease. *N Engl J Med* 2002; **347**: 417-429 [PMID: 12167685 DOI: 10.1056/NEJMra020831]

4 **Abraham C**, Medzhitov R. Interactions between the host innate immune system and microbes in inflammatory bowel disease. *Gastroenterology* 2011; **140**: 1729-1737 [PMID: 21530739 DOI: 10.1053/j.gastro.2011.02.012]

5 **Barnich N**, Darfeuille-Michaud A. Role of bacteria in the etiopathogenesis of inflammatory bowel disease. *World J Gastroenterol* 2007; **13**: 5571-5576 [PMID: 17948930]

6 **De Hertogh G**, Aerssens J, Geboes KP, Geboes K. Evidence for the involvement of infectious agents in the pathogenesis of Crohn's disease. *World J Gastroenterol* 2008; **14**: 845-852 [PMID: 18240341 DOI: 10.3748/wjg.14.845]

7 **Sartor RB**. Microbial influences in inflammatory bowel diseases. *Gastroenterology* 2008; **134**: 577-594 [PMID: 18242222 DOI: 10.1053/j.gastro.2007.11.059]

8 **Harper PH**, Lee EC, Kettlewell MG, Bennett MK, Jewell DP. Role of the faecal stream in the maintenance of Crohn's colitis. *Gut* 1985; **26**: 279-284 [PMID: 3972275 DOI: 10.1136/gut.26.3.279]

9 **Rutgeerts P**, Goboes K, Peeters M, Hiele M, Penninckx F, Aerts R, Kerremans R, Vantrappen G. Effect of faecal stream diversion on recurrence of Crohn's disease in the neoterminal ileum. *Lancet* 1991; **338**: 771-774 [PMID: 1681159 DOI: 10.1016/0140-6736(91)90663-A]

10 **D'Haens GR**, Geboes K, Peeters M, Baert F, Penninckx F, Rutgeerts P. Early lesions of recurrent Crohn's disease caused by infusion of intestinal contents in excluded ileum. *Gastroenterology* 1998; **114**: 262-267 [PMID: 9453485 DOI: 10.1016/S0016-5085(98)70476-7]

11 **Sellon RK**, Tonkonogy S, Schultz M, Dieleman LA, Grenther W, Balish E, Rennick DM, Sartor RB. Resident enteric bacteria are necessary for development of spontaneous colitis and immune system activation in interleukin-10-deficient mice. *Infect Immun* 1998; **66**: 5224-5231 [PMID: 9784526]

12 **Swidsinski A**, Ladhoff A, Pernthaler A, Swidsinski S, Loening-Baucke V, Ortner M, Weber J, Hoffmann U, Schreiber S, Dietel M, Lochs H. Mucosal flora in inflammatory bowel disease. *Gastroenterology* 2002; **122**: 44-54 [PMID: 11781279 DOI: 10.1053/gast.2002.30294]

13 **Swidsinski A**, Loening-Baucke V, Herber A. Mucosal flora in Crohn's disease and ulcerative colitis - an overview. *J Physiol Pharmacol* 2009; **60** Suppl 6: 61-71 [PMID: 20224153]

14 **Sokol H**, Pigneur B, Watterlot L, Lakhdari O, Bermúdez-Humarán LG, Gratadoux JJ, Blugeon S, Bridonneau C, Furet JP, Corthier G, Grangette C, Vasquez N, Pochart P, Trugnan G, Thomas G, Blottière HM, Doré J, Marteau P, Seksik P, Langella P. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. *Proc Natl Acad Sci USA* 2008; **105**: 16731-16736 [PMID: 18936492 DOI: 10.1073/pnas.0804812105]

15 **Sokol H**, Seksik P, Furet JP, Firmesse O, Nion-Larmurier I, Beaugerie L, Cosnes J, Corthier G, Marteau P, Doré J. Low counts of Faecalibacterium prausnitzii in colitis microbiota. *Inflamm Bowel Dis* 2009; **15**: 1183-1189 [PMID: 19235886 DOI: 10.1002/ibd.20903]

16 **Ogura Y**, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH, Achkar JP, Brant SR, Bayless TM, Kirschner BS, Hanauer SB, Nuñez G, Cho JH. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001; **411**: 603-606 [PMID: 11385577 DOI: 10.1038/35079114]

17 **Hugot JP**, Chamaillard M, Zouali H, Lesage S, Cézard JP, Belaiche J, Almer S, Tysk C, O'Morain CA, Gassull M, Binder V, Finkel Y, Cortot A, Modigliani R, Laurent-Puig P, Gower-Rousseau C, Macry J, Colombel JF, Sahbatou M, Thomas G. Association of NOD2 leucine-rich repeat variants with susceptibility to Crohn's disease. *Nature* 2001; **411**: 599-603 [PMID: 11385576 DOI: 10.1038/35079107]

18 **Wehkamp J**, Harder J, Weichenthal M, Schwab M, Schäffeler E, Schlee M, Herrlinger KR, Stallmach A, Noack F, Fritz P, Schröder JM, Bevins CL, Fellermann K, Stange EF. NOD2 (CARD15) mutations in Crohn's disease are associated with diminished mucosal alpha-defensin expression. *Gut* 2004; **53**: 1658-1664 [PMID: 15479689 DOI: 10.1136/gut.2003.032805]

19 **Rogler G**. The effects of NOD2/CARD15 mutations on the function of the intestinal barrier. *J Crohns Colitis* 2007; **1**: 53-60 [PMID: 21172185 DOI: 10.1016/j.crohns.2007.08.008]

20 **Strober W**, Kitani A, Fuss I, Asano N, Watanabe T. The molecular basis of NOD2 susceptibility mutations in Crohn's disease. *Mucosal Immunol* 2008; **1** Suppl 1: S5-S9 [PMID: 19079230 DOI: 10.1038/mi.2008.42]

21 **Cho JH**, Brant SR. Recent insights into the genetics of inflammatory bowel disease. *Gastroenterology* 2011; **140**: 1704-1712 [PMID: 21530736 DOI: 10.1053/j.gastro.2011.02.046]

22 **Duchmann R**, Kaiser I, Hermann E, Mayet W, Ewe K, Meyer zum Büschenfelde KH. Tolerance exists towards resident intestinal flora but is broken in active inflammatory bowel disease (IBD) *Clin Exp Immunol* 1995; **102**: 448-455 [PMID: 8536356 DOI: 10.1111/j.1365-2249.1995.tb03836.x]

23 **Hoentjen F**, Harmsen HJ, Braat H, Torrice CD, Mann BA, Sartor RB, Dieleman LA. Antibiotics with a selective aerobic or anaerobic spectrum have different therapeutic activities in various regions of the colon in interleukin 10 gene deficient mice. *Gut* 2003; **52**: 1721-1727 [PMID: 14633949 DOI: 10.1136/gut.52.12.1721]

24 **Dieleman LA**, Goerres MS, Arends A, Sprengers D, Torrice C, Hoentjen F, Grenther WB, Sartor RB. Lactobacillus GG prevents recurrence of colitis in HLA-B27 transgenic rats after antibiotic treatment. *Gut* 2003; **52**: 370-376 [PMID: 12584218 DOI: 10.1136/gut.52.3.370]

25 **Madsen KL**, Doyle JS, Tavernini MM, Jewell LD, Rennie RP, Fedorak RN. Antibiotic therapy attenuates colitis in interleukin 10 gene-deficient mice. *Gastroenterology* 2000; **118**: 1094-1105 [PMID: 10833484 DOI: 10.1016/S0016-5085(00)70362-3]

26 **Rath HC**, Schultz M, Freitag R, Dieleman LA, Li F, Linde HJ, Schölmerich J, Sartor RB. Different subsets of enteric bacteria induce and perpetuate experimental colitis in rats and mice. *Infect Immun* 2001; **69**: 2277-2285 [PMID: 11254584 DOI: 10.1128/IAI.69.4.2277-2285.2001]

27 **Hammer HF**. Gut microbiota and inflammatory bowel disease. *Dig Dis* 2011; **29**: 550-553 [PMID: 22179210 DOI: 10.1159/000332981]

28 **Sartor RB**. Therapeutic manipulation of the enteric microflora in inflammatory bowel diseases: antibiotics, probiotics, and prebiotics. *Gastroenterology* 2004; **126**: 1620-1633 [PMID: 15168372 DOI: 10.1053/j.gastro.2004.03.024]

29 **Prantera C**. What role do antibiotics have in the treatment of IBD? *Nat Clin Pract Gastroenterol Hepatol* 2008; **5**: 670-671 [PMID: 18825144 DOI: 10.1038/ncpgasthep1273]

30 **Prantera C**, Scribano ML. Antibiotics and probiotics in inflammatory bowel disease: why, when, and how. *Curr Opin Gastroenterol* 2009; **25**: 329-333 [PMID: 19444096 DOI: 10.1097/MOG.0b013e32832b20bf]

31 **Khan KJ**, Ullman TA, Ford AC, Abreu MT, Abadir A, Marshall JK, Talley NJ, Moayyedi P. Antibiotic therapy in inflammatory bowel disease: a systematic review and meta-analysis. *Am J Gastroenterol* 2011; **106**: 661-673 [PMID:

21407187 DOI: 10.1038/ajg.2011.72]
32 **Dignass A**, Van Assche G, Lindsay JO, Lémann M, Söderholm J, Colombel JF, Danese S, D'Hoore A, Gassull M, Gomollón F, Hommes DW, Michetti P, O'Morain C, Oresland T, Windsor A, Stange EF, Travis SP. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Current management. *J Crohns Colitis* 2010; **4**: 28-62 [PMID: 21122489 DOI: 10.1016/j.crohns.2009.12.002]
33 **Chiodini RJ**, Van Kruiningen HJ, Thayer WR, Merkal RS, Coutu JA. Possible role of mycobacteria in inflammatory bowel disease. I. An unclassified Mycobacterium species isolated from patients with Crohn's disease. *Dig Dis Sci* 1984; **29**: 1073-1079 [PMID: 6499624 DOI: 10.1007/BF01317078]
34 **Graham DY**, Markesich DC, Yoshimura HH. Mycobacteria as the cause of Crohn's disease. *Gastroenterology* 1989; **97**: 1354-1356 [PMID: 2792667]
35 **Shanahan F**, O'Mahony J. The mycobacteria story in Crohn' s disease. *Am J Gastroenterol* 2005; **100**: 1537-1538 [PMID: 15984977 DOI: 10.1111/j.1572-0241.2005.50358.x]
36 **Prantera C**. Mycobacteria and Crohn's disease: the endless story. *Dig Liver Dis* 2007; **39**: 452-454 [PMID: 17379592 DOI: 10.1016/j.dld.2007.02.002]
37 **Van Kruiningen HJ**. Where are the weapons of mass destruction − the Mycobacterium paratuberculosis in Crohn' s disease? *J Crohns Colitis* 2011; **5**: 638-644 [PMID: 22115388 DOI: 10.1016/j.crohns.2011.08.004]
38 **Borgaonkar MR**, MacIntosh DG, Fardy JM. A meta-analysis of antimycobacterial therapy for Crohn's disease. *Am J Gastroenterol* 2000; **95**: 725-729 [PMID: 10710065 DOI: 10.1111/j.1572-0241.2000.01842.x]
39 **Selby W**, Pavli P, Crotty B, Florin T, Radford-Smith G, Gibson P, Mitchell B, Connell W, Read R, Merrett M, Ee H, Hetzel D. Two-year combination antibiotic therapy with clarithromycin, rifabutin, and clofazimine for Crohn's disease. *Gastroenterology* 2007; **132**: 2313-2319 [PMID: 17570206 DOI: 10.1053/j.gastro.2007.03.031]
40 **Peyrin-Biroulet L**, Neut C, Colombel JF. Antimycobacterial therapy in Crohn's disease: game over? *Gastroenterology* 2007; **132**: 2594-2598 [PMID: 17570230 DOI: 10.1053/j.gastro.2007.04.027]
41 **Lipton JE**, Barash DP. Flawed Australian CD study does not end MAP controversy. *Gastroenterology* 2007; **133**: 1742; author reply 1745-1746 [PMID: 17983825 DOI: 10.1053/j.gastro.2007.09.015]
42 **Kuenstner JT**. The Australian antibiotic trial in Crohn's disease: alternative conclusions from the same study. *Gastroenterology* 2007; **133**: 1742-1743; author reply 1745-1746 [PMID: 17983824 DOI: 10.1053/j.gastro.2007.09.012]
43 **Gitlin L**, Biesecker J. Australian Crohn's antibiotic study opens new horizons. *Gastroenterology* 2007; **133**: 1743-1744; author reply 1745-1746 [PMID: 17983826 DOI: 10.1053/j.gastro.2007.09.014]
44 **Chamberlin W**. Importance of the Australian Crohn's disease antibiotic study. *Gastroenterology* 2007; **133**: 1744-1745; author reply 1745-1746 [PMID: 17983827 DOI: 10.1053/j.gastro.2007.09.013]
45 **Chiodini RJ**, Chamberlin WM, Sarosiek J, McCallum RW. Crohn's disease and the mycobacterioses: a quarter century later. Causation or simple association? *Crit Rev Microbiol* 2012; **38**: 52-93 [PMID: 22242906 DOI: 10.3109/1040841X.2011.638273]
46 **Rhodes JM**. The role of Escherichia coli in inflammatory bowel disease. *Gut* 2007; **56**: 610-612 [PMID: 17440180 DOI: 10.1136/gut.2006.111872]
47 **Barnich N**, Darfeuille-Michaud A. Adherent-invasive Escherichia coli and Crohn's disease. *Curr Opin Gastroenterol* 2007; **23**: 16-20 [PMID: 17133079 DOI: 10.1097/MOG.0b013e3280105a38]
48 **Sepehri S**, Khafipour E, Bernstein CN, Coombes BK, Pilar AV, Karmali M, Ziebell K, Krause DO. Characterization of Escherichia coli isolated from gut biopsies of newly diagnosed patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2011; **17**: 1451-1463 [PMID: 21674703 DOI: 10.1002/ibd.21509]
49 **Sasaki M**, Sitaraman SV, Babbin BA, Gerner-Smidt P, Ribot EM, Garrett N, Alpern JA, Akyildiz A, Theiss AL, Nusrat A, Klapproth JM. Invasive Escherichia coli are a feature of Crohn's disease. *Lab Invest* 2007; **87**: 1042-1054 [PMID: 17660846 DOI: 10.1038/labinvest.3700661]
50 **Nguyen DD**. Are E. coli the culprit? *Inflamm Bowel Dis* 2008; **14**: 575-576 [PMID: 18088052 DOI: 10.1002/ibd.20350]
51 **Leiper K**, Martin K, Ellis A, Watson AJ, Morris AI, Rhodes JM. Clinical trial: randomized study of clarithromycin versus placebo in active Crohn's disease. *Aliment Pharmacol Ther* 2008; **27**: 1233-1239 [PMID: 18315579 DOI: 10.1111/j.1365-2036.2008.03661.x]
52 **Blichfeldt P**, Blomhoff JP, Myhre E, Gjone E. Metronidazole in Crohn's disease. A double blind cross-over clinical trial. *Scand J Gastroenterol* 1978; **13**: 123-127 [PMID: 345410 DOI: 10.3109/00365527809179816]
53 **Ursing B**, Alm T, Bárány F, Bergelin I, Ganrot-Norlin K, Hoevels J, Huitfeldt B, Järnerot G, Krause U, Krook A, Lindström B, Nordle O, Rosén A. A comparative study of metronidazole and sulfasalazine for active Crohn's disease: the cooperative Crohn's disease study in Sweden. II. Result. *Gastroenterology* 1982; **83**: 550-562 [PMID: 6124474]
54 **Ambrose NS**, Allan RN, Keighley MR, Burdon DW, Youngs D, Barnes P, Lennard-Jones JE. Antibiotic therapy for treatment in relapse of intestinal Crohn's disease. A prospective randomized study. *Dis Colon Rectum* 1985; **28**: 81-85 [PMID: 3882364 DOI: 10.1007/BF02552649]
55 **Sutherland L**, Singleton J, Sessions J, Hanauer S, Krawitt E, Rankin G, Summers R, Mekhjian H, Greenberger N, Kelly M. Double blind, placebo controlled trial of metronidazole in Crohn's disease. *Gut* 1991; **32**: 1071-1075 [PMID: 1916494 DOI: 10.1136/gut.32.9.1071]
56 **Turunen U**, Farkkila M, Hakala K, Seppl K, Sivonen A, Ogren M, Vuoristo M, Valtonen VV, Miettinen TA. Ciprofloxacin treatment combined with conventional therapy in Crohn's disease: a prospective, double blind, placebo controlled study. *Gut* 1995; **37** Suppl 2: A193
57 **Prantera C**, Zannoni F, Scribano ML, Berto E, Andreoli A, Kohn A, Luzi C. An antibiotic regimen for the treatment of active Crohn's disease: a randomized, controlled clinical trial of metronidazole plus ciprofloxacin. *Am J Gastroenterol* 1996; **91**: 328-332 [PMID: 8607501]
58 **Colombel JF**, Lémann M, Cassagnou M, Bouhnik Y, Duclos B, Dupas JL, Notteghem B, Mary JY. A controlled trial comparing ciprofloxacin with mesalazine for the treatment of active Crohn's disease. Groupe d'Etudes Thérapeutiques des Affections Inflammatoires Digestives (GETAID). *Am J Gastroenterol* 1999; **94**: 674-678 [PMID: 10086650 DOI: 10.1111/j.1572-0241.1999.935_q.x]
59 **Arnold GL**, Beaves MR, Pryjdun VO, Mook WJ. Preliminary study of ciprofloxacin in active Crohn's disease. *Inflamm Bowel Dis* 2002; **8**: 10-15 [PMID: 11837933 DOI: 10.1097/00054725-200201000-00002]
60 **Steinhart AH**, Feagan BG, Wong CJ, Vandervoort M, Mikolainis S, Croitoru K, Seidman E, Leddin DJ, Bitton A, Drouin E, Cohen A, Greenberg GR. Combined budesonide and antibiotic therapy for active Crohn's disease: a randomized controlled trial. *Gastroenterology* 2002; **123**: 33-40 [PMID: 12105831 DOI: 10.1053/gast.2002.34225]
61 **Issa M**, Vijayapal A, Graham MB, Beaulieu DB, Otterson MF, Lundeen S, Skaros S, Weber LR, Komorowski RA, Knox JF, Emmons J, Bajaj JS, Binion DG. Impact of Clostridium difficile on inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2007; **5**: 345-351 [PMID: 17368234 DOI: 10.1016/j.cgh.2006.12.028]
62 **Rodemann JF**, Dubberke ER, Reske KA, Seo da H, Stone

CD. Incidence of Clostridium difficile infection in inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2007; **5**: 339-344 [PMID: 17368233 DOI: 10.1016/j.cgh.2006.12.027]

63 **Prantera C**, Lochs H, Campieri M, Scribano ML, Sturniolo GC, Castiglione F, Cottone M. Antibiotic treatment of Crohn's disease: results of a multicentre, double blind, randomized, placebo-controlled trial with rifaximin. *Aliment Pharmacol Ther* 2006; **23**: 1117-1125 [PMID: 16611272 DOI: 10.1111/j.1365-2036.2006.02879.x]

64 **Prantera C**, Lochs H, Grimaldi M, Danese S, Scribano ML, Gionchetti P. Rifaximin-extended intestinal release induces remission in patients with moderately active Crohn's disease. *Gastroenterology* 2012; **142**: 473-481 [PMID: 22155172 DOI: 10.1053/j.gastro.2011.11.032]

65 **Patrick Basu P**, Dinani A, Rayapudi K, Pacana T, Shah NJ, Hampole H, Krishnaswamy NV, Mohan V. Rifaximin therapy for metronidazole-unresponsive Clostridium difficile infection: a prospective pilot trial. *Therap Adv Gastroenterol* 2010; **3**: 221-225 [PMID: 21180604 DOI: 10.1177/1756283X10372985]

66 **Marchese A**, Salerno A, Pesce A, Debbia EA, Schito GC. In vitro activity of rifaximin, metronidazole and vancomycin against Clostridium difficile and the rate of selection of spontaneously resistant mutants against representative anaerobic and aerobic bacteria, including ammonia-producing species. *Chemotherapy* 2000; **46**: 253-266 [PMID: 10859431 DOI: 10.1159/000007297]

67 **Rutgeerts P**, Hiele M, Geboes K, Peeters M, Penninckx F, Aerts R, Kerremans R. Controlled trial of metronidazole treatment for prevention of Crohn's recurrence after ileal resection. *Gastroenterology* 1995; **108**: 1617-1621 [PMID: 7768364 DOI: 10.1016/0016-5085(95)90121-3]

68 **Rutgeerts P**, Van Assche G, Vermeire S, D'Haens G, Baert F, Noman M, Aerden I, De Hertogh G, Geboes K, Hiele M, D'Hoore A, Penninckx F. Ornidazole for prophylaxis of postoperative Crohn's disease recurrence: a randomized, double-blind, placebo-controlled trial. *Gastroenterology* 2005; **128**: 856-861 [PMID: 15825069 DOI: 10.1053/j.gastro.2005.01.010]

69 **D'Haens GR**, Vermeire S, Van Assche G, Noman M, Aerden I, Van Olmen G, Rutgeerts P. Therapy of metronidazole with azathioprine to prevent postoperative recurrence of Crohn's disease: a controlled randomized trial. *Gastroenterology* 2008; **135**: 1123-1129 [PMID: 18727929 DOI: 10.1053/j.gastro.2008.07.010]

70 **Van Assche G**, Dignass A, Reinisch W, van der Woude CJ, Sturm A, De Vos M, Guslandi M, Oldenburg B, Dotan I, Marteau P, Ardizzone A, Baumgart DC, D'Haens G, Gionchetti P, Portela F, Vucelic B, Söderholm J, Escher J, Koletzko S, Kolho KL, Lukas M, Mottet C, Tilg H, Vermeire S, Carbonnel F, Cole A, Novacek G, Reinshagen M, Tsianos E, Herrlinger K, Oldenburg B, Bouhnik Y, Kiesslich R, Stange E, Travis S, Lindsay J. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Special situations. *J Crohns Colitis* 2010; **4**: 63-101 [PMID: 21122490 DOI: 10.1016/j.crohns.2009.09.009]

71 **Bernstein LH**, Frank MS, Brandt LJ, Boley SJ. Healing of perineal Crohn's disease with metronidazole. *Gastroenterology* 1980; **79**: 357-365 [PMID: 7399243]

72 **Brandt LJ**, Bernstein LH, Boley SJ, Frank MS. Metronidazole therapy for perineal Crohn's disease: a follow-up study. *Gastroenterology* 1982; **83**: 383-387 [PMID: 7084615]

73 **Jakobovits J**, Schuster MM. Metronidazole therapy for Crohn's disease and associated fistulae. *Am J Gastroenterol* 1984; **79**: 533-540 [PMID: 6741906]

74 **Thia KT**, Mahadevan U, Feagan BG, Wong C, Cockeram A, Bitton A, Bernstein CN, Sandborn WJ. Ciprofloxacin or metronidazole for the treatment of perianal fistulas in patients with Crohn's disease: a randomized, double-blind, placebo-controlled pilot study. *Inflamm Bowel Dis* 2009; **15**: 17-24 [PMID: 18668682 DOI: 10.1002/ibd.20608]

P- Reviewers Veres G, Yamamoto T **S- Editor** Lv S
L- Editor A **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i5.654

World J Gastroenterol 2013 February 7; 19(5): 654-664
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

MINIREVIEWS

Clinical impact of hepatitis B and C virus envelope glycoproteins

Hélène Jeulin, Aurélie Velay, John Murray, Evelyne Schvoerer

Hélène Jeulin, Aurélie Velay, Virology Laboratory, Centre Hospitalier Universitaire de Nancy, Vandoeuvre-lès-Nancy, F-54511, France
Hélène Jeulin, EA RHEM 4369, Université de Lorraine, F-54000 Nancy, France
John Murray, School of Mathematics and Statistics, University of New South Wales, Sydney, NSW 2052, Australia
John Murray, The Kirby Institute, University of New South Wales, Sydney, NSW 2052, Australia
Evelyne Schvoerer, Virology Laboratory, Centre Hospitalier Universitaire de Nancy, F-54511 Vandoeuvre-lès-Nancy, France
Author contributions: Jeulin H and Velay A contributed equally to this work, generated the figures and wrote the manuscript; Murray J contributed to the writing of the manuscript; Schvoerer E designed the aim of the editorial and wrote the manuscript.
Correspondence to: Evelyne Schvoerer, MD, PhD, Professor, Virology Laboratory, Centre Hospitalier Universitaire de Nancy, F-54511 Vandoeuvre-lès-Nancy, France. eschvoerer@gmail.com
Telephone: +33-38-3155269 Fax: +33-38-3153474
Received: September 14, 2012 Revised: October 29, 2012
Accepted: December 15, 2012
Published online: February 7, 2013

Abstract

Chronic infection by either hepatitis B virus (HBV) or hepatitis C virus (HCV) share epidemiological characteristics with risks for development of severe complications such as liver cirrhosis and hepatocellular carcinoma. HBV and HCV also share a high genetic variability. Among highly variable regions, viral genes encoding surface proteins (hepatitis B surface antigen, E1/E2 HCV glycoproteins) play key roles in the stimulation of the host-related immune response and viral entry into hepatocytes. Specific segments of HBV envelope proteins (preS1, "a" determinant) are crucial in the entry process into permissive cells. HCV entry is a complex multistep process involving multiple cell cofactors (glycosaminoglycans, low density lipoprotein receptor, SR-B1, CD81, claudin-1, occludin, EGFR, EphA2) in the interaction with HCV E1/E2 envelope glycoproteins. *In vitro* both viruses can be controlled by antibody-mediated neutralization targeting viral envelope, also essential in preventing HBV infection *in vivo* as observed through successful vaccination using HBs antigen. But preventive vaccination and/or therapeutic pressure can influence HBV and HCV variability. For HBV, the patterns of antiviral drug resistance in chronic hepatitis are complex and the original *pol*/*S* gene overlap has to be taken into account. Treatment-induced HBV mutations in pol could indeed generate S mutants with subsequent modified antigenicity or increased cancer induction. Variability of HBV and HCV envelope proteins combining high exposure to selective pressures and crucial functional roles require investigation in the context of diagnostic, vaccination and treatment tools. In this editorial a synthesis is performed of HBV and HCV envelope properties at the entry step and as antigenic proteins, and the subsequent clinical impact.

© 2013 Baishideng. All rights reserved.

Key words: Hepatitis B; Hepatitis C; Viral envelope glycoproteins; Clinical outcome

Jeulin H, Velay A, Murray J, Schvoerer E. Clinical impact of hepatitis B and C virus envelope glycoproteins. *World J Gastroenterol* 2013; 19(5): 654-664 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i5/654.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i5.654

INTRODUCTION

Approximately 400 million people are currently living with chronic infection due to hepatitis B virus (HBV) and the number is approaching 160 million for chronic hepatitis C virus (HCV)-related infection[1]. Besides sharing many epidemiological features, HBV and HCV can both provoke chronic infections after an acute phase, due to a failure of humoral and cellular immune host responses[2-5].

HBV and HCV also share some biological similarities, especially the high frequency of mutations occuring dur-

ing viral replication. As a result both viruses can develop mutations leading to drug failure, especially for drugs with low potency and a low genetic barrier to resistance such as with anti-HBV lamivudine monotherapy. It is expected this will be less likely during treatment by entecavir or tenofovir which exhibit potent anti-HBV activity and high genetic barriers to resistance.

Viral dynamics are rapid with daily production of $10^{12\text{-}13}$ virions for HBV and 10^{12} for HCV. The half-life of free viral particles is short, less than 4 h for HBV[6,7] and 2-3 h for HCV[8]. Although both HBV and HCV can produce consistently high levels of virus in serum, they do so using viral templates with very different life-spans. The template for HBV replication, covalently closed circular DNA (cccDNA), resides in the nucleus of infected hepatocytes and is very stable in chronic infection, likely persisting for months[9], if not longer. On the other hand the intracytoplasmic minus strand RNA HCV template only persists for a few hours. Correspondingly HBV eradication is more difficult and only occurs after several years of viral suppression by therapy. Another characteristic of HBV is that its genome consists of overlapping reading frames: as a consequence, mutation rates to functional virus are lower for HBV compared to HCV which does not contain any overlapping gene. The counterpart of this molecular HBV characteristic is that changes at one position may affect the structure and function of more than one viral protein: the polymerase gene overlaps with the envelope gene, and consequently mutations have yet been published in polymerase gene under treatment pressure can produce mutations in the envelope *S* gene[10].

Despite the availability of an effective vaccine against HBV, new infections are still highly frequent worldwide. Hence, a careful examination of circulating viral strains must be considered in order to possibly adapt the antigenic vaccine content. To date however no effective vaccine is available against HCV. For both viruses, surface envelope glycoproteins are the external antigens first encountered by host-related immune responses during primary infection. These proteins contain both highly variable and conserved regions, the latter providing possible targets for preventive immunization and/or complementary therapeutic approaches.

The aim of this editorial is to give an overview of the current knowledge on hepatitis B and hepatitis C envelope glycoproteins, however with a particular emphasis on HBV. Structural and functional data will be described on the role of these proteins in the viral entry step into hepatocytes and in the stimulation of host-related responses and clinical impacts for HBV will also be described.

GENERAL PRESENTATION OF HBV ENVELOPE GLYCOPROTEINS AND ANIMAL/CELL CULTURES SYSTEMS SUITABLE FOR THEIR INVESTIGATION

For more than 20 years, primary human hepatocytes (PHH) from surgically excised liver specimens have been the only available *in vitro* HBV infection model. The transfection of hepatocyte-related cells (based on primary hepatocytes or hepatic cell lines which are easier to handle) with replication-competent HBV genomes allowed the production of secreted infectious virions. Regardless of the difficulty in maintaining these *in vitro* models, PHH cultures have for a long time been the only way to study viral infectivity. Due to poor efficiency of HBV infection in this model, virus amplification required the use of dimethylsulfoxide (DMSO) and of 4% polyethylene glycol during cultivation and infection, respectively. Primary hepatocyte cultures from *Tupaia belangeri* can also be infected with HBV, as efficiently as PHH cultures, but with fewer restrictions. Among human hepatic cell lines, the optimal system for various experiments was shown to be HepaRG cells, recently established from a liver tumor, and which become susceptible to HBV upon treatment with DMSO and hydrocortisone[11,12] (Table 1).

Many discoveries about the viral replication cycle, persistence and clearance have been obtained using animal models such as chimpanzee, gibbon or tupaia infected with HBV[13,14]. Moreover, humanized uPA/SCID mice have been recently used to study HBV/hepatitis D virus coinfection and represent an interesting model allowing us to investigate new antiviral drugs[15]. Experimental systems have also been provided by other *Hepadnaviridae* such as the duck HBV transmissible to many duck species or the woodchuck hepatitis virus, restricted to the North-Eastern American woodchuck[13].

Hepadnaviruses are characterized by specific liver tropism and high species specificity, restricting *in vivo* infection to their natural host or closely related species and contributing to the difficulty in developing suitable models of HBV infection[11].

HBV envelope glycoproteins are key viral elements in the viral cycle and in the stimulation of host-related immunity. Within virions, HBV surface envelope antigen (HBsAg) includes three viral surface glycoproteins, named large, medium, and small proteins (LHBs, MHBs and SHBs). They exhibit various distributions either in virions or in subviral particles. Their expression is directed by one stop codon and three start codons in a single open reading frame for translation into preS1, preS2 and S proteins[16]. The preS1 protein [108 or 119 amino acids (aa) depending on the genotype] is present only in LHBs; the preS2 protein (55 aa) is present in LHBs and MHBs; and the S protein (226 aa) is shared by LHBs, MHBs and SHBs[11] (Figure 1).

The three HBV surface preS1, preS2 and S proteins have different functions. Within the preS1 domain, amino acids 3-77 of the Large protein are essential for infectivity as is the myristoylation of glycine at position 2. The preS2-domain is present in both M- and L-proteins but because of the cytosolic orientation of the preS1-domain in the L-protein, it is N-glycosylated only in the M-protein. Although antibodies against the N-terminal part of preS2 can inhibit HBV infection *in vitro*, the Medium protein of HBV is believed to play an accessory

Table 1 Cell culture systems and *in vitro* models developed to study hepatitis B virus and hepatitis C virus envelope glycoproteins

	In vitro model/cell culture system	Step of the viral cycle	Benefits and major findings	Drawbacks
HBV	Primary hepatocyte cultures PHH PTH	Replication	No need for DMSO and hydrocortisone for PTH system	Not easy to handle Cells cannot be propagated *in vitro* Addition of growth factors
	Hepatic cell lines HepG2 HepaRG	Binding and infection (HBV and HDV)	Specific binding and uptake Cellular determinants of hepatocyte differentiation and their influence on HBV infection	No productive infection Addition of DMSO and hydrocortisone
HCV	Recombinant E2 glycoprotein: Truncated soluble form of recombinant E2 glycoprotein	Entry process	Identification of two major receptors CD81 and SR-BI Interaction with heparan sulfate proteoglycans	Different behavior from E1 E2 heterodimers Binding to various cell lines different from hepatocytes
	VLPs: Self assembly of HCV structural proteins produced in insect or mammalian cells using a recombinant virus	Entry process	E1–E2 heterodimers at virion surface Cell attachment Attractive vaccine candidate	Difference in glycosylation status Difficult to prepare Non replicative
	HCVpp: Unmodified HCV envelope glycoproteins assembled onto retroviral or lentiviral core particles	Entry process	Study of infectivity and neutralization	Only the very early steps of viral cycle No association with lipoproteins No budding at the ER
	HCVcc: Transfection of one HCV strain sequence (JFH1) from a Japanese patient with fulminant hepatitis, in Huh 7 cell line.	Entire life cycle	Entry process +++ Replication Virus production Screening of antiviral molecules	Restricted to Huh-7 cell line Restricted to JFH1 non structural proteins sequence

HBV: Hepatitis B virus; HCV: Hepatitis C virus; VLP: Virus-like particles; HCVpp: HCV pseudotype particles; HCVcc: Cell culture derived HCV; HDV: Hepatitis D virus; DMSO: Dimethyl sulfoxide; SR-BI: scavenger receptor class B type I ; ER: Endoplasmic reticulum; PHH: Primary human hepatocytes; PTH: Primary *Tupaia belangeri* hepatocytes; DMSO: Dimethylsulfoxide.

role in infectivity. While the preS-domains form linear epitopes, the S-domain forms multiprotein complexes using inter- and intramolecular disulfide bonds with the eight Cys residues within the antigenic loop. A crucial function of the S-domain is viral morphogenesis, but the first transmembrane sequence also plays a role in viral entry into hepatocytes[11]. Moreover, two different transmembrane topologies have been described for the L surface protein: L chains with an internal N-terminal preS1 part are required in virion morphogenesis, whereas the L molecules which expose their preS1 domain on the viral particle surface probably link to a putative virus receptor and could determine the species specificity and viral tropism[16]. In addition to the preS1 domain, a second infectivity determinant, located in the antigenic loop of the S domain, is also required for infectivity[17].

HBsAg plays a central role in stimulating and, because of its variability, also oppositely thwarting the host-related immune response. The main antigenic part of HBsAg, namely the "a" determinant which is the core part of the major hydrophilic region (amino acid residues 99-169 of HBsAg), is a target of neutralizing antibodies. Immunogenic T cell epitopes are also present in the HBs Ag : P1 (aa 16-33) and P4 (aa 213-226) are the dominant epitopes in vaccine immunization[18].

INTERACTION WITH PUTATIVE CELLULAR RECEPTOR(S)

HBV envelope glycoproteins are the key components contributing to the attachment to the hepatocyte plasma membrane, thus defining organ and species specificity of HBV. Viral tropism is essentially restricted to the liver[14]. Although there is considerable evidence that a HBV receptor exists on hepatocyte membranes, it still remains to be identified. Convergent data place the receptor-binding site in the preS1 segment of HBV envelope proteins that play a key role at the early entry step for further viral infectivity, as described above. The preS1 (21-47) region of the envelope protein was demonstrated to be the dominant binding site to hepatoblastoma cell line (HepG2). More recently, the region consisting of amino acids 9-18 of the preS1 domain was identified as a conserved crucial attachment site while amino acids 28-48 as an accessory binding site[19]. The receptor for the preS1 peptide seems to be a single major peptide of molecular weight 31 kD[20]. The asialoglycoprotein receptor was described as playing a role in the attachment process[21], and numerous other cellular partners have been suggested to also contribute, such as heparin or annexin V, earlier referred to as Endonexin Ⅱ[22-26]. Interestingly, heparan sulfate proteoglycans emerged as potential major components in the HBV entry step[27]. However HBV entry and different steps of this process are not characterized in detail, while it is substantially known for HCV.

HOST-RELATED IMMUNE RESPONSES

During viral infection, immunological reactivity usually begins with an innate response in infected cells, where viral replication induces activation of intracellular an-

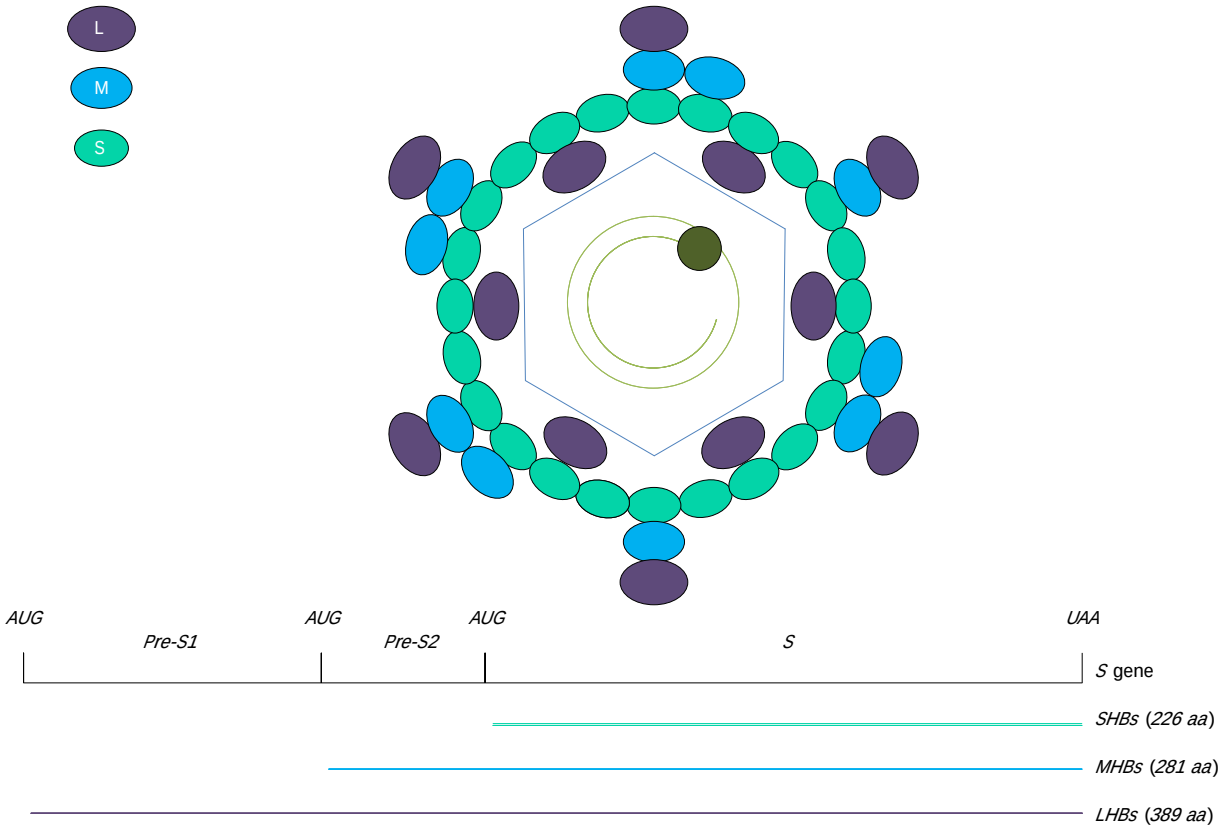


Figure 1 Schematic representation of hepatitis B virus envelope proteins and genome. Hepatitis B virus (HBV) surface envelope antigen includes three viral surface glycoproteins, named large, medium, and small proteins (LHBs, MHBs and SHBs). Their expression is directed by the *S* gene including three start codons and one stop codon in a single open reading frame.

tiviral mechanisms through transcription of interferon (IFN) inducible genes. However, in acutely HBV infected chimpanzees no specific pattern of cellular genes expression was observed during widespread expansion of HBV in the liver, revealing an absence of innate response by infected cells[28]. Interestingly, this could be thwarted by using IFN-α which has been described to inhibit HBV transcription and replication in cell culture and in humanized mice by targeting the nuclear cccDNA[29]. The adaptive immune response is responsible for viral clearance as well as HBV-related diseases pathogenesis. In patients with acute hepatitis, the T cell response is vigorous and multispecific. The CD8+ T cell response, under the control of CD4+ T cells, acts on viral clearance by triggering direct hepatocyte apoptosis and by secreting IFN-γ which inhibits HBV replication. In chronically infected patients, a weak and focused T cell response is observed (Figure 2). This seems less related to sufficient numbers of CD8+ T cells, but more to their functional alteration[30]. Human leukocyte antigen (HLA) class I and HLA class Ⅱ restricted T-cell epitopes have been recently reviewed in Desmond 2008[31]. Several HBV epitopes recognized by CD4+ T cells were described in the surface protein, including $S2_{109-134}$, $S_{200-214}$ and $S_{337-357}$, with $S_{179-194}$, considered as an immunodominant HLA-DR1-restricted epitope[32].

Regulatory T (Treg) lymphocytes, known to suppress the activation, differentiation and proliferation of immune cells, could be major actors in the inadequate immune responses observed during chronic HBV infection. The Treg response protects the host by limiting liver immunopathology while at the same time favouring the virus by inhibiting a protective T-cell response. The timing of the Treg response is vital; it needs to be not too late otherwise resulting in the excessive destruction of hepatocytes by effector T-cells, and not too early which would favour the establishment of chronic infection by blocking antiviral T-cell activity[33]. As suggested by Manigold *et al*[33], a higher frequency of Treg cells has been described in patients with chronic infection and correlated with greater HBV DNA concentration in serum.

The humoral immune response is based on anti-envelope protective antibodies. On the one hand, antibodies complex with free particles, removing them from circulation and on the other hand they prevent HBV attachment to hepatocytes, thus playing a critical role in viral clearance. Both viral and host factors can delay or inhibit antibody-mediated neutralization of HBV. As it will be further described, viral variability contributes to the failure of the humoral response. Other factors such as HIV coinfection[34] or immunosuppression from a variety of causes can also negatively modulate humoral reactivity[35]. Additionally, the development of an immune response, usually characterized by the strong appearance

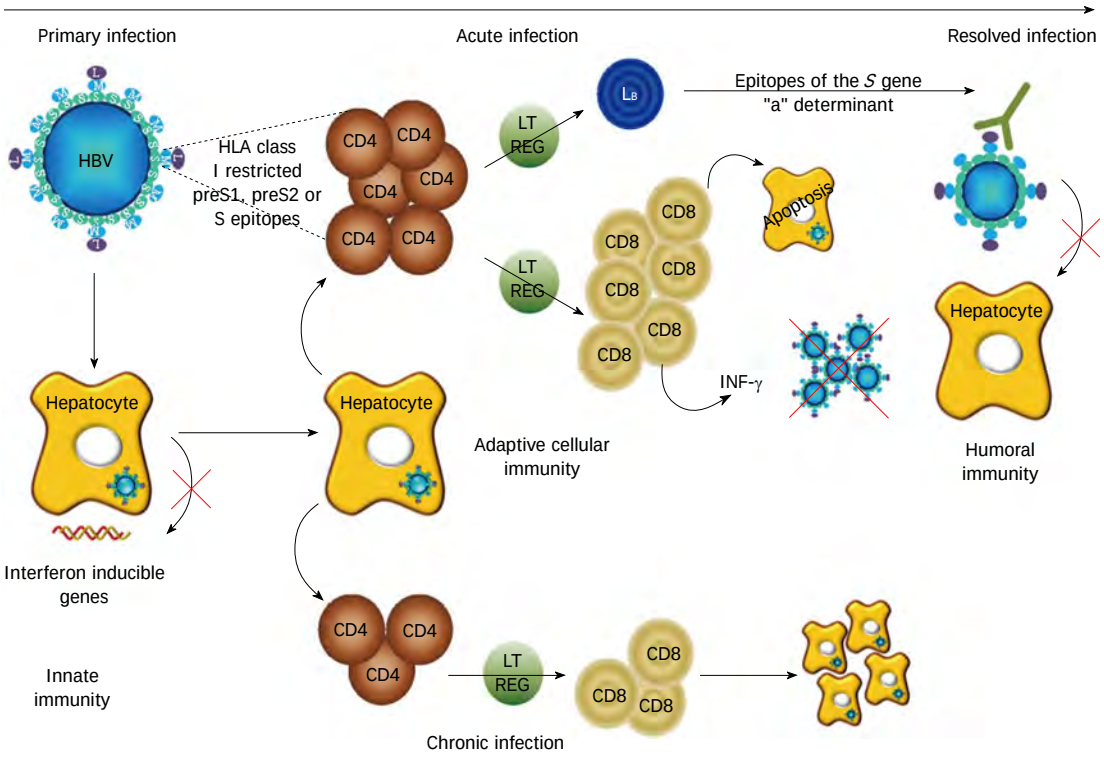


Figure 2 Role of the hepatitis B surface protein in the immune response to hepatitis B infection. Hepatitis B virus (HBV) primary infection does not induce activation of intracellular antiviral mechanisms through transcription of interferon inducible genes. After acute infection, the evolution to resolved hepatitis B is promoted by a vigorous and multispecific T cell response (CD4+ and CD8+ T cell) through hepatocyte apoptosis and interferon (IFN)-γ secretion, while evolution to chronicity is characterized by a weak and focused T cell response. Regulatory T cells (Treg) cells (LT REG) limit the function of effector T cells preventing excessive auto-destructive disease. A protective immunity is due in part to humoral and cellular anti-envelope response, preventing HBV attachment to hepatocytes, thus playing a critical role in prevention of infection after classical vaccination and in viral clearance in resolved hepatitis. HLA: Human leukocyte antigen.

of anti-HBs antibodies, may be delayed by social stress, as identified during HBV vaccination of students under exam stress[36].

GENERAL PRESENTATION OF HCV ENVELOPE GLYCOPROTEINS AND RELEVANT ANIMAL/CELL CULTURE MODELS

HCV envelope glycoprotein E1 and E2 represent crucial elements in the viral life cycle. Indeed, they are involved in virus cellular entry and are also targeted by host immune components. Thus characterization of these two proteins is essential to better understand virus-host interactions.

E1 and E2 are transmembrane glycoproteins containing an important N terminal ectodomain and a C terminal hydrophobic anchor domain. The E2 protein displays an amphipathic helix that forms a stem region, linking the ectodomain and the transmembrane region. The two glycoproteins form a non-covalent heterodimer at the viral particle surface, interacting with each other during virus entry into target cells. E1 and E2 are both stabilized by numerous disulfide bonds. Krey *et al*[37] described the connectivity of the E2 disulfide bonds and suggested that E2 could display a tertiary structure similar to that of class Ⅱ fusion proteins, and containing three domains termed DI, DII and DIII. Their model reveals the distribution of E2 amino acids among the different domains. E1 and E2 are highly glycosylated proteins and glycosylation is needed for correct glycoprotein processing, folding and/or cellular entry.

The E2 glycoprotein contains three hypervariable regions, termed hypervariable region (HVR) 1, HVR2 and HVR3 and conformational binding sites to certain cellular co-receptors (CD81 binding site as an example). E1 and E2 glycoproteins are targets of both humoral and cellular immune responses generated during HCV infection and several specific epitopes have been described in E1 and E2.

Our knowledge of the HCV viral cycle and of the structural and functional characteristics of envelope glycoproteins has been hampered by the lack of reliable *in vivo* and *in vitro* models. Chimpanzee infection has long been the only available animal model, but with many limitations including ethical considerations, high cost, and clinical differences with human HCV infection. Other small animal models, such as humanized mice, are in development to overcome these problems.

Some *in vitro* models, such as soluble glycoproteins, virus like particles, and HCV pseudoparticles, have been developed that allow investigation of the viral entry process. Despite their ability to duplicate the early steps

of HCV entry, none of these models support the entire HCV replication cycle or efficient production of infectious particles. In this regard an important breakthrough was achieved with the cell culture system HCVcc, which is capable of producing infectious particles. The most useful *in vitro* models that allow the study of HCV glycoproteins are detailed in Table 1.

INTERACTIONS OF HCV ENVELOPE GLYCOPROTEINS WITH CELLULAR RECEPTORS

The main target cells of HCV are hepatocytes. HCV enters into target cells in a complex multistep process involving the following entry host factors: tetraspanin CD81, scavenger receptor class B member I (SR-BI), Heparan Sulfate and the tight junction proteins Claudin-1 and Occludin. Other partners in HCV entry were recently characterized, such as EGFR and EphA2[38].

Highly sulfated heparan sulfate structures are cell surface factors mediating initial virus attachment prior to virus interaction with CD81 and SR-BI, which directly interact with HCV envelope glycoproteins. SR-BI may define the attachment of the virion in the form of virolipoparticles to the cell surface and that then favours particle interactions with CD81 and Claudin coreceptors with a role of EGFR and EphA2 activated kinases in the CD81-Claudin binary complex. CD81 and Claudin-1 are essential for the clathrin dependant particle internalization, and the engagement of CD81 promotes internalization. CD81 associates with HCV and mediates entry *via* Claudin-1 and occludin complexes. Claudin-1/CD81 complexes seem to localize at the basolateral surface of polarized hepatoma cells. After uncoating and internalization by clathrin-dependent endocytosis, the viral genome is released into the cytoplasm leading to its translation and replication.

INTERPLAY BETWEEN HCV ENVELOPE GLYCOPROTEINS AND THE HOST IMMUNE RESPONSE

Persistent viral infection can be explained by the fact that HCV escapes both innate and adaptative immune responses or interferes with host defense mechanisms. Despite broadly reactive neutralizing and multispecific T cell responses generated during chronic infection, in most cases viral clearance is not achieved.

HCV RNA can be detected in patient serum between one and two weeks following infection whereas the appearance of HCV specific T-lymphocytes and antibodies is delayed up to several weeks after infection. In the early phase of infection neutralizing antibodies are rapidly induced by patients who subsequently clear the virus or control viral infection whereas they are absent or of low quantity in patients who progress to chronic HCV infection[39]. Both cellular and humoral responses fail to control HCV infection during the very early phase of infection. If viral clearance is characterized by a broad vigorous and persistent T cell response, cellular immunity is weak, narrow and transient in patients chronically infected (Figure 3). Some mutations occurring in epitopes targeted by CD4+ or CD8+ T lymphocytes can show a strong inhibition of immune reactivity, such as HCV E1 226-240 and HCV E2 436-450[3]. Patients who do not clear the virus present high quantity and even cross-neutralizing antibodies during the chronic phase, but these antibodies are unable to control HCV infection.

The failure of the humoral response is mainly due to the rapid onset of mutations in HVR1 of E2 and in other epitopes of the E1 and E2 proteins. However, HVR1, the most variable region of the HCV genome, is one of the targets of the neutralizing immune response. HVR1 may function as an "immunological decoy", stimulating an ineffective immune response for viral clearance but inducing selection of viral variants[40].

The E2-CD81 interaction has been shown to modulate B and T cell function. During HCV infection, the E2-CD81 interaction induces hypermutation of the heavy chain immunoglobulin in B cells. Hypermutation may lower the affinity and specificity of HCV-specific antibodies, enabling HCV to escape immune surveillance. Additionally, engagement of CD81 on natural killer cells by the E2 protein has been shown to inhibit the antiviral function of NK cells early in the infection process.

GENES AND PROTEINS VARIABILITY, ESPECIALLY IN VIRAL ENVELOPE GLYCOPROTEINS

Despite the compact arrangement of its DNA genome and the overlapping open reading frames that limit genomic plasticity, HBV is evidently diverse on a global scale as indicated by the eight referenced genotypes (A-H). HCV is also characterized by high genetic variability given that six (and probably a seventh) major genotypes have been described, each including several subtypes. Intragenomic variability of HBV and HCV is further reflected in the quasispecies distribution defined by the genetic heterogeneity of the viral pool found in any infected subject at a given time point[41,42]. For both viruses, this genetic variability is a consequence firstly of the high spontaneous error rate associated with the lack of a proofreading mechanism of viral polymerases, and secondly of the structural and/or functional constraints exerted on the virus by host immunity, immunoprophylaxis or antiviral therapy pressure.

Due to the structural constraints of its genomic organization, the emergence of HBV mutants occurs more slowly than for HCV. Since the preS1 and preS2 domains overlap with the region of the polymerase gene encoding for the spacer domain, deletions in preS can occur without affecting the enzymatic properties of HBV

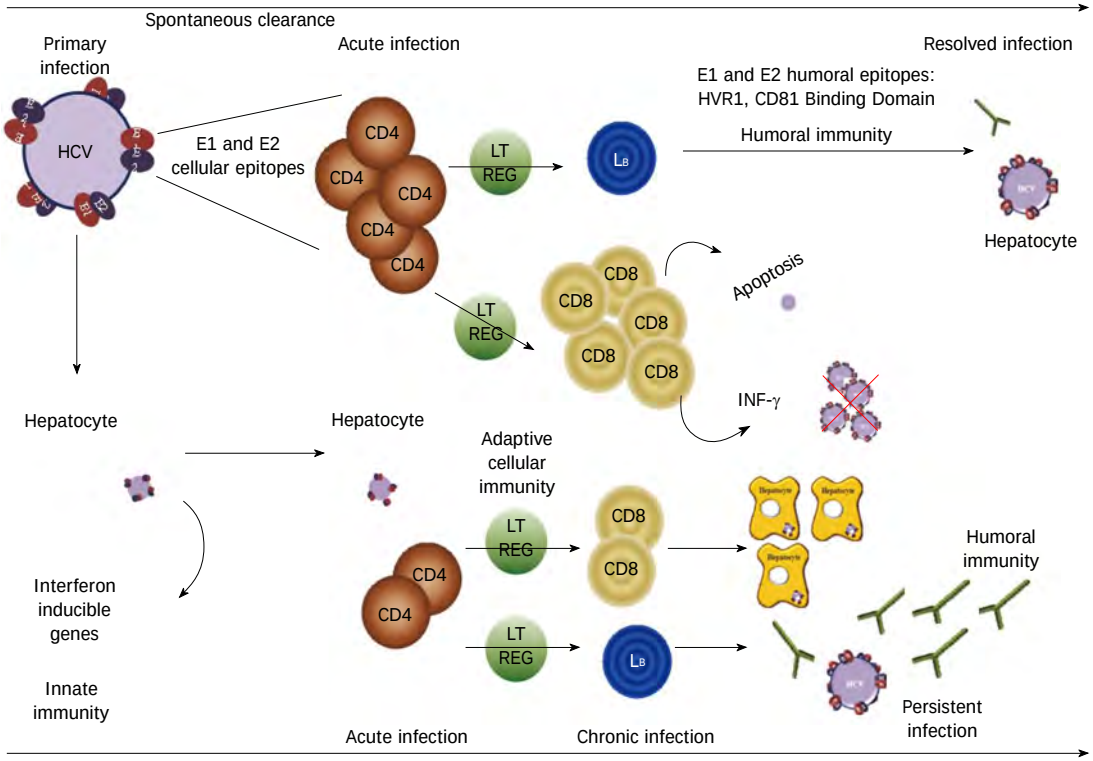


Figure 3 Role of hepatitis C glycoproteins in the immune response to hepatitis C infection. Following hepatitis C virus (HCV) infection, innate host response is initiated by hepatocytes producing an antiviral state by stimulating interferon inducible genes expression. In case of spontaneous clearance or resolved infection, a strong cellular response, due to CD4+ and CD8+ T lymphocytes (denoted as CD4 and CD8 in the figure), is observed in the early phase of infection. Moreover Regulatory T cells (Treg) cells (LT REG) limit the function of effector T cells. Then neutralizing antibodies are rapidly expressed. In most cases, the host adaptative immunity is unable to control HCV infection evolving to a chronic phase. The cellular response (CD4+ and CD8+ T lymphocytes and B lymphocytes, mentioned as L_B in the figure) is weak and transient. The appearance of neutralizing antibodies is delayed and the humoral response not efficient. IFN: Interferon.

polymerase. However, although preS1 deleted mutants are able to replicate they usually need a helper virus to infect new hepatocytes[43]. The surface S gene seems to vary less than the preS1 and preS2 regions[44]. Functional mutations that have been described in the HBV envelope genome consist of preS1 and preS2 deletions, preS2 start codon mutations, and C-terminally truncated or "a" determinant mutated S proteins. These viral variants exhibit modified replication capacities and antigenic characteristics; on the other hand they may be resistant to antiviral therapies and/or contribute to escape from host-related immune surveillance. They can also alter the sensitivity of diagnostic immunoassays. In HepG2 cells, replication of preS/S variants leads to decreased HBsAg secretion, retention of envelope proteins in the endoplasmic reticulum, less efficient virion secretion and higher amounts of cccDNA in the nucleus. In patients with HBV-related chronic liver disease, the emergence of preS/S variants appears to provoke the loss of correlation between HBV DNA replication and HBsAg synthesis/secretion[45].

Briefly for HCV, the HCV genome contains both highly conserved and highly variable regions. The most variable regions are located in NS5A and in parts of the E2 glycoprotein (HVR)[46,47]. Some mutations have been described in E2 showing an impact on HCV infectivity and/or its accessibility to antibodies or cellular response mediated by CD4+ and CD8+ T cells[3,48-51].

PREVENTIVE OPTIONS AND THE RISK OF ESCAPE MUTANTS

Vaccination using a recombinant HBV surface antigen is the most important prophylactic measure available for the prevention of new HBV infections. Immunoprophylaxis with vaccine or immunoglobulins is also recommended after exposure to blood from HBV-infected patients, in new born children from HBV-infected mothers, and to prevent recurrent HBV infection in patients receiving liver transplants for end-stage hepatitis B liver disease.

HBV surface gene variants can be selected under immune pressure after preventive immunization. This is illustrated by report of several surveys in Taiwan describing an increase in the prevalence of *S* gene "a" determinant mutants during a vaccination campaign[52]. In HBV DNA positive children from four sequential surveys in Taiwan, the prevalence of hepatitis B surface gene "a" determinant mutants increased from 7.8% before the vaccination program, to 19.6%, 28.1% and 23.1% at five, 10 and 15 years after the immunization period. However, lower infectivity of the G145R mutant virus, the use of a recombinant vaccine, and mutant loss with older age seem to decrease the "a" mutant prevalence in an immunized population over time[53]. In Europe and North America, mutations in the HBV *S* gene are mostly observed in infants born from HBV-infected mothers,

in a small proportion of occult HBV infections, and in liver transplant recipients[54]. Among variants which were described, the Arg-145 and Arg-129 mutants show the lowest binding ability to monoclonal antibodies. In addition, variants epitopes in HBs antigen induced decreased or a lack of T-cell reactivity[18]. As an illustration, HBV replication was detected in vaccinated chimpanzees challenged with an HBV strain containing polymerase/envelope overlapping mutations[55].

The considerable global diversity of HCV has hampered the development of any successful vaccine. Consequently, neither vaccine, nor specific immunoglobulins are currently available for clinical practice. Current HCV vaccine research therefore follows two main pathways: (1) prophylactic; and (2) therapeutic to increase virological response to antiviral treatment and to reduce the duration of therapy. Although numerous approaches have been developed only a few of these have progressed to human trials, especially for potential therapeutic vaccines. HCV envelope proteins that induce antibodies against conserved domain involved in cell binding are considered as a promising target for vaccine research[56]. However, several mechanisms make antibody-mediated neutralization difficult: interference by interfering antibodies, glycans or lipids, the quasispecies distribution of HCV, and difficult to access cell-to-cell transfer[57].

TREATMENT AND RISK OF ESCAPE MUTANTS

The treatment of chronic hepatitis B aims to reduce liver cell inflammation, prevent the progression to cirrhosis and hepatocellular carcinoma through the suppression of viral replication, and ultimately to clear the infection. HBV treatments use the immunomodulatory agents IFN-α or its pegylated form as well as direct-acting antiviral molecules. IFN-α binding to type 1 IFN receptors activates an anti-viral state in cells by inducing stimulation of *IFN* genes. IFN also stimulates the cellular immune response and production of IFN-γ by CD4+ and CD8+ T cells[58]. On the other hand, nucleos(t)ide analogues block HBV polymerase functions[59]. A sustained virological response to antiviral therapy is defined by plasma HBV DNA < 2000 IU/mL six months after discontinuation of therapy, while a complete response is defined by the disappearance of both HBV DNA and HBsAg with or without appearance of anti-HBs antibodies[58]. During treatment various escape mutants can be detected. Well-characterized mutations in the reverse transcriptase (rt) gene have been described (*e.g.*, rtM204V for lamivudine and rtN236T for adefovir). HBV resistance can develop after a single mutation with some agents or only after multiple mutations for others. Entecavir exhibits low rates of resistance, with fewer than 1% of patients on monotherapy developing resistant mutants after 5 years of treatment[59], if patients strictly respect treatment recommendations. Similarly tenofovir has a very high genetic barrier. Due to the structure of the HBV genome, mutations in the rt domains can affect the amino acid sequence of other HBV proteins, as demonstrated for the rtM204V + rtV173L + rtL180M mutations that lead to envelope changes behaving as mutants escaping vaccination *in vitro*[59]. In addition, studies analyzing HBV quasispecies demonstrated that resistance mutations to nucleos(t)ide analogues can cause amino acid changes within epitopes of the HBV preS/S or core antigen leading to changes in HBV immunogenicity[60].

Treatment of HCV chronic infection consists of a combination of pegylated IFN-α and ribavirin, and this combination remains the basis of gold standard treatments including the use of anti-protease molecules. The rate of sustained virological response (undetectable HCV viral load six months after the end of the therapy, detection threshold at 12-15 IU/mL) depends on the genotype of HCV with lower rates for genotype 1 and is correlated to pre-treatment HCV load and several host-related factors. Both IFN and ribavirin have broad spectrum non specific antiviral activity without direct action on viral genomes[46]. IFN produce an antiviral state by the up-regulation of IFN stimulated genes, leading to the expression of several antiviral proteins including 2', 5' oligoadenylate synthase, and the Mx protein. Four putative mechanisms of action have been proposed to explain the effect of ribavirin on HCV infection: modulation of the host adaptive antiviral response, HCV RNA mutagenesis, action on the intracellular GTP pool necessary for RNA synthesis and inhibition of HCV polymerase. Thus resistance to treatment is a complex mechanism involving host- and virus-related features.

Several HCV proteins (E2, core, NS5A, NS5B) are suspected to be linked with resistance mechanisms to antiviral therapy[46,61,62]. In relation to HCV envelope glycoproteins, treatment outcome can be influenced by amino acid substitutions within CD81 binding sites and HVR2 of E2[63] as well as theoretically through mutations in the PKR/eIF-2α phosphorylation homology domain of E2[61]. A lower HVR1 heterogeneity among viral variants in an individual before antiviral therapy seems to be associated with higher rates of virological response to IFN-based treatment[46,64]. HCV genetic variability in regions such as E2 HVR1 has been associated with antiviral treatment failure[46,65]. In a clinical context, Aurora *et al*[66] described frequent covarying amino acids positions, most often located in E1 and E2, that linked to response to treatment.

CONCLUSION

For both HBV and HCV, viral envelope glycoproteins play a key role in viral entry into hepatocytes and are exposed to host-related immune responses. Even though data that fully elucidates HBV entry into permissive cells are still lacking, specific segments of HBV envelope proteins (preS1, "a" determinant) are instrumental in the process. For HCV, numerous experimental results have recently demonstrated that viral entry is a com-

plex multistep process involving multiple cell cofactors (glycosaminoglycans, low density lipoprotein receptor, SR-B1, CD81, claudin-1, occludin, EGFR, EphA2) in the interaction with HCV E1/E2 envelope glycoproteins. *In vitro* both viruses can be controlled by antibody-mediated neutralization targeting viral envelope. Neutralizing antibodies are also essential in preventing HBV infection *in vivo* as is well characterized through successful vaccination using HBs antigen.

Several factors, such as preventive vaccination and/or therapeutic pressure, can influence HBV and HCV variability. Moreover, for HBV, the patterns of antiviral drug resistance in chronic hepatitis are complex and the original pol/S gene overlap has to be taken into account in the analysis of public health significance of emerging genomic mutations in infected populations. Treatment-induced HBV mutations in pol could indeed generate S mutants with subsequent modified antigenicity or pathogenesis such as increased cancer induction[10].

Variability of HBV and HCV envelope proteins combining high exposure to selective pressures and crucial functional roles require investigation for the adaptation of diagnostic, vaccination and treatment tools. In this context, improved therapy for chronic HCV infection has emerged from a better understanding of the viral replication cycle, with HCV entry providing a large number of therapeutic targets[67,68]. Besides IFN-α and nucleos(t)idic analogues, complementary therapies for chronic HBV infection are desperately needed and the HBV entry process, when better characterized, can offer potential therapeutic opportunities.

REFERENCES

1 **Lavanchy D**. Evolving epidemiology of hepatitis C virus. *Clin Microbiol Infect* 2011; **17**: 107-115 [PMID: 21091831 DOI: 10.1111/j.1469-0691.2010.03432.x]

2 **Thimme R**, Bukh J, Spangenberg HC, Wieland S, Pemberton J, Steiger C, Govindarajan S, Purcell RH, Chisari FV. Viral and immunological determinants of hepatitis C virus clearance, persistence, and disease. *Proc Natl Acad Sci USA* 2002; **99**: 15661-15668 [PMID: 12441397 DOI: 10.1073/pnas.202608]

3 **von Hahn T**, Yoon JC, Alter H, Rice CM, Rehermann B, Balfe P, McKeating JA. Hepatitis C virus continuously escapes from neutralizing antibody and T-cell responses during chronic infection in vivo. *Gastroenterology* 2007; **132**: 667-678 [PMID: 17258731 DOI: 10.1053/j.gastro.2006.12.008]

4 **Chen Y**, Qian F, Yuan Q, Li X, Wu W, Guo X, Li L. Mutations in hepatitis B virus DNA from patients with coexisting HBsAg and anti-HBs. *J Clin Virol* 2011; **52**: 198-203 [PMID: 21840251 DOI: 10.1016/j.jcv.2011.07.011]

5 **Baumert TF**, Thimme R, von Weizsäcker F. Pathogenesis of hepatitis B virus infection. *World J Gastroenterol* 2007; **13**: 82-90 [PMID: 17206757]

6 **Murray JM**, Purcell RH, Wieland SF. The half-life of hepatitis B virions. *Hepatology* 2006; **44**: 1117-1121 [PMID: 17058221 DOI: 10.1002/hep.21364]

7 **Dandri M**, Murray JM, Lutgehetmann M, Volz T, Lohse AW, Petersen J. Virion half-life in chronic hepatitis B infection is strongly correlated with levels of viremia. *Hepatology* 2008; **48**: 1079-1086 [PMID: 18697217 DOI: 10.1002/hep.22469]

8 **Neumann AU**, Lam NP, Dahari H, Davidian M, Wiley TE, Mika BP, Perelson AS, Layden TJ. Differences in viral dynamics between genotypes 1 and 2 of hepatitis C virus. *J Infect Dis* 2000; **182**: 28-35 [PMID: 10882578 DOI: 10.1086/315661]

9 **Zhu Y**, Yamamoto T, Cullen J, Saputelli J, Aldrich CE, Miller DS, Litwin S, Furman PA, Jilbert AR, Mason WS. Kinetics of hepadnavirus loss from the liver during inhibition of viral DNA synthesis. *J Virol* 2001; **75**: 311-322 [PMID: 11119601 DOI: 10.1128/JVI.75.1.311-322.2001]

10 **Warner N**, Locarnini S. The antiviral drug selected hepatitis B virus rtA181T/sW172* mutant has a dominant negative secretion defect and alters the typical profile of viral rebound. *Hepatology* 2008; **48**: 88-98 [PMID: 18537180 DOI: 10.1002/hep.22295]

11 **Glebe D**, Urban S. Viral and cellular determinants involved in hepadnaviral entry. *World J Gastroenterol* 2007; **13**: 22-38 [PMID: 17206752]

12 **Gripon P**, Rumin S, Urban S, Le Seyec J, Glaise D, Cannie I, Guyomard C, Lucas J, Trepo C, Guguen-Guillouzo C. Infection of a human hepatoma cell line by hepatitis B virus. *Proc Natl Acad Sci USA* 2002; **99**: 15655-15660 [PMID: 12432097 DOI: 10.1073/pnas.232137699]

13 **Zoulim F**, Berthillon P, Guerhier FL, Seigneres B, Germon S, Pichoud C, Cheng YC, Trepo C. Animal models for the study of HBV infection and the evaluation of new anti-HBV strategies. *J Gastroenterol Hepatol* 2002; **17** Suppl: S460-S463 [PMID: 12534778 DOI: 10.1046/j.1440-1746.17.s4.10.x]

14 **De Meyer S**, Gong ZJ, Suwandhi W, van Pelt J, Soumillion A, Yap SH. Organ and species specificity of hepatitis B virus (HBV) infection: a review of literature with a special reference to preferential attachment of HBV to human hepatocytes. *J Viral Hepat* 1997; **4**: 145-153 [PMID: 9181523 DOI: 10.1046/j.1365-2893.1997.00126.x]

15 **Lütgehetmann M**, Mancke LV, Volz T, Helbig M, Allweiss L, Bornscheuer T, Pollok JM, Lohse AW, Petersen J, Urban S, Dandri M. Humanized chimeric uPA mouse model for the study of hepatitis B and D virus interactions and preclinical drug evaluation. *Hepatology* 2012; **55**: 685-694 [PMID: 22031488 DOI: 10.1002/hep.24758]

16 **Bruss V**, Gerhardt E, Vieluf K, Wunderlich G. Functions of the large hepatitis B virus surface protein in viral particle morphogenesis. *Intervirology* 1996; **39**: 23-31 [PMID: 8957666 DOI: 10.1159/000150471]

17 **Salisse J**, Sureau C. A function essential to viral entry underlies the hepatitis B virus "a" determinant. *J Virol* 2009; **83**: 9321-9328 [PMID: 19570861 DOI: 10.1128/JVI.00678-09]

18 **Bauer T**, Weinberger K, Jilg W. Variants of two major T cell epitopes within the hepatitis B surface antigen are not recognized by specific T helper cells of vaccinated individuals. *Hepatology* 2002; **35**: 455-465 [PMID: 11826423 DOI: 10.1053/jhep.2002.30903]

19 **Glebe D**. Attachment sites and neutralising epitopes of hepatitis B virus. *Minerva Gastroenterol Dietol* 2006; **52**: 3-21 [PMID: 16554703]

20 **Dash S**, Rao KV, Panda SK. Receptor for pre-S1(21-47) component of hepatitis B virus on the liver cell: role in virus cell interaction. *J Med Virol* 1992; **37**: 116-121 [PMID: 1629710 DOI: 10.1002/jmv.1890370208]

21 **Stockert RJ**. The asialoglycoprotein receptor: relationships between structure, function, and expression. *Physiol Rev* 1995; **75**: 591-609 [PMID: 7624395]

22 **Hertogs K**, Leenders WP, Depla E, De Bruin WC, Meheus L, Raymackers J, Moshage H, Yap SH. Endonexin II, present on human liver plasma membranes, is a specific binding protein of small hepatitis B virus (HBV) envelope protein. *Virology* 1993; **197**: 549-557 [PMID: 8249278 DOI: 10.1006/viro.1993.1628]

23 **de Bruin WC**, Leenders WP, Moshage H, van Haelst UJ. Species specificity for HBsAg binding protein endonexin II. *J Hepatol* 1996; **24**: 265-270 [PMID: 8778191 DOI: 10.1016/S0168-8278(96)80003-1]

24 **De Meyer S**, Gong ZJ, Hertogs K, Depla E, van Pelt JF, Ros-

kams T, Maertens G, Yap SH. Influence of the administration of human annexin V on in vitro binding of small hepatitis B surface antigen to human and to rat hepatocytes and on in vitro hepatitis B virus infection. *J Viral Hepat* 2000; **7**: 104-114 [PMID: 10760040 DOI: 10.1046/j.1365-2893.1999.00167.x]

25 **Yamada M**, Oeda A, Jung J, Iijima M, Yoshimoto N, Niimi T, Jeong SY, Choi EK, Tanizawa K, Kuroda S. Hepatitis B virus envelope L protein-derived bio-nanocapsules: mechanisms of cellular attachment and entry into human hepatic cells. *J Control Release* 2012; **160**: 322-329 [PMID: 22100387 DOI: 10.1016/j.1conrel.2011.11.004]

26 **Leistner CM**, Gruen-Bernhard S, Glebe D. Role of glycosaminoglycans for binding and infection of hepatitis B virus. *Cell Microbiol* 2008; **10**: 122-133 [PMID: 18086046 DOI: 10.1111/j.1462-5822.2007.01023.x]

27 **Schulze A**, Gripon P, Urban S. Hepatitis B virus infection initiates with a large surface protein-dependent binding to heparan sulfate proteoglycans. *Hepatology* 2007; **46**: 1759-1768 [PMID: 18046710 DOI: 10.1002/hep.21896]

28 **Chisari FV**, Isogawa M, Wieland SF. Pathogenesis of hepatitis B virus infection. *Pathol Biol (Paris)* 2010; **58**: 258-266 [PMID: 20116937 DOI: 10.1016/j.patbio.2009.11.001]

29 **Belloni L**, Allweiss L, Guerrieri F, Pediconi N, Volz T, Pollicino T, Petersen J, Raimondo G, Dandri M, Levrero M. IFN-α inhibits HBV transcription and replication in cell culture and in humanized mice by targeting the epigenetic regulation of the nuclear cccDNA minichromosome. *J Clin Invest* 2012; **122**: 529-537 [PMID: 22251702 DOI: 10.1172/JCI58847]

30 **Maini MK**, Bertoletti A. How can the cellular immune response control hepatitis B virus replication? *J Viral Hepat* 2000; **7**: 321-326 [PMID: 10971819 DOI: 10.1046/j.1365-2893.2000.00234.x]

31 **Desmond CP**, Bartholomeusz A, Gaudieri S, Revill PA, Lewin SR. A systematic review of T-cell epitopes in hepatitis B virus: identification, genotypic variation and relevance to antiviral therapeutics. *Antivir Ther* 2008; **13**: 161-175 [PMID: 18505168]

32 **Pajot A**, Michel ML, Mancini-Bourgine M, Ungeheuer MN, Ojcius DM, Deng Q, Lemonnier FA, Lone YC. Identification of novel HLA-DR1-restricted epitopes from the hepatitis B virus envelope protein in mice expressing HLA-DR1 and vaccinated human subjects. *Microbes Infect* 2006; **8**: 2783-2790 [PMID: 17045504 DOI: 10.1016/j.micinf.2006.08.009]

33 **Manigold T**, Racanelli V. T-cell regulation by CD4 regulatory T cells during hepatitis B and C virus infections: facts and controversies. *Lancet Infect Dis* 2007; **7**: 804-813 [PMID: 18045563 DOI: 10.1016/S1473-3099(07)70289-X]

34 **van den Berg R**, van Hoogstraten I, van Agtmael M. Non-responsiveness to hepatitis B vaccination in HIV seropositive patients; possible causes and solutions. *AIDS Rev* 2009; **11**: 157-164 [PMID: 19654857]

35 **Sjogren MH**. Prevention of hepatitis B in nonresponders to initial hepatitis B virus vaccination. *Am J Med* 2005; **118** Suppl 10A: 34S-39S [PMID: 16271539 DOI: 10.1016/j.amjmed.2005.07.012]

36 **Glaser R**, Kiecolt-Glaser JK, Bonneau RH, Malarkey W, Kennedy S, Hughes J. Stress-induced modulation of the immune response to recombinant hepatitis B vaccine. *Psychosom Med* 1992; **54**: 22-29 [PMID: 1553399]

37 **Krey T**, d'Alayer J, Kikuti CM, Saulnier A, Damier-Piolle L, Petitpas I, Johansson DX, Tawar RG, Baron B, Robert B, England P, Persson MA, Martin A, Rey FA. The disulfide bonds in glycoprotein E2 of hepatitis C virus reveal the tertiary organization of the molecule. *PLoS Pathog* 2010; **6**: e1000762 [PMID: 20174556 DOI: 10.1371/journal.ppat.1000762]

38 **Lupberger J**, Zeisel MB, Xiao F, Thumann C, Fofana I, Zona L, Davis C, Mee CJ, Turek M, Gorke S, Royer C, Fischer B, Zahid MN, Lavillette D, Fresquet J, Cosset FL, Rothenberg SM, Pietschmann T, Patel AH, Pessaux P, Doffoël M, Raffelsberger W, Poch O, McKeating JA, Brino L, Baumert TF. EGFR and EphA2 are host factors for hepatitis C virus entry and possible targets for antiviral therapy. *Nat Med* 2011; **17**: 589-595 [PMID: 21516087 DOI: 10.1038/nm.2341]

39 **Pestka JM**, Zeisel MB, Bläser E, Schürmann P, Bartosch B, Cosset FL, Patel AH, Meisel H, Baumert J, Viazov S, Rispeter K, Blum HE, Roggendorf M, Baumert TF. Rapid induction of virus-neutralizing antibodies and viral clearance in a single-source outbreak of hepatitis C. *Proc Natl Acad Sci USA* 2007; **104**: 6025-6030 [PMID: 17392433 DOI: 10.1073/pnas.0607026104]

40 **Bartosch B**, Verney G, Dreux M, Donot P, Morice Y, Penin F, Pawlotsky JM, Lavillette D, Cosset FL. An interplay between hypervariable region 1 of the hepatitis C virus E2 glycoprotein, the scavenger receptor BI, and high-density lipoprotein promotes both enhancement of infection and protection against neutralizing antibodies. *J Virol* 2005; **79**: 8217-8229 [PMID: 15956567 DOI: 10.1128/JVI.79.13.8217-8229.2005]

41 **Panjaworayan N**, Brown CM. Effects of HBV Genetic Variability on RNAi Strategies. *Hepat Res Treat* 2011; **2011**: 367908 [PMID: 21760994 DOI: 10.1155/2011/367908]

42 **Simmonds P**. Genetic diversity and evolution of hepatitis C virus--15 years on. *J Gen Virol* 2004; **85**: 3173-3188 [PMID: 15483230 DOI: 10.1099/vir.0.80401-0]

43 **Kay A**, Zoulim F. Hepatitis B virus genetic variability and evolution. *Virus Res* 2007; **127**: 164-176 [PMID: 17383765 DOI: 10.1016/j.virusres.2007.02.021]

44 **Bowyer SM**, Sim JG. Relationships within and between genotypes of hepatitis B virus at points across the genome: footprints of recombination in certain isolates. *J Gen Virol* 2000; **81**: 379-392 [PMID: 10644836]

45 **Pollicino T**, Amaddeo G, Restuccia A, Raffa G, Alibrandi A, Cutroneo G, Favaloro A, Maimone S, Squadrito G, Raimondo G. Impact of hepatitis B virus (HBV) preS/S genomic variability on HBV surface antigen and HBV DNA serum levels. *Hepatology* 2012; **56**: 434-443 [PMID: 22271491 DOI: 10.1002/hep.25592]

46 **Wohnsland A**, Hofmann WP, Sarrazin C. Viral determinants of resistance to treatment in patients with hepatitis C. *Clin Microbiol Rev* 2007; **20**: 23-38 [PMID: 17223621 DOI: 10.1128/CMR.00010-06]

47 **Chayama K**, Hayes CN. Hepatitis C virus: How genetic variability affects pathobiology of disease. *J Gastroenterol Hepatol* 2011; **26** Suppl 1: 83-95 [PMID: 21199518 DOI: 10.1111/j.1440-1746.2010.06550.x]

48 **Grove J**, Nielsen S, Zhong J, Bassendine MF, Drummer HE, Balfe P, McKeating JA. Identification of a residue in hepatitis C virus E2 glycoprotein that determines scavenger receptor BI and CD81 receptor dependency and sensitivity to neutralizing antibodies. *J Virol* 2008; **82**: 12020-12029 [PMID: 18829747 DOI: 10.1128/JVI.01569-08]

49 **Zhong J**, Gastaminza P, Chung J, Stamataki Z, Isogawa M, Cheng G, McKeating JA, Chisari FV. Persistent hepatitis C virus infection in vitro: coevolution of virus and host. *J Virol* 2006; **80**: 11082-11093 [PMID: 16956932 DOI: 10.1128/JVI.01307-06]

50 **Keck ZY**, Xia J, Wang Y, Wang W, Krey T, Prentoe J, Carlsen T, Li AY, Patel AH, Lemon SM, Bukh J, Rey FA, Foung SK. Human monoclonal antibodies to a novel cluster of conformational epitopes on HCV E2 with resistance to neutralization escape in a genotype 2a isolate. *PLoS Pathog* 2012; **8**: e1002653 [PMID: 22511875]

51 **Gal-Tanamy M**, Keck ZY, Yi M, McKeating JA, Patel AH, Foung SK, Lemon SM. In vitro selection of a neutralization-resistant hepatitis C virus escape mutant. *Proc Natl Acad Sci USA* 2008; **105**: 19450-19455 [PMID: 19052239 DOI: 10.1073/pnas.0809879105]

52 **Chang MH**. Hepatitis B virus mutation in children. *Indian J Pediatr* 2006; **73**: 803-807 [PMID: 17006039 DOI: 10.1007/BF02790390]

53 **Hsu HY**, Chang MH, Ni YH, Chiang CL, Chen HL, Wu JF,

Chen PJ. No increase in prevalence of hepatitis B surface antigen mutant in a population of children and adolescents who were fully covered by universal infant immunization. *J Infect Dis* 2010; **201**: 1192-1200 [PMID: 20210630 DOI: 10.1086/651378]

54 **Tabor E**. Infections by hepatitis B surface antigen gene mutants in Europe and North America. *J Med Virol* 2006; **78** Suppl 1: S43-S47 [PMID: 16622882 DOI: 10.1002/jmv.20606]

55 **Kamili S**, Sozzi V, Thompson G, Campbell K, Walker CM, Locarnini S, Krawczynski K. Efficacy of hepatitis B vaccine against antiviral drug-resistant hepatitis B virus mutants in the chimpanzee model. *Hepatology* 2009; **49**: 1483-1491 [PMID: 19274751 DOI: 10.1002/hep.22796]

56 **Torresi J**, Johnson D, Wedemeyer H. Progress in the development of preventive and therapeutic vaccines for hepatitis C virus. *J Hepatol* 2011; **54**: 1273-1285 [PMID: 21236312 DOI: 10.1016/j.jhep.2010.09.040]

57 **Di Lorenzo C**, Angus AG, Patel AH. Hepatitis C virus evasion mechanisms from neutralizing antibodies. *Viruses* 2011; **3**: 2280-2300 [PMID: 22163345 DOI: 10.3390/v3112280]

58 **Takkenberg RB**, Weegink CJ, Zaaijer HL, Reesink HW. New developments in antiviral therapy for chronic hepatitis B. *Vox Sang* 2010; **98**: 481-494 [PMID: 19951307 DOI: 10.1111/j.1423-0410.2009.01282.x]

59 **Bhattacharya D**, Thio CL. Review of hepatitis B therapeutics. *Clin Infect Dis* 2010; **51**: 1201-1208 [PMID: 20954965 DOI: 10.1086/656624]

60 **Tang YZ**, Liu L, Pan MM, Wang YM, Deng GH. Evolutionary pattern of full hepatitis B virus genome during sequential nucleos(t)ide analog therapy. *Antiviral Res* 2011; **90**: 116-125 [PMID: 21440005 DOI: 10.1016/j.antiviral.2011.03.183]

61 **Maekawa S**, Enomoto N. Viral factors influencing the response to the combination therapy of peginterferon plus ribavirin in chronic hepatitis C. *J Gastroenterol* 2009; **44**: 1009-1015 [PMID: 19756352 DOI: 10.1007/s00535-009-0126-7]

62 **Kurbanov F**, Tanaka Y, Matsuura K, Sugauchi F, Elkady A, Khan A, Hasegawa I, Ohno T, Tokuda H, Mizokami M. Positive selection of core 70Q variant genotype 1b hepatitis C virus strains induced by pegylated interferon and ribavirin. *J Infect Dis* 2010; **201**: 1663-1671 [PMID: 20420509 DOI: 10.1086/652500]

63 **Hofmann WP**, Sarrazin C, Kronenberger B, Schönberger B, Bruch K, Zeuzem S. Mutations within the CD81-binding sites and hypervariable region 2 of the envelope 2 protein: correlation with treatment response in hepatitis C virus-infected patients. *J Infect Dis* 2003; **187**: 982-987 [PMID: 12660945 DOI: 10.1086/368221]

64 **Chambers TJ**, Fan X, Droll DA, Hembrador E, Slater T, Nickells MW, Dustin LB, Dibisceglie AM. Quasispecies heterogeneity within the E1/E2 region as a pretreatment variable during pegylated interferon therapy of chronic hepatitis C virus infection. *J Virol* 2005; **79**: 3071-3083 [PMID: 15709027 DOI: 10.1128/JVI.79.5.3071-3083.2005]

65 **Gaudy C**, Moreau A, Veillon P, Temoin S, Lunel F, Goudeau A. Significance of pretreatment analysis of hepatitis C virus genotype 1b hypervariable region 1 sequences to predict antiviral outcome. *J Clin Microbiol* 2003; **41**: 3615-3622 [PMID: 12904364 DOI: 10.1128/JCM.41.8.3615-3622.2003]

66 **Aurora R**, Donlin MJ, Cannon NA, Tavis JE. Genome-wide hepatitis C virus amino acid covariance networks can predict response to antiviral therapy in humans. *J Clin Invest* 2009; **119**: 225-236 [PMID: 19104147 DOI: 10.1172/JCI37085]

67 **Zeisel MB**, Fofana I, Fafi-Kremer S, Baumert TF. Hepatitis C virus entry into hepatocytes: molecular mechanisms and targets for antiviral therapies. *J Hepatol* 2011; **54**: 566-576 [PMID: 21146244 DOI: 10.1016/j.jhep.2010.10.014]

68 **Ploss A**, Dubuisson J. New advances in the molecular biology of hepatitis C virus infection: towards the identification of new treatment targets. *Gut* 2012; **61** Suppl 1: i25-i35 [PMID: 22504917 DOI: 10.1136/gutjnl-2012-302048]

P- Reviewers Rippe RA, Yamagiwa S **S- Editor** Gou SX
L- Editor A **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i5.665

World J Gastroenterol 2013 February 7; 19(5): 665-672
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

MINIREVIEWS

Burning mouth syndrome

Grigoriy E Gurvits, Amy Tan

Grigoriy E Gurvits, Division of Gastroenterology, New York University School of Medicine/Langone Medical Center, New York, NY 10016, United States
Amy Tan, New York University School of Medicine, New York, NY 10016, United States
Author contributions: Gurvits GE and Tan A contributed equally to this work.
Correspondence to: Grigoriy E Gurvits, MD, Division of Gastroenterology, New York University School of Medicine/Langone Medical Center, 530 First Ave, SKI - 9N, New York, NY 10016, United States. dr.gurvits@hotmail.com
Telephone: +1-212-2633095 Fax: +1-212-2633096
Received: November 2, 2012 Revised: December 4, 2012
Accepted: December 25, 2012
Published online: February 7, 2013

Abstract

Burning mouth syndrome is a debilitating medical condition affecting nearly 1.3 million of Americans. Its common features include a burning painful sensation in the mouth, often associated with dysgeusia and xerostomia, despite normal salivation. Classically, symptoms are better in the morning, worsen during the day and typically subside at night. Its etiology is largely multifactorial, and associated medical conditions may include gastrointestinal, urogenital, psychiatric, neurologic and metabolic disorders, as well as drug reactions. BMS has clear predisposition to peri-/post menopausal females. Its pathophysiology has not been fully elucidated and involves peripheral and central neuropathic pathways. Clinical diagnosis relies on careful history taking, physical examination and laboratory analysis. Treatment is often tedious and is aimed at correction of underlying medical conditions, supportive therapy, and behavioral feedback. Drug therapy with alpha lipoic acid, clonazepam, capsaicin, and antidepressants may provide symptom relief. Psychotherapy may be helpful. Short term follow up data is promising, however, long term prognosis with treatment is lacking. BMS remains an important medical condition which often places a recognizable burden on the patient and health care system and requires appropriate recognition and treatment.

© 2013 Baishideng. All rights reserved.

Key words: Burning mouth syndrome; Glossodynia; Glossopyrosis; Burning tongue

Gurvits GE, Tan A. Burning mouth syndrome. *World J Gastroenterol* 2013; 19(5): 665-672 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i5/665.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i5.665

INTRODUCTION

Burning mouth syndrome (BMS) is a chronic pain disorder characterized by burning, stinging, and/or itching of the oral cavity in the absence of any organic disease. It lasts at least 4 to 6 mo and most often involves the tongue with or without extension to the lips and oral mucosa[1,2]. BMS can be accompanied by dysgeusia (distortion in sense of taste) and subjective xerostomia (dry mouth). Its onset is spontaneous and the syndrome has a clear predisposition to peri-/postmenopausal women. Its secondary form has been associated with a variety of conditions including thyroid disease, psychiatric illnesses, oral infections, drug use, dental treatment, vitamin/mineral deficiencies, and others[3,4].

First described in mid nineteenth century, this condition was further characterized in the early twentieth century by Butlin and Oppenheim as glossodynia[5]. Over the ensuing years, BMS has been referred to as glossopyrosis, oral dysesthesia, sore tongue, stomatodynia, and stomatopyrosis[6]. It was first categorized as a distinct disease in 2004 by the International Headache Society, which defined primary BMS as "an intraoral burning sensation for which no medical or dental cause can be found." Current diagnostic criteria consist of daily persistent pain in the mouth with normal oral mucosa after exclusion of local and systemic diseases[7]. Its etiology is thought to be multifactorial, involving various local, sys-

temic, and/or psychogenic causes. Female gender, perimenopause, depression and anxiety, Parkinson's disease, and chronic medical conditions including gastrointestinal and urogenital diseases are risk factors for developing BMS[1,8-11].

BMS is an important clinical condition with aggravating symptoms, directly and indirectly impacting the quality of life, which often places a recognizable burden on the patient and health care system. Clinical consultations with a practicing gastroenterologist are common practice in today's medicine for the patients with BMS. This review focuses on various aspects of BMS, including its epidemiology, pathophysiology, etiology, clinical presentation, differential diagnosis, classification, clinical diagnosis, current treatment, and general prognosis.

EPIDEMIOLOGY

The estimated prevalence of BMS in the general population varies widely in the literature. Tammiala-Salonen *et al*[12] reported a rate of 15% of burning mouth symptoms in Finnish adult population, though half of the patients had visible oral mucosal lesions. In a cross-sectional analysis of over 1000 randomly selected Swedish patients from Public Dental Health Service registers, 3.7% of subjects were diagnosed with BMS after reporting burning mouth symptoms and undergoing a subsequent physical examination[8]. In contrast, Lipton *et al*[13] reported a prevalence of 0.7% based solely on self-reported symptoms from over 45 000 American households. Haberland *et al*[14] noted that 10% of new patients observed in his practice were diagnosed with BMS. Most recently, a large retrospective study of over 3000 Brazilian patients referred to an oral pathology service reported a prevalence of about 1%[1]. These highly variable rates are attributed to the wide age disparities of the examined population (typically, prevalence of BMS dramatically increases with age) as well as previous lack of universally accepted diagnostic criteria for BMS. Although further studies are needed with satisfactory criteria to determine the true prevalence of BMS, this data illustrates that the disease is an important medical condition that may be often encountered in the clinical practice.

BMS has a clear predisposition to gender and age. Women are 2.5 to 7 times more commonly affected than men[1,8,10]. Furthermore, up to 90% of female patients with BMS are perimenopausal women with typical onset from 3 prior- to 12 years post- the beginning of menopause[9]. BMS may affect any age group, with patients age ranging from 27 to 87 years of age, and a reported mean age of 61 years. Recent analysis by the same group showed an increased likelihood of gastrointestinal and urogenital disease in patients with BMS, with estimated odds ratio of 3.5 and 2.9, respectively, compared to control subjects. Patients with BMS had a statistically higher intake of medications for gastric disease compared to control group as well[1].

ANATOMY

The oral cavity is primary responsible for the ingestion and mastication of food. Its anatomical boundaries include the lips anteriorly, the cheeks laterally, the oropharynx posteriorly, the palate superiorly, and the floor of the mouth inferiorly[15]. The oral cavity contains several structures, including upper and lower dentition, the tongue, salivary glands, and mucosal glands[16]. It is lined by non-keratinized stratified squamous epithelium, which is moistened by secretions from various salivary glands[17].

The neuroanatomy of the oral cavity, particularly somatosensory innervation, has been implicated in the pathophysiology of BMS. The maxillary (V2) and mandibular (V3) branches of the trigeminal nerve supply most of the somatosensory innervation in the oral cavity, with some contribution from the glossopharyngeal nerve (cranial nerve Ⅸ). In regards to the tongue, the most commonly affected area in BMS, the lingual branch of V3 innervates the anterior two-thirds of the tongue while the glossopharyngeal nerve innervates the posterior third of the tongue. Together, they innervate the receptors on the papillae of the tongue, which are sensitive to mechanical, thermal, and tactile stimuli[17].

Alterations in taste and quantity of salivation are commonly reported in BMS. The chorda tympani branch of the facial nerve (Ⅶ) supplies chemoreceptors for taste in the anterior two-thirds of the tongue. The glossopharyngeal nerve (Ⅸ) provides taste sensation for the posterior third of the tongue. There are also taste receptors on the soft palate supplied by the greater superficial petrosal nerve branch of Ⅶ and on the larynx from the superior laryngeal nerve of the vagus nerve (Ⅹ). The salivary reflex begins with afferent inputs from taste and mechanoreceptors in the mouth that reach the brainstem salivatory centers. Parasympathetic and sympathetic fibers then supply the efferent fibers that act on the salivary glands[15].

PATHOPHYSIOLOGY

The pathophysiology of BMS has not been fully elucidated. Various studies have shown significant differences in thermal and nociception thresholds of patient with BMS compared to control subjects[18,19]. Thus, a neuropathic mechanism for BMS is currently favored. However, controversy remains over whether a peripheral or central dysfunction is responsible for BMS.

Evidence in the literature links BMS to a peripheral neuropathy. Superficial biopsies of the anterolateral tongue from BMS patients showed a significantly lower density of epithelial and subpapillary nerve fibers than controls. Morphologic changes were consistent with axonal degeneration. This supports a trigeminal small-fiber sensory neuropathy or axonopathy[20]. Moreover, Borelli *et al*[21] found increased levels of nerve growth factor, a neuropeptide vital to nociceptive function in adults, in the saliva of BMS subjects. Other histopathologic studies of patients with BMS have shown increased density

of TRPV1 ion channels and $P2X_3$ receptors on scattered nerve fibers, a finding previously linked to hypersensitivity and neuropathic pain symptoms in various models of human pain conditions[22]. Additionally, dysfunction of the chorda tympani branch of the facial nerve may be involved in the pathogenesis of BMS. Patients with BMS will report improved symptoms with eating, suggesting that stimulation of the gustatory system decreases pain sensation. Finally, increased excitability or inhibition of the trigeminal system has been implicated as patients with BMS have greater alterations in blink reflexes compared to normal subjects[23-28].

However, recent evidence indicates that dysfunction in the central nervous system can also cause BMS. Albuquerque *et al*[29] showed that BMS patients process thermal and pain stimulation in the brain differently than pain-free individuals as demonstrated by functional magnetic resonance imaging of the thalamus. Additionally, the dysregulation of the nigrostriatal dopaminergic system has been implicated in BMS[30]. Patients with Parkinson's disease are reportedly five times more likely to have BMS than the general population[31]. Finally, hospital anxiety and depression scores were significantly higher in the patients with central BMS[22].

As evident from these studies, the pathophysiology of BMS is highly complex, likely involving neural pathways at different levels of neuraxis. A recent double blind, randomized cross-over study of postmenopausal women showed heterogeneity of the response of BMS symptoms to lingual nerve block with lidocaine. If fact, it may lead to an effective increase, decrease, or an unchanged burning pain in patients, an effect attributed to variation in central, peripheral, or combined neurological pathways in pathogenesis of BMS[32]. The symptoms of BMS can result from subclinical insults at various points in the nervous system, presenting with instant or gradual bilateral distribution. Recent classification suggests a possible overlap of three distinct subclasses in BMS: a peripheral oral small fiber neuropathy (50%-60% of cases), subclinical major trigeminal neuropathy (20%-25%), and central deficiency in dopaminergic top-down inhibition (20%-40%)[23].

ETIOLOGY

The exact etiology of BMS remains imprecise and is likely multifactorial, including neuropsychiatric, endocrine, immunologic, nutritional, infectious, and iatrogenic causes. The disorder has been associated with several psychiatric diseases[9,33-35]. Depression or anxiety occurs in more than 50% of BMS patients, with depression predominating[34]. Personality disorders are also linked to BMS, affecting 86% of sufferers compared to 24% of normal individuals, with significant predilection to Cluster A disorders[36]. Most recently, a cross-sectional controlled study showed that BMS patients have a significantly higher frequency of past or present major depressive disorder, general anxiety disorder, hypochondria, and cancerophobia[37]. Although psychiatric disease was initially considered as a primary cause of BMS, it is now considered a concurrent or secondary factor as there is no definite correlation between the onset of BMS and stressful events and many other causes of BMS have been identified[9,33,35,38,39].

As described previously, BMS most commonly affects perimenopausal women, a finding that is attributed to dryness of mucosal membranes from age-related reduction in estrogen and progesterone levels and increased frequency of psychological disorders in middle-aged and elderly women[40]. Woda *et al*[41] has suggested that the fall in neuroprotective gonadal and adrenal steroids during menopause leads to a concomitant decrease in neuroactive steroids, leading to degeneration of oral mucosal small nerve fibers and brain areas involved in oral somatic sensations. These changes can become irreversible, resulting in burning pain and associated symptoms. Gao *et al*[42] showed that peri-/post-menopausal patients suffering from BMS may have lower levels of estradiol and increased levels of follicle stimulating hormone compared to healthy controls. Other endocrine conditions implicated in BMS may include diabetes mellitus and hypothyroidism[8].

Evidence also exists for an immunologic etiology. Allergic reactions have been demonstrated in BMS patients to dietary antigens. These include sorbic acid, cinnamon, nicotinic acid, propylene glycol, and benzoic acid[43,44]. Other allergens identified by patch testing are dental metals such as zinc, cobalt, mercury, gold, and palladium[45]. Sodium lauryl sulfate, a detergent in toothpaste known to cause dry mouth, may also be involved in the development of BMS[46]. Finally, autoimmune connective tissue disorders such as Sjogren's syndrome and systemic lupus erythematosus, are also associated with BMS[47].

BMS has also been linked to nutritional deficiencies including vitamins B1, B2, B6 and B12 as well as folic acid[2]. Most recently, zinc deficiency was shown to be a possible cause of BMS, with patients reporting improved symptoms after zinc replacement therapy[48].

A potential relationship between smoking and development of BMS has been described, with an estimated odd ratio of 12.6 in a recent study[43].

Certain oral infections have been implicated in BMS, particularly candidiasis. Patients with BMS have a higher intraoral prevalence of *Candida* species and coliforms like *Enterobacter* and *Klebsiella*[49,50]. Although this finding may be related to xerostomia and prosthetic dental wearing, a possible infectious origin of BMS is suggested by reports of remission after oral antifungal therapy[18].

Drug-associated BMS has also been reported in the literature. ACE inhibitors and angiotensin receptor blockers may trigger development of BMS, possibly due to increased levels of kallikrein in the saliva of BMS patients leading to increased inflammation in the oral cavity[9,51-53]. Nevirapine and efavirenz have been reported to cause BMS *via* an unknown mechanism[54,55]. Levodopa may play a role in the development of BMS in patients with Parkinson disease[31]. Finally, a case report of topiramate causing burning mouth-like symptoms has been de-

scribed, with symptom resolution upon discontinuation of the medication[56].

CLINICAL PRESENTATION

A typical patient with BMS is a peri- or post-menopausal woman with various medical comorbidities who complains of the classic triad of unremitting oral mucosal burning pain associated with dysgeusia and xerostomia in nearly two thirds of the cases with no visible disease in the oral mucosa for 4-6 mo duration. Clinical presentations may vary as some patients can be oligosymptomatic (pain and dysgeusia or xerostomia) or monosymptomatic (pain only)[10]. In general, 63% of patients report accompanying dry mouth, 60% bitter/metallic taste, and 35% altered taste perception[9]. The pain is described as burning, scalding, tingling, or numbness. It is of moderate to severe intensity and can decrease during eating. It is commonly bilateral and most often involves the tongue followed by the palate and lower lip. In contrast, the buccal mucosa and floor of the mouth are rarely affected[9]. The onset is spontaneous, though some BMS patients report antecedent dental procedures, initiation of medications, or other illnesses[10,57]. Xerostomia may be subjective however some patients have demonstrated alterations in saliva quantity and quality[10]. Vertical visual analogue scale (VAS, 0-10 cm)[58] is commonly used to describe pain intensity in BMS.

Review of systems may be remarkable for headache, chronic fatigue, gastrointestinal and urogenital symptoms, insomnia, mood changes, irritability, anxiety, and depression[4,57]. Other observed clinical conditions may include gastroesophageal reflux disease, hypertension, hematological disorders, nutritional deficiencies, diabetes mellitus, thyroid disorders, Parkinson's disease, Sjogren's syndrome and other autoimmune diseases[51,59]. Finally, parafunctional habits such as lip and cheek biting, bruxism, tooth grinding and clenching, tongue thrusting, and lip licking are observed with BMS[10].

Physical examination and laboratory analysis are classically unremarkable in primary BMS. However, they can be abnormal in secondary BMS. Oral findings potentially include areas of erythema, geographic tongue, candidiasis, atrophic glossitis, lichen planus, and xerostomia. Laboratory evaluation may reveal positive fungal oral cultures, elevated fasting blood sugar, decreased levels of vitamin B1, B2, B6, B12, folate, iron, and zinc, abnormal thyroid function studies, and positive serum autoantibodies[4,49].

MIMICKERS OF BMS

BMS presents with a main complaint of an intraoral sensation of burning, tingling, or stinging and sometimes accompanied by taste disturbances or dry mouth. The mimickers of BMS may include stomatitis, atypical facial pain, atypical odontalgia, idiopathic facial arthromyalgia, pemphigoid, pemphigus, neoplastic lesions in the oral cavity, acoustic neuroma, denture design or tooth restoration failures, herpes simplex or herpes zoster, trauma to lingual or mandibular nerves after dental surgery[10,57]. Detailed history and physical exam is crucial to differentiate above medical conditions.

CLASSIFICATION

Two classification schemes have been proposed based on either etiology or clinical symptoms. When classifying by etiology, primary BMS is the idiopathic form for which organic causes cannot be identified while secondary BMS results from local or systemic pathological conditions[10]. The other scheme divides BMS cases into three types based on diurnal fluctuations of symptoms. Patients with type 1 BMS (35%) are symptom-free upon awakening with worsening symptoms throughout the day and variable symptoms at night. Type 2 BMS (55%) is defined by continuous symptoms in the day but none at night. Patients with type 3 BMS (10%) have intermittent symptoms interspersed with symptom-free days[34,60]. Type 1 BMS is linked to nutritional deficiencies and diabetes, type 2 to chronic anxiety, and type 3 to dietary or prosthetic allergies[2,18,44,61].

CLINICAL DIAGNOSIS

The diagnosis of BMS remains challenging as diagnostic criteria are not sufficiently defined or universally accepted, several confounding diagnoses exist, and the clinical picture is often variable. Scala *et al*[10] proposed the following fundamental criteria: (1) daily and deep bilateral burning sensation of the oral mucosa; (2) burning sensation for at least 4 to 6 mo; (3) constant intensity, or increasing intensity during the day; (4) no worsening but possible improvement on eating or drinking; and (5) no interference with sleep. Additional supportive criteria are, (1) dysgeusia and/or xerostomia; (2) sensory or chemosensory alterations; and (3) mood changes or psychopathological alterations.

Since primary BMS is a diagnosis of exclusion, thorough investigation for local and systemic factors associated with secondary BMS is essential. Careful review of recent mood disturbances, dietary habits, history of dental procedures, use of dental prosthetics, nutritional deficiencies, and changes in medication is necessary in the evaluation of BMS. Physical examination primarily consists of detailed study of the oral cavity, including dental inspection. Laboratory analyses must include hematological assessment of nutritional deficiencies, blood glucose levels, autoimmune markers, estrogen and progesterone concentrations, patch testing for specific allergies[10]. Measurement of salivary flow rates should be employed[62].

Additional studies may warrant oral cultures and scrapings to evaluate for a bacterial or fungal origin of symptoms. Tongue biopsy is not required if the tongue appears normal on clinical exam and is only indicated when a particular lesion is visualized. In general, the diagnosis of BMS remains a major challenge, requiring extensive clinical and laboratory evaluation with a particular atten-

tion to details of patient's history and physical exam.

TREATMENT

The first step in management is contingent on the specific type of BMS, primary versus secondary. The goal of therapy for secondary BMS should initially be directed at treating the causative local or systemic disease and withdrawing offending medications (such as ACE inhibitors). This etiology-directed therapy typically yields a good response. The cure for primary BMS, however, remains elusive despite attempts with different classes of medication. The variable response rate to medical therapy is likely due to the multifactorial pathophysiology of idiopathic BMS, including irreversible processes. Treatment is aimed at management this disease as a type of chronic neuropathy. Investigated strategies include benzodiazepines, antidepressants, topical capsaicin, alpha-lipoic acid, hormone replacement therapy, anticonvulsants, biofeedback technique to modify parafunctional habits, and psychosocial therapies.

Early studies by Italian researchers supported the use of alpha lipoic acid (ALA, a potent antioxidant) in 600 mg daily dose over two months in patients with BMS[63]. This data was not replicated by subsequent analysis in Brazilian patients[64], possibly due to the multivitamin compound of the ALA supplements or longer duration of the original therapy. Interestingly, same group suggested added benefit of ALA in patients with thyroid disease[65].

Recent randomized double blind placebo controlled trial showed that use of gabapentin alone (300 mg daily) or in combination with ALA (600 mg daily) was beneficial in reducing symptoms in 50% and 70% of patients with BMS, respectively, compared to placebo (15%)[66]. In line with the observed predisposition of BMS to peri-/post- menopausal women, a hormone replacement therapy may be intuitive, but short of initial promise with local oral administration, it is largely not effective when given systemically[67] possibly due to the irreversible nature of the neuropathic changes.

Clonazepam lozenges (oral dissolution of 1-mg tablets for 3 min with subsequent expectoration three times a day) are beneficial in patients with predominantly peripheral BMS. A double blind, randomized controlled study of topical clonazepam reported a reduction in pain intensity in 66% of patients after 2 wk and 29% after 6 mo[68]. Similar clinical benefit was replicated in follow up studies[33].

Short term use of oral Chlordiazepoxide (5-10 mg three times a day) to treat BMS has been reported, however, its long term effects were not significant[69].

Efficacy of capsaicin, a desensitizer of receptors for neurogenic inflammation, has been evaluated in several studies. Systemic capsaicin 0.25% capsules three times daily showed dramatic improvement (93%) in patients with severe BMS (VAS scale 8-10) at 1 mo[70]. Side effects including gastric pains in 32% of the patients were cumulative, and may preclude long term use of this medication. Local capsaicin rinse may be beneficial in treating BMS, with reported improvement of symptoms in over 75% of the patients after 8 wk of therapy without significant side effects[71].

Use of proton pump inhibitors (PPI), although clearly increased in the patients with BMS[1], may not necessarily be of benefit. Recent study measuring oropharyngeal pH found no significant correlation between laryngopharyngeal reflux and intraoral burning sensation in the examined population[72]. Anecdotal evidence exists in case reports of symptom relief with twice daily omeprazole in patients with endoscopy proven gastroesophageal reflux disease[73]. Whether there is a casual relationship between use of PPI and clinical primary BMS, an observation phenomenon, a true response, or simply a failed therapy remains to be established. Treatment of secondary BMS in the setting of gastroesophageal reflux disease is, however, warranted. Larger studies are needed to address potential benefit (or lack of it) of PPI in treatment of BMS.

Medical management of BMS may also include antidepressants and antipsychotics. In a prospective, open-label, noncomparative study of selective serotonin reuptake inhibitor (SSRI) paroxetine, 80% of patients experienced overall pain reduction including 36% of patients who reported complete remission after 12 wk of incremental treatment. Both treatment effects and adverse events were found to be dose dependent[74]. However, prophylactic use of domperidone for nausea in selected study patients may have had a potential confounding effect on the results. Another single-blinded non-placebo controlled study comparing paroxetine (20 mg daily), sertraline (50 mg daily), and an atypical antipsychotic amisulpride (50 mg daily) demonstrated a nearly 70% improvement in VAS score after 8 wk of therapy in each of the three groups[75]. A group from Spain reported complete remission in all female patients after 8 wk of amisulpride use, an effect lasting up to 24 wk with continuous administration of the medication[76].

Additionally, modifications of parafunctional habits may offer some symptom relief. Tongue protectors (worn 15 min three times a day) were shown to significantly improve pain scales in BMS patients after two months of treatment, however sample sizes were small and placebo effect could have been introduced[77].

Finally, psychiatric interventions show great promise in treating patients with BMS. Weekly one-hour sessions of cognitive behavioral therapy lasting for 12-15 wk significantly reduced BMS symptoms in all study patients compared to placebo control group, with an estimated 27% of patients remaining symptom-free at 6 mo follow up (none in placebo group)[78]. Weekly group psychotherapy administered for three consecutive months achieved symptom improvement in 70% of the patients[52]. Femiano *et al*[79] noted a statistically significant symptom improvement with cognitive psychotherapy (40%), alpha lipoic acid (81%), combination therapy (90%) compared to pill placebo control group (13%) of patients with BMS.

Anecdotal use of diphenhydramine/pectin swish and spit elixir[18] as well as oral pramipexole in BMS patients with Parkinson's disease have been reported[57].

Perceived variation in the results of the published data may stem from lack of effective differentiation between predominantly peripheral and predominantly central pathways in the pathogenesis of BMS. Therefore, improvement in future clinical diagnosis and discrimination between such groups of patients may improve clinical response rate to targeted local and systemic therapies.

For now, BMS remains a challenging medical condition to treat, and further research is required to determine the true efficacy of current management strategies for patients with this disorder. Future blinded randomized control trials with large sample size are necessary to provide new insight for use of various treatment modalities in BMS.

PROGNOSIS

Although the short term follow up studies may show potential symptomatic improvement with treatment in patients with BMS, the long-term outcomes for BMS remain unclear. Early observational report by Gilpin[5] in 1936 may provide a closer look at the natural history of the disease that follows a "rule of 3's": up to one third of cases would enter spontaneous remission, another third would show moderate improvement, and finally the last third would show no improvement or even worsening of the symptoms. Prospective clinical and pharmaceutical advances may have significantly changed the landscape of BMS, as recent study showed nearly 10% of spontaneous remission, 26% of moderate improvement, 37% of no significant change, and finally 26% of worsening of symptoms in patients receiving no therapy with an estimated follow up of at least 18 mo. Therapy may be effective in 29% of the patients, with 56% reporting no changes, and 15% admitting worsening of the pain[62]. In perspective, complete understanding of the etiology and pathogenesis is imperative to the development of novel and efficacious therapeutic strategies, and will guide overall prognosis of the disease in the future.

CONCLUSION

BMS is a relatively common chronic intraoral pain disorder classically characterized by intractable burning that may be associated with dysgeusia and xerostomia. Its pathogenesis relates to complex interlay of central and/or peripheral neural pain pathways. Etiology of BMS is multifactorial and a secondary form of BMS should be diligently sought for and treated. Multidisciplinary approach, including medical and psychosocial therapy may be effective in symptom relief in patients with BMS, however, further studies are necessary to establish long term prognosis. BMS remains an important medical condition which often places a significant burden on the patient and health care system, and requires diligent recognition and treatment.

REFERENCES

1 **Netto FO**, Diniz IM, Grossmann SM, de Abreu MH, do Carmo MA, Aguiar MC. Risk factors in burning mouth syndrome: a case-control study based on patient records. *Clin Oral Investig* 2011; **15**: 571-575 [PMID: 20440632]

2 **Lamey PJ**, Lamb AB. Prospective study of aetiological factors in burning mouth syndrome. *Br Med J* (Clin Res Ed) 1988; **296**: 1243-1246 [PMID: 3133028]

3 **Yilmaz Z**, Renton T, Yiangou Y, Zakrzewska J, Chessell IP, Bountra C, Anand P. Burning mouth syndrome as a trigeminal small fibre neuropathy: Increased heat and capsaicin receptor TRPV1 in nerve fibres correlates with pain score. *J Clin Neurosci* 2007; **14**: 864-871 [PMID: 17582772]

4 **Grushka M**, Epstein JB, Gorsky M. Burning mouth syndrome: differential diagnosis. *Dermatol Ther* 2002; **15**: 287-291 [DOI: 10.1046/j.1529-8019.2002.01535]

5 **Gilpin SF**. Glossodynia. *JAMA* 1936; **106**: 1722-1724

6 **Zakrzewska JM**, Forssell H, Glenny AM. Interventions for the treatment of burning mouth syndrome. *Cochrane Database Syst Rev* 2005; (1): CD002779 [PMID: 15674897]

7 **Headache Classification Subcommittee of the International Headache Society**. The International Classification of Headache Disorders: 2nd edition. *Cephalalgia* 2004; **24** Suppl 1: 9-160 [PMID: 14979299]

8 **Bergdahl M**, Bergdahl J. Burning mouth syndrome: prevalence and associated factors. *J Oral Pathol Med* 1999; **28**: 350-354 [PMID: 10478959]

9 **Grushka M**. Clinical features of burning mouth syndrome. *Oral Surg Oral Med Oral Pathol* 1987; **63**: 30-36 [PMID: 3468464]

10 **Scala A**, Checchi L, Montevecchi M, Marini I, Giamberardino MA. Update on burning mouth syndrome: overview and patient management. *Crit Rev Oral Biol Med* 2003; **14**: 275-291 [PMID: 12907696]

11 **Schiavone V**, Adamo D, Ventrella G, Morlino M, De Notaris EB, Ravel MG, Kusmann F, Piantadosi M, Pollio A, Fortuna G, Mignogna MD. Anxiety, depression, and pain in burning mouth syndrome: first chicken or egg? *Headache* 2012; **52**: 1019-1025 [PMID: 22607629]

12 **Tammiala-Salonen T**, Hiidenkari T, Parvinen T. Burning mouth in a Finnish adult population. *Community Dent Oral Epidemiol* 1993; **21**: 67-71 [PMID: 8485972]

13 **Lipton JA**, Ship JA, Larach-Robinson D. Estimated prevalence and distribution of reported orofacial pain in the United States. *J Am Dent Assoc* 1993; **124**: 115-121 [PMID: 8409001]

14 **Haberland CM**, Allen CM, Beck FM. Referral patterns, lesion prevalence, and patient care parameters in a clinical oral pathology practice. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 1999; **87**: 583-588 [PMID: 10348517]

15 **Standring S**, Gray H. Gray's anatomy: the anatomical basis of clinical practice. 40th ed. Edinburgh: Churchill Livingstone, 2008: 499-525

16 **German RZ**, Palmer JB. Anatomy and development of oral cavity and pharynx. *GI Motility online* 2006 [DOI: 10.1038/gimo5]

17 **Schunke M**. Thieme atlas of anatomy: head and neuroanatomy. Stuttgart: Georg Thieme Verlag, 2007: 102-114

18 **Ito M**, Kurita K, Ito T, Arao M. Pain threshold and pain recovery after experimental stimulation in patients with burning mouth syndrome. *Psychiatry Clin Neurosci* 2002; **56**: 161-168 [PMID: 11952919]

19 **Grushka M**, Sessle BJ, Howley TP. Psychophysical assessment of tactile, pain and thermal sensory functions in burning mouth syndrome. *Pain* 1987; **28**: 169-184 [PMID: 3822502]

20 **Lauria G**, Majorana A, Borgna M, Lombardi R, Penza P, Padovani A, Sapelli P. Trigeminal small-fiber sensory neu-

ropathy causes burning mouth syndrome. *Pain* 2005; **115**: 332-337 [PMID: 15911160]
21 **Borelli V**, Marchioli A, Di Taranto R, Romano M, Chiandussi S, Di Lenarda R, Biasotto M, Zabucchi G. Neuropeptides in saliva of subjects with burning mouth syndrome: a pilot study. *Oral Dis* 2010; **16**: 365-374 [PMID: 20233324]
22 **Jääskeläinen SK**. Pathophysiology of primary burning mouth syndrome. *Clin Neurophysiol* 2012; **123**: 71-77 [PMID: 22030140]
23 **Patton LL**, Siegel MA, Benoliel R, De Laat A. Management of burning mouth syndrome: systematic review and management recommendations. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2007; **103** Suppl: S39.e1-S39.13 [PMID: 17379153]
24 **Eliav E**, Kamran B, Schaham R, Czerninski R, Gracely RH, Benoliel R. Evidence of chorda tympani dysfunction in patients with burning mouth syndrome. *J Am Dent Assoc* 2007; **138**: 628-633 [PMID: 17473041]
25 **Grushka M**. Burning mouth syndrome and oral dysesthesias. *Can J Diag* 2000; **17**: 99-109
26 **Nasri-Heir C**, Gomes J, Heir GM, Ananthan S, Benoliel R, Teich S, Eliav E. The role of sensory input of the chorda tympani nerve and the number of fungiform papillae in burning mouth syndrome. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2011; **112**: 65-72 [PMID: 21601494]
27 **Jääskeläinen SK**, Forssell H, Tenovuo O. Abnormalities of the blink reflex in burning mouth syndrome. *Pain* 1997; **73**: 455-460 [PMID: 9469537]
28 **Forssell H**, Jääskeläinen S, Tenovuo O, Hinkka S. Sensory dysfunction in burning mouth syndrome. *Pain* 2002; **99**: 41-47 [PMID: 12237182]
29 **Albuquerque RJ**, de Leeuw R, Carlson CR, Okeson JP, Miller CS, Andersen AH. Cerebral activation during thermal stimulation of patients who have burning mouth disorder: an fMRI study. *Pain* 2006; **122**: 223-234 [PMID: 16632202]
30 **Jääskeläinen SK**, Rinne JO, Forssell H, Tenovuo O, Kaasinen V, Sonninen P, Bergman J. Role of the dopaminergic system in chronic pain -- a fluorodopa-PET study. *Pain* 2001; **90**: 257-260 [PMID: 11207397]
31 **Clifford TJ**, Warsi MJ, Burnett CA, Lamey PJ. Burning mouth in Parkinson's disease sufferers. *Gerodontology* 1998; **15**: 73-78 [PMID: 10530180]
32 **Grémeau-Richard C**, Dubray C, Aublet-Cuvelier B, Ughetto S, Woda A. Effect of lingual nerve block on burning mouth syndrome (stomatodynia): a randomized crossover trial. *Pain* 2010; **149**: 27-32 [PMID: 20083352]
33 **Lamey PJ**, Lewis MA. Oral medicine in practice: burning mouth syndrome. *Br Dent J* 1989; **167**: 197-200 [PMID: 2789896]
34 **Rojo L**, Silvestre FJ, Bagan JV, De Vicente T. Psychiatric morbidity in burning mouth syndrome. Psychiatric interview versus depression and anxiety scales. *Oral Surg Oral Med Oral Pathol* 1993; **75**: 308-311 [PMID: 8469540]
35 **Bogetto F**, Maina G, Ferro G, Carbone M, Gandolfo S. Psychiatric comorbidity in patients with burning mouth syndrome. *Psychosom Med* 1998; **60**: 378-385 [PMID: 9625229]
36 **Maina G**, Albert U, Gandolfo S, Vitalucci A, Bogetto F. Personality disorders in patients with burning mouth syndrome. *J Pers Disord* 2005; **19**: 84-93 [PMID: 15899722]
37 **de Souza FT**, Teixeira AL, Amaral TM, dos Santos TP, Abreu MH, Silva TA, Kummer A. Psychiatric disorders in burning mouth syndrome. *J Psychosom Res* 2012; **72**: 142-146 [PMID: 22281456]
38 **Eli I**, Kleinhauz M, Baht R, Littner M. Antecedents of burning mouth syndrome (glossodynia)--recent life events vs. psychopathologic aspects. *J Dent Res* 1994; **73**: 567-572 [PMID: 8120222]
39 **Minor JS**, Epstein JB. Burning mouth syndrome and secondary oral burning. *Otolaryngol Clin North Am* 2011; **44**: 205-219, vii [PMID: 21093630]
40 **Fischer MJ**. Amine coupling through EDC/NHS: a practical approach. *Methods Mol Biol* 2010; **627**: 55-73 [PMID: 20217613]
41 **Woda A**, Dao T, Gremeau-Richard C. Steroid dysregulation and stomatodynia (burning mouth syndrome). *J Orofac Pain* 2009; **23**: 202-210 [PMID: 19639097]
42 **Gao J**, Chen L, Zhou J, Peng J. A case-control study on etiological factors involved in patients with burning mouth syndrome. *J Oral Pathol Med* 2009; **38**: 24-28 [PMID: 19192047]
43 **Lamey PJ**, Lamb AB, Hughes A, Milligan KA, Forsyth A. Type 3 burning mouth syndrome: psychological and allergic aspects. *J Oral Pathol Med* 1994; **23**: 216-219 [PMID: 8046659]
44 **Haustein UF**. Burning mouth syndrome due to nicotinic acid esters and sorbic acid. *Contact Dermatitis* 1988; **19**: 225-226 [PMID: 3191688]
45 **Steele JC**, Bruce AJ, Davis MD, Torgerson RR, Drage LA, Rogers RS. Clinically relevant patch test results in patients with burning mouth syndrome. *Dermatitis* 2012; **23**: 61-70 [PMID: 22653121]
46 **Jensen JL**, Barkvoll P. Clinical implications of the dry mouth. Oral mucosal diseases. *Ann N Y Acad Sci* 1998; **842**: 156-162 [PMID: 9599305]
47 **Grushka M**, Ching V, Epstein J. Burning mouth syndrome. *Adv Otorhinolaryngol* 2006; **63**: 278-287 [PMID: 16733345]
48 **Cho GS**, Han MW, Lee B, Roh JL, Choi SH, Cho KJ, Nam SY, Kim SY. Zinc deficiency may be a cause of burning mouth syndrome as zinc replacement therapy has therapeutic effects. *J Oral Pathol Med* 2010; **39**: 722-727 [PMID: 20618611]
49 **Huang W**, Rothe MJ, Grant-Kels JM. The burning mouth syndrome. *J Am Acad Dermatol* 1996; **34**: 91-98 [PMID: 8543701]
50 **Samaranayake LP**, Lamb AB, Lamey PJ, MacFarlane TW. Oral carriage of Candida species and coliforms in patients with burning mouth syndrome. *J Oral Pathol Med* 1989; **18**: 233-235 [PMID: 2671340]
51 **Klasser GD**, Epstein JB, Villines D. Diagnostic dilemma: the enigma of an oral burning sensation. *J Can Dent Assoc* 2011; **77**: b146 [PMID: 22260803]
52 **Miziara ID**, Filho BC, Oliveira R, Rodrigues dos Santos RM. Group psychotherapy: an additional approach to burning mouth syndrome. *J Psychosom Res* 2009; **67**: 443-448 [PMID: 19837207]
53 **Loeb LM**, Naffah-Mazzacoratti MG, Porcionatto MA, Martins JR, Kouyoumdjian M, Weckx LM, Nader HB. Chondroitin sulfate and kallikrein in saliva: markers for glossodynia. *Int Immunopharmacol* 2008; **8**: 1056-1058 [PMID: 18486918]
54 **Moura MD**, Senna MI, Madureira DF, Fonseca LM, Mesquita RA. Oral adverse effects due to the use of Nevirapine. *J Contemp Dent Pract* 2008; **9**: 84-90 [PMID: 18176653]
55 **Borrás-Blasco J**, Belda A, Rosique-Robles JD, Casterá MD, Abad FJ. Burning mouth syndrome due to efavirenz therapy. *Ann Pharmacother* 2006; **40**: 1471-1472 [PMID: 16822896]
56 **Friedman DI**. Topirimate-induced burning mouth syndrome. *Headache* 2010; **50**: 1383-1385 [PMID: 20561063]
57 **Speciali JG**, Stuginski-Barbosa J. Burning mouth syndrome. *Curr Pain Headache Rep* 2008; **12**: 279-284 [PMID: 18625105]
58 **Scott J**, Huskisson EC. Graphic representation of pain. *Pain* 1976; **2**: 175-184 [PMID: 1026900]
59 **Ship JA**, Grushka M, Lipton JA, Mott AE, Sessle BJ, Dionne RA. Burning mouth syndrome: an update. *J Am Dent Assoc* 1995; **126**: 842-853 [PMID: 7629360]
60 **López-Jornet P**, Camacho-Alonso F, Andujar-Mateos P, Sánchez-Siles M, Gómez-Garcia F. Burning mouth syndrome: an update. *Med Oral Patol Oral Cir Bucal* 2010; **15**: e562-e568 [PMID: 20038880]
61 **Lamb AB**, Lamey PJ, Reeve PE. Burning mouth syndrome: psychological aspects. *Br Dent J* 1988; **165**: 256-260 [PMID: 3179130]
62 **Sardella A**, Lodi G, Demarosi F, Bez C, Cassano S, Carrassi A. Burning mouth syndrome: a retrospective study investi-

gating spontaneous remission and response to treatments. *Oral Dis* 2006; **12**: 152-155 [PMID: 16476036]
63 **Femiano F**, Scully C. Burning mouth syndrome (BMS): double blind controlled study of alpha-lipoic acid (thioctic acid) therapy. *J Oral Pathol Med* 2002; **31**: 267-269 [PMID: 12110042]
64 **Cavalcanti DR**, da Silveira FR. Alpha lipoic acid in burning mouth syndrome--a randomized double-blind placebo-controlled trial. *J Oral Pathol Med* 2009; **38**: 254-261 [PMID: 19175713]
65 **Femiano F**, Lanza A, Buonaiuto C, Gombos F, Nunziata M, Cuccurullo L, Cirillo N. Burning mouth syndrome and burning mouth in hypothyroidism: proposal for a diagnostic and therapeutic protocol. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2008; **105**: e22-e27 [PMID: 18155597]
66 **López-D'alessandro E**, Escovich L. Combination of alpha lipoic acid and gabapentin, its efficacy in the treatment of Burning Mouth Syndrome: a randomized, double-blind, placebo controlled trial. *Med Oral Patol Oral Cir Bucal* 2011; **16**: e635-e640 [PMID: 20711135]
67 **Eliav E**, Nasri-Heir C. Critical commentary 2: steroid dysregulation and stomatodynia (burning mouth syndrome). *J Orofac Pain* 2009; **23**: 214-215; discussion 219-220 [PMID: 19639099]
68 **Gremeau-Richard C**, Woda A, Navez ML, Attal N, Bouhassira D, Gagnieu MC, Laluque JF, Picard P, Pionchon P, Tubert S. Topical clonazepam in stomatodynia: a randomised placebo-controlled study. *Pain* 2004; **108**: 51-57 [PMID: 15109507]
69 **Kempfle M**. Relaxation spectra of two-step mechanisms as measured by the NADH-binding to glutamate dehydrogenase. *Bull Math Biol* 1977; **39**: 297-310 [PMID: 192398]
70 **Petruzzi M**, Lauritano D, De Benedittis M, Baldoni M, Serpico R. Systemic capsaicin for burning mouth syndrome: short-term results of a pilot study. *J Oral Pathol Med* 2004; **33**: 111-114 [PMID: 14720197]
71 **Marino R**, Torretta S, Capaccio P, Pignataro L, Spadari F. Different therapeutic strategies for burning mouth syndrome: preliminary data. *J Oral Pathol Med* 2010; **39**: 611-616 [PMID: 20701667]
72 **Becker S**, Schmidt C, Berghaus A, Tschiesner U, Olzowy B, Reichel O. Does laryngopharyngeal reflux cause intraoral burning sensations? A preliminary study. *Eur Arch Otorhinolaryngol* 2011; **268**: 1375-1381 [PMID: 21359587]
73 **Garcia-Bravatti M**. Re: Burning mouth syndrome. *Am J Gastroenterol* 1996; **91**: 1281-1282 [PMID: 8651200]
74 **Yamazaki Y**, Hata H, Kitamori S, Onodera M, Kitagawa Y. An open-label, noncomparative, dose escalation pilot study of the effect of paroxetine in treatment of burning mouth syndrome. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 2009; **107**: e6-11 [PMID: 18996028]
75 **Maina G**, Vitalucci A, Gandolfo S, Bogetto F. Comparative efficacy of SSRIs and amisulpride in burning mouth syndrome: a single-blind study. *J Clin Psychiatry* 2002; **63**: 38-43 [PMID: 11838624]
76 **Rodriguez-Cerdeira C**, Sanchez-Blanco E. Treatment of burning mouth syndrome with amisulpride. *J Clin Med Res* 2012; **4**: 167-171 [PMID: 22719802]
77 **López-Jornet P**, Camacho-Alonso F, Andujar-Mateos P. A prospective, randomized study on the efficacy of tongue protector in patients with burning mouth syndrome. *Oral Dis* 2011; **17**: 277-282 [PMID: 20860765]
78 **Bergdahl J**, Anneroth G, Perris H. Cognitive therapy in the treatment of patients with resistant burning mouth syndrome: a controlled study. *J Oral Pathol Med* 1995; **24**: 213-215 [PMID: 7616460]
79 **Femiano F**, Gombos F, Scully C. Burning Mouth Syndrome: open trial of psychotherapy alone, medication with alpha-lipoic acid (thioctic acid), and combination therapy. *Med Oral* 2004; **9**: 8-13 [PMID: 14704612]

P- Reviewers Aghakhani A, Elena V, Mignogna MD
S- Editor Wen LL **L- Editor** A **E- Editor** Li JY



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i5.673

World J Gastroenterol 2013 February 7; 19(5): 673-681
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

ORIGINAL ARTICLE

Desferrioxamine in warm reperfusion media decreases liver injury aggravated by cold storage

Peter G Arthur, Xian-Wa Niu, Wen-Hua Huang, Bastiaan DeBoer, Ching Tat Lai, Enrico Rossi, John Joseph, Gary P Jeffrey

Peter G Arthur, Ching Tat Lai, School of Biomedical, Biomolecular and Chemical Sciences, the University of Western Australia, Crawley, WA 6009, Australia
Xian-Wa Niu, Gary P Jeffrey, School of Medicine and Pharmacology, the University of Western Australia, Crawley, WA 6009, Australia
Xian-Wa Niu, Gary P Jeffrey, Gastroenterology/Hepatology, Sir Charles Gairdner Hospital, Nedlands, WA 6009, Australia
Wen-Hua Huang, WA Liver and Kidney Transplantation Services, QEII Medical Centre, Hospital Avenue, Nedlands, WA 6009, Australia
Bastiaan DeBoer, Division of Anatomical Pathology, PathWest, QEII Medical Centre, Hospital Avenue, Nedlands, WA 6009, Australia
Enrico Rossi, John Joseph, Department of Biochemistry, PathWest, QEII Medical Centre, Hospital Avenue, Nedlands, WA 6009, Australia
Author contributions: Arthur PG and Niu XW designed the study, analysed and interpreted the data, prepared and wrote the manuscript; Niu XW and Huang WH performed the experiments; DeBoer B, Joseph J and Rossi E analysed the samples; Lai CT provided statistical analysis of the data; Jeffrey GP oversaw the study.
Correspondence to: Dr. Xian-Wa Niu, School of Medicine and Pharmacology, the University of Western Australia, 35 Stirling Highway, Crawley, WA 6009, Australia. xianwa.niu@uwa.edu.au
Telephone: +61-8-92876061 Fax: +61-8-93462816
Received: August 24, 2012 Revised: December 11, 2012
Accepted: December 20, 2012
Published online: February 7, 2013

Abstract

AIM: To evaluate whether desferrioxamine decreases ischemia and perfusion injury aggravated by cold storage (CS) in a rat liver perfusion model.

METHODS: Isolated rat livers were kept in CS in University of Wisconsin Solution for 20 h at 4 ℃, then exposed to 25 min of warm ischemia (WI) at 37 ℃ followed by 2 h of warm perfusion (WP) at 37 ℃ with oxygenated (95% oxygen and 5% carbon dioxide) Krebs-Henseleit buffer. Desferrioxamine (DFO), an iron chelator, was added at different stages of storage, ischemia and perfusion: in CS only, in WI only, in WP only, in WI and perfusion, or in all stages. Effluent samples were collected after CS and after WI. Perfusate samples and bile were collected every 30 min (0, 0.5, 1, 1.5 and 2 h) during liver perfusion. Cellular injury was assessed by the determination of lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) in the effluent and perfusate samples. Total iron was analysed in the perfusate samples. After WP, the liver was collected for the determination of liver swelling (wet to dry ratio) and liver morphological examination (hematoxylin and eosin staining).

RESULTS: Increased CS time caused increased liver dysfunction during WP. After 2 h of WP, liver injury was indicated by increased release of AST (0.5 h CS: 9.4 ± 2.2 U/g liver *vs* 20 h CS: 45.9 ± 10.8 U/g liver, $P < 0.05$) and LDH (0.5 h CS: 59 ± 14 U/g liver *vs* 20 h CS: 297 ± 71 U/g liver, $P < 0.05$). There was an associated increase in iron release into the perfusate (0.5 h CS: 0.11 ± 0.03 μmoL/g liver *vs* 20 h CS: 0.58 ± 0.10 μmoL/g liver, $P < 0.05$) and reduction in bile flow (0.5 h CS: 194 ± 12 μL/g *vs* 20 h CS: 71 ± 8 μL/g liver, $P < 0.05$). When DFO was added during WI and WP following 20 h of CS, release of iron into the perfusate was decreased (DFO absent 0.58 ± 0.10 μmoL/g liver *vs* DFO present 0.31 ± 0.06 μmoL/g liver, $P < 0.05$), and liver function substantially improved with decreased release of AST (DFO absent 45.9 ± 10.8 U/g liver *vs* DFO present 8.1 ± 0.9 U/g liver, $P < 0.05$) and LDH (DFO absent 297 ± 71 U/g liver *vs* DFO present 56 ± 7 U/g liver, $P < 0.05$), and increased bile flow (DFO absent 71 ± 8 μL/g liver *vs* DFO present 237 ± 36 μL/g liver, $P < 0.05$). DFO was also shown to improve liver morphology after WP. Cellular injury (the release of LDH and AST) was significantly reduced with the addition of

DFO in CS medium but to a lesser extent compared to the addition of DFO in WP or WI and perfusion. There was no effect on liver swelling or bile flow when DFO was only added to the CS medium.

CONCLUSION: DFO added during WI and perfusion decreased liver perfusion injury aggravated by extended CS.

© 2013 Baishideng. All rights reserved.

Key words: Iron chelation; Ischemia and perfusion injury; Liver; Organ preservation; Rat

Arthur PG, Niu XW, Huang WH, DeBoer B, Lai CT, Rossi E, Joseph J, Jeffrey GP. Desferrioxamine in warm reperfusion media decreases liver injury aggravated by cold storage. *World J Gastroenterol* 2013; 19(5): 673-681 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i5/673.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i5.673

INTRODUCTION

Hypothermia is used extensively to preserve many types of donor organs for transplantation and protects organs, in part, by reduced requirements for metabolic energy[1]. Donor livers are flushed and then stored at 4 ℃ in specialized storage solutions (*e.g.*, University Wisconsin solution). However, preservation injury can cause primary graft dysfunction and increased morbidity and mortality in liver transplant recipients[2]. When cold storage (CS) time is extended beyond 10 h, there is a significant increase in primary graft dysfunction and long term biliary complications following liver transplantation[3-6].

The mechanisms responsible for donor liver dysfunction after preservation are not fully understood but one important potential contributor to liver damage is redox active iron. Redox active iron has been identified as a contributor to isolated hepatocyte death and liver damage during hypothermia[7-10]. Redox active iron can react with hydrogen peroxide and initiate radical reactions which damage cellular macromolecules, such as DNA, proteins and membrane lipids. Chelation of iron by specialised proteins usually prevents the generation of radical species *in vivo*. However, when cells[7,9,11] or organs[12-14] are cooled there is an increase in redox active iron and this increase has been linked to cell death during hypothermia[7,9,10].

One approach to abrogating the activity of redox active iron is to chelate free iron with iron chelators such as desferrioxamine (DFO)[7,9,15]. When DFO was added to CS media there was reduced hepatocyte and endothelial cell death during CS, and liver damage on re-perfusion[7,11,14,16]. However, it is unlikely that DFO is taken up intracellularly by liver cells during storage at 4 ℃[7,17,18], so intracellular redox active iron has the potential to predispose the donor liver to injury during implantation [warm ischemia (WI)] and subsequent re-perfusion[14]. DFO enters tissue by endocytosis at 37 ℃ and therefore the presence of DFO during WI and re-perfusion may offer additional protection from CS injury. To test this hypothesis, isolated rat livers were perfused after CS, with DFO added to the preservation media during CS, WI and WP. This strategy significantly protected livers from CS damage.

MATERIALS AND METHODS

Materials

Krebs-Henseleit buffer (KHB), DFO and reduced glutathione were purchased from SIGMA and Aldrich, Sydney, Australia. Hartmann's solution was obtained from Baxter Health Care Pty Ltd, Old Toongabbie, Australia, University of Wisconsin solution (UWS) from Bristol-Myers Squibb Company, New York, United States, gentamicin from Pfizer, West Ryde, Australia and Actrapid human insulin from Novo Nordisk Pharmaceuticals Pty Ltd, Baulkham Hills, Australia. Dexamethasone sodium phosphate was from DBL, Rowville, Australia.

Liver isolation, CS and WI

The use of animals was approved by the Animal Ethics Committee of the University of Western Australia. All animals received food and water before graft retrieval. Adult male rats (Strain PVG, average weight 272 ± 5 g, n = 35) were anesthetized with halothane. The gastroduodenal vein, splenic vein, right renal artery, right and left adrenal veins were ligated and the bile duct was cannulated. The liver was flushed with 20 mL of cold Hartmann's solution (control) or UWS with supplements (reduced glutathione, dexamethasone, insulin and gentamicin, pH 7.35-7.4 on ice) and heparin (5 U/mL) *via* the aorta. The liver was excised and placed in UWS with supplements for 20 h at 2-4 ℃. After CS the suprahepatic vena cava (outlet) and portal vein (inlet) were cannulated, and the liver was connected to a rat liver perfusion system while it was in the CS medium. In the control group, after the liver was excised, the liver was connected to a rat liver perfusion system while it was in cold saline for up to 0.5 h (CS0.5 h). The liver was then transferred to the perfusion chamber (37 ℃) and flushed with 20 mL of Hartmann's solution and heparin (5 U/mL). The first 6.5 mL of effluent and the CS medium were collected. To simulate WI during liver implantation, the liver was covered with damp gauze and left at 37 ℃ chamber for 25 min.

Liver WP

After WI, the liver was perfused using a water-jacketed perfusion system (Radnoti Glass Technology, Inc., Monrovia, United States) with oxygenated (95% oxygen and 5% carbon dioxide) KHB at 20 mL/min at 37 ℃ and the first 6.5 mL of perfusate was collected. After 7.5 min of perfusion, the perfusate was re-circulated and filtered through a pre-filter and filter (0.8 μm/0.2 μm, 32 mm OD, Pall Life Sciences, Australia) and the liver was then perfused for 2 h. During liver perfusion, perfusate (1.5 mL) and bile were collected at 0, 0.5, 1, 1.5 and 2 h and

Table 1 Details of experimental groups

Experimental group	Number per group	CS time (h) in UWS	Addition of DFO		
			CS	WI	WP
CS0.5 h	5	-	-	-	-
CS20 h	5	20	-	-	-
DFO (CS)	5	20	DFO	-	-
DFO (WI)	5	20	-	DFO	-
DFO (WP)	5	20	-	-	DFO
DFO (WIP)	5	20	-	DFO	DFO
DFO (CS WIP)	5	20	DFO	DFO	DFO

DFO: Desferrioxamine; CS: Cold storage; UWS: University of Wisconsin solution; WI: Warm ischemia; WP: Warm perfusion; WIP: Warm ischemia and perfusion.

kept on ice. Perfusates were centrifuged at 10 000 *g* for 10 min at 4 ℃ and the supernatant was analysed for lactate dehydrogenase (LDH), aspartate aminotransferase (AST) and total iron.

After 2 h of perfusion, the liver was pat-dried and weighed. Tissue (approximately 0.5 g) was dried at 80 ℃ for 72 h for the calculation of wet to dry ratio and a portion of tissue was snap frozen in liquid nitrogen for subsequent analysis. The remaining liver was fixed in 10% formaldehyde for histopathological examination.

Assays

Perfusate LDH levels were measured by the method of Passonneau[19]. AST enzyme activity was measured by automated biochemical analyser (Hitachi 917, Roche Diagnostics). Total iron levels were measured by Inductively Coupled Plasma-Mass Spectrometry (Varian 820, California, United States) with aqueous standards used for the calibration curve. LDH, AST and total iron were expressed relative to dry liver weight.

Liver histopathology

Liver sections were stained with hematoxylin and eosin and then examined independently by a pathologist (B DeBoer) and a hepatologist (GP Jeffrey). A scale for morphological classification of hepatic injury was used as previously described[20]. The 9 point scale was: (1) normal rectangular structure; (2) round hepatocytes with an increase of sinusoidal spaces; (3) vacuolization in zone 3; (4) vacuolization in zone 2; (5) vacuolization in zone 1; (6) vacuolization and nuclear pyknosis in zone 3; (7) vacuolization and nuclear pyknosis in zone 2; (8) vacuolization and nuclear pyknosis in zone 1; and (9) necrosis.

DFO addition to media

There were seven experimental groups and each group consisted of 5 rats. DFO (1 mmol/L) was tested in five experimental groups with different combinations of DFO during CS, WI, WP and perfusion (WIP), or CS, WI and perfusion (CS WIP) (Table 1). The required amount of DFO was weighed and added to the media prior to use and the concentration (1 mmol/L) was based on our previous isolated rat hepatocyte study[7].

Statistical analysis

All analyses were carried out using R 2.10.1[21], using the package base and agricolae[22]. A one-way between ANOVA was used to compare the outcome of each measurement (the levels of LDH, AST, liver wet to dry ratio and bile flow) and treatment condition (Table 1). Least Square Difference (LSD) was used for Post Hoc testing. Differences are considered to be significant if $P < 0.05$. Data is reported as mean ± SE.

RESULTS

Effect of extended CS

The degree to which liver damage occurred during CS, WI and WP was measured by the release of LDH and AST. Liver damage was evident immediately after extended CS. The LDH level was 7.9 ± 0.6 U/g liver and the AST level was 1.9 ± 0.1 U/g liver after 20 h of CS compared to 0.7 ± 0.1 U/g liver and 0.1 ± 0.0 U/g liver in the controls (CS0.5 in Figure 1). Increased damage was also evident after WI, with 9 fold higher LDH and 8 fold higher AST release in extended CS, relative to the controls (CS0.5 in Figure 1). The bulk (more than 95%) of LDH and AST release occurred during WP of livers subjected to extended CS (Figure 1). After 2 h of perfusion, LDH levels were 297 ± 71 U/g liver and AST levels were 45.9 ± 10.8 U/g liver in livers subjected to 20 h of CS compared to 59 ± 14 U/g liver and 9.4 ± 2.2 U/g liver respectively in livers of the control group. Twenty hours of CS resulted in a 14% increase in liver swelling and a 63% decrease in bile flow (Figure 1). Taken together, these data indicate that 20 h of CS caused a detrimental effect on liver function during subsequent WI and WP.

Protective effect of DFO

DFO was first added to CS medium only. There was no significant improvement in LDH levels or AST levels during WI. However during WP there was a 40% reduction of LDH and AST levels after 2 h of perfusion compared to livers that had not been exposed to DFO during CS (Figure 2). There was no effect on liver swelling or bile flow (Figure 2).

To test if either redox active iron was not completely chelated by DFO during CS, or additional redox active iron release occurred during WI and WP, DFO was added to solutions during all stages of the experiment. This resulted in a significant reduction of AST levels from 6.7 ± 1.3 U/g liver to 3.4 ± 0.5 U/g liver compared with no DFO during WI ($P < 0.05$). LDH levels were not significantly affected (DFO absent 39.9 ± 7.9 U/g liver *vs* DFO present 24.4 ± 2.5 U/g liver). In contrast, after 2 h of WP there was a large reduction of AST levels and LDH levels from 45.9 ± 10.8 U/g liver to 8.1 ± 0.9 U/g liver and 297 ± 71 U/g liver to 56 ± 7 U/g liver respectively when compared to no DFO (Figure 2). These levels were similar to the controls (CS0.5 in Figure 1). Consistent DFO effects were observed with improved liver swelling, decreased from 4.01 ± 0.17 to 3.37 ± 0.05 (wet weight to dry weight ratio) and bile flow increased from 71 ± 8

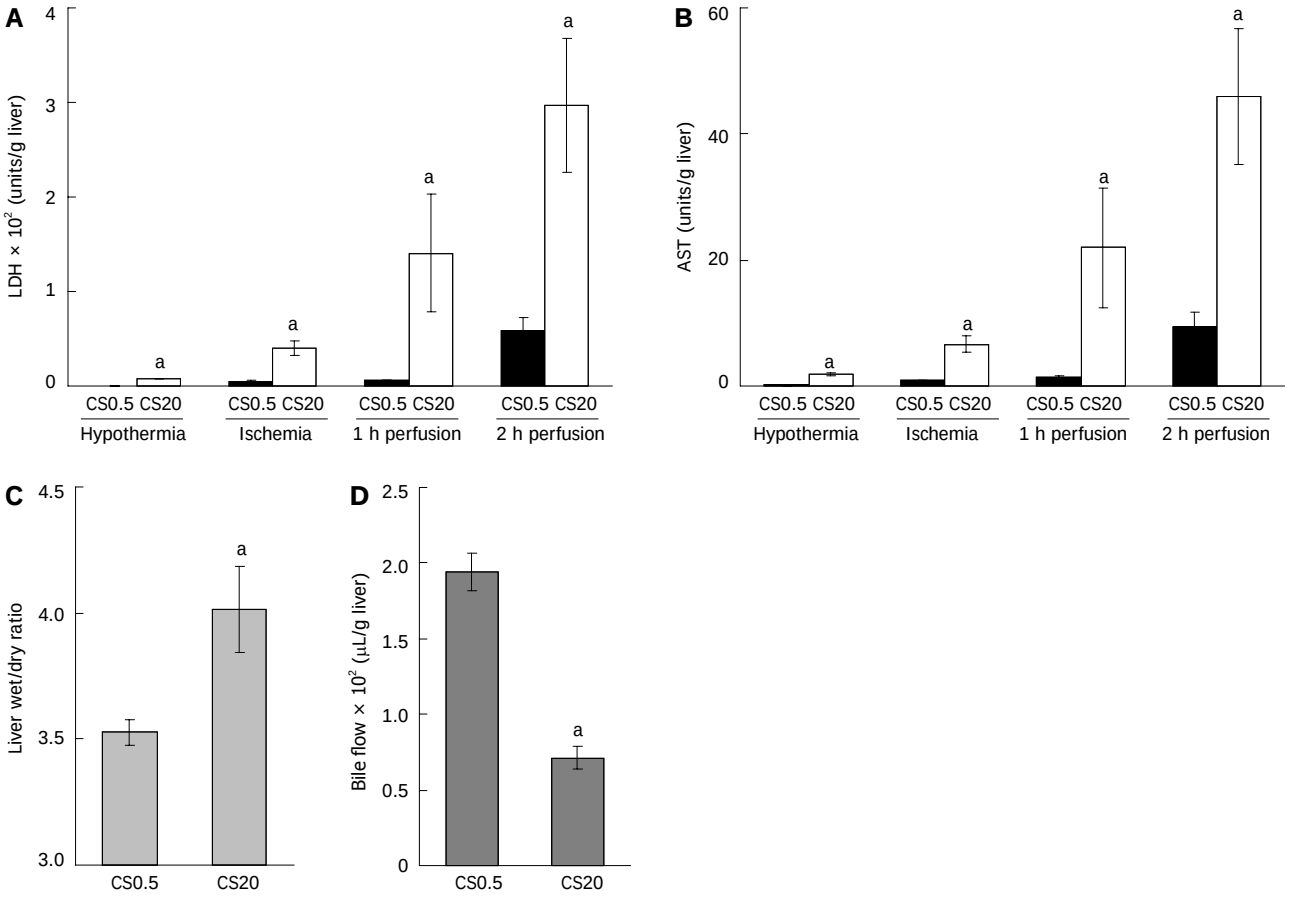


Figure 1 Liver function following cold storage, warm ischemia and perfusion. A, B: Liver damage was assessed by measuring lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) in media from control livers after 0.5 h of cold storage (CS0.5) and from the livers after 20 h of extended cold storage (CS20). LDH and AST were measured in media flushed from livers following cold storage, in media flushed from livers following 25 min of warm ischemia and in media at 1 and 2 h of warm perfusion; C, D: Liver health was assessed by liver swelling (liver wet to dry ratio) after 2 h of perfusion and bile flow over 2 h of perfusion. Significantly different from the controls (mean ± SE, $n = 5$, $^{a}P < 0.05$).

μL/g liver to 237 ± 36 μL/g liver (Figure 2).

To test the possibility that DFO had its predominant protective effect during WI and/or WP, DFO was added to either WI or WP media alone. DFO had no effect when added during WI alone, however there was a protective effect when it was added to perfusion medium alone (Figure 2). There was a reduction of LDH from 297 ± 71 U/g liver to 72 ± 15 U/g liver and AST from 45.9 ± 10.8 U/g liver to 13.4 ± 2.2 U/g liver compared to livers not treated with DFO (Figure 2). Furthermore, liver swelling was prevented and bile flow was maintained (Figure 2). Of note, the protective effect was comparable to that observed when DFO was present during all three stages of the experiment and this is consistent with the concept that redox active iron continued to be a major cause of liver damage during WI and WP following extended CS.

To further assess the protective effect of DFO, liver morphology was examined following 2 h of WP and assessed using a nine point scale ranging from 1 (normal structure) to 9 (necrosis) (Figure 3). Relative to the controls (CS0.5 h, morphological classification of 3.0 ± 0.1, mean ± SE, $n = 4$), extended CS resulted in extensive vacuolization and marked pyknosis (morphological classification of 5.5 ± 0.9, mean ± SE, $n = 4$, $P < 0.05$). DFO was largely effective in preventing changes to liver morphology (morphological classification of 3.0 ± 0.4, mean ± SE, $n = 4$, $P < 0.05$ relative to no DFO).

Iron release during CS and perfusion

The effectiveness of DFO in preventing liver damage indicated redox active iron was likely a major contributor to liver damage. To further investigate this, iron release was measured following WI and during perfusion. Following WI and 20 h of CS there was 0.15 ± 0.02 μmoL iron/g liver compared with 0.02 ± 0.01 μmoL iron/g liver in the controls (CS0.5 in Figure 4, $P < 0.05$). After 2 h of WP, iron release after 20 h of CS was 0.58 ± 0.10 μmoL iron/g liver which was significantly higher than 0.11 ± 0.03 μmoL/g liver in the controls (CS0.5 in Figure 4, $P < 0.05$). Iron concentrations were significantly correlated with LDH release and AST release during WI (Figure 5). During WP, the concentration of iron was also correlated with LDH release and AST release (Figure 5). The significant correlation between iron and the damage markers LDH and AST was consistent with the hypothesis that redox active iron was causing liver damage.

The presence of DFO altered iron concentrations in

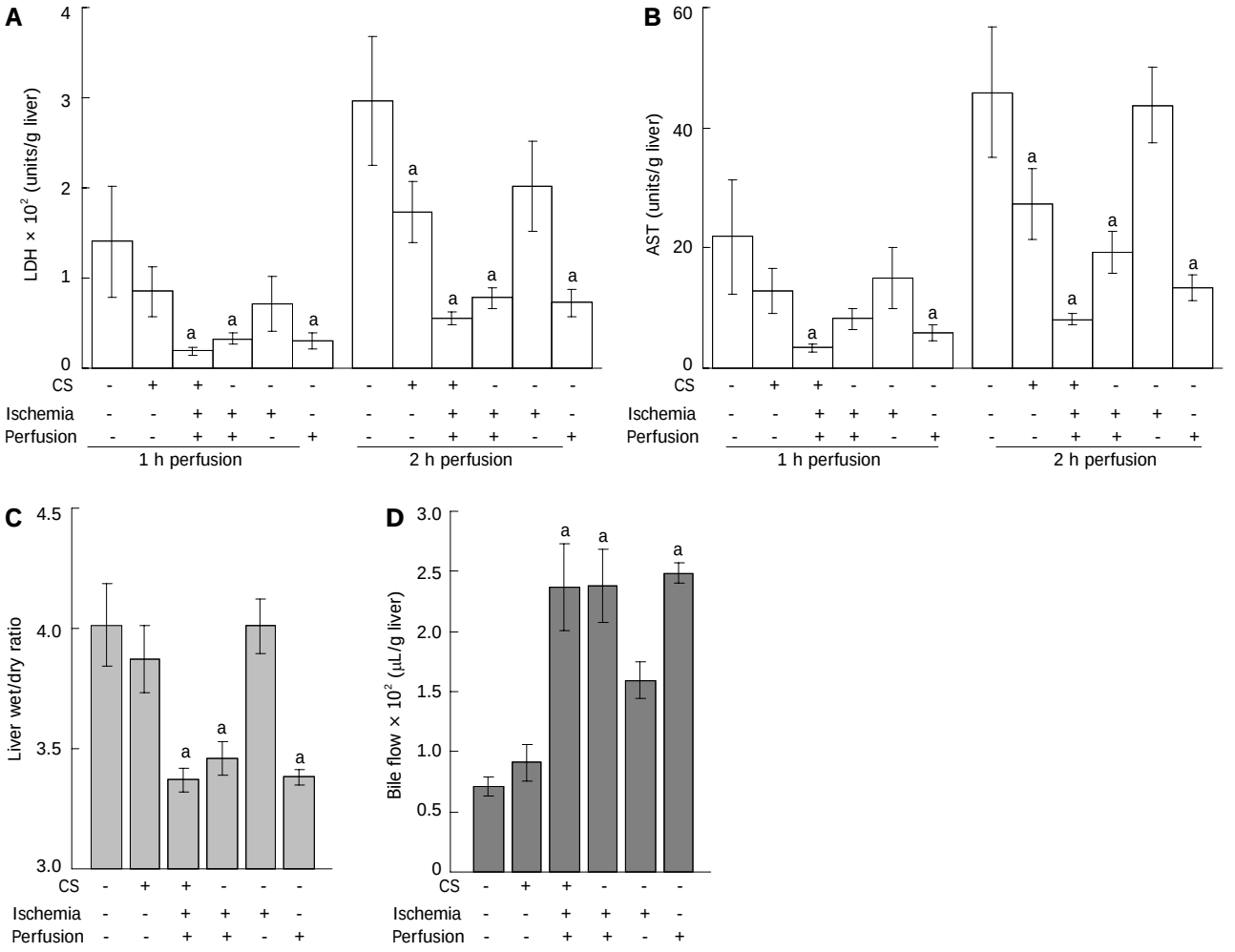


Figure 2 The effect of desferrioxamine added during cold storage, warm ischemia and/or perfusion on liver function following 20 h of cold storage. A,B: Desferrioxamine was added (indicated by +) to cold storage (CS), warm ischemia and perfusion media. Liver damage was assessed by measuring lactate dehydrogenase (LDH) and aspartate aminotransferase (AST) in media from livers during perfusion at 1 and 2 h; C, D: Liver health was assessed by liver swelling (liver wet to dry ratio) after 2 h of perfusion and bile flow over 2 h of perfusion. Data for extended (20 h) CS are shown. Significantly different from 20 h of CS (mean ± SE, $n = 5$, $^{a}P < 0.05$).

WP media. When DFO was added during CS, WI and perfusion, iron concentrations were significantly decreased from 0.58 ± 0.10 μmoL/g liver (no DFO) to 0.31 ± 0.06 μmoL/g liver (with DFO, $P < 0.05$) (Figure 4). There was also a significant relationship between the concentration of iron and liver damage during both WI and WP (Figure 5). However, the slope of the line describing the relationship was significantly lower in the presence of DFO compared to the absence of DFO during WP (Figure 5). Consequently, for the same concentration of iron, there was less damage in the presence of DFO relative to absence of DFO. One explanation for this observation may be that by chelating iron during WI or perfusion, DFO prevented ongoing liver damage caused by redox active iron.

DISCUSSION

It is well established that extended CS causes increased liver damage and that the presence of iron chelators in storage media can substantially protect hepatocytes and livers from damage during cold incubation or storage[9,14,23,24]. In this study, extended CS also aggravated liver damage during the simulated transplantation protocol involving WI (mimicking implantation) and WP. The presence of DFO during CS only partial decreased the subsequent vulnerability of livers to the simulated transplantation protocol. The novel finding of this study was that the presence of DFO during WI and WP could substantially decrease the vulnerability of livers to extended CS.

Liver damage during WP was related to events occurring during CS, such that extended CS sensitised the liver to subsequent WI and perfusion injury. Redox active iron has been identified as a contributor to hepatocyte death[10] and liver damage during CS[7,9,10]. Data from this study provided evidence that redox active iron was involved in sensitising the liver during extended CS, as the presence of DFO during CS did partially protect against subsequent liver damage. There is evidence that DFO is not able to cross membranes during incubation at 4 ℃[7,18], so by what mechanism did DFO partially protect the liver in this present model? Iron is present in UWS solu-

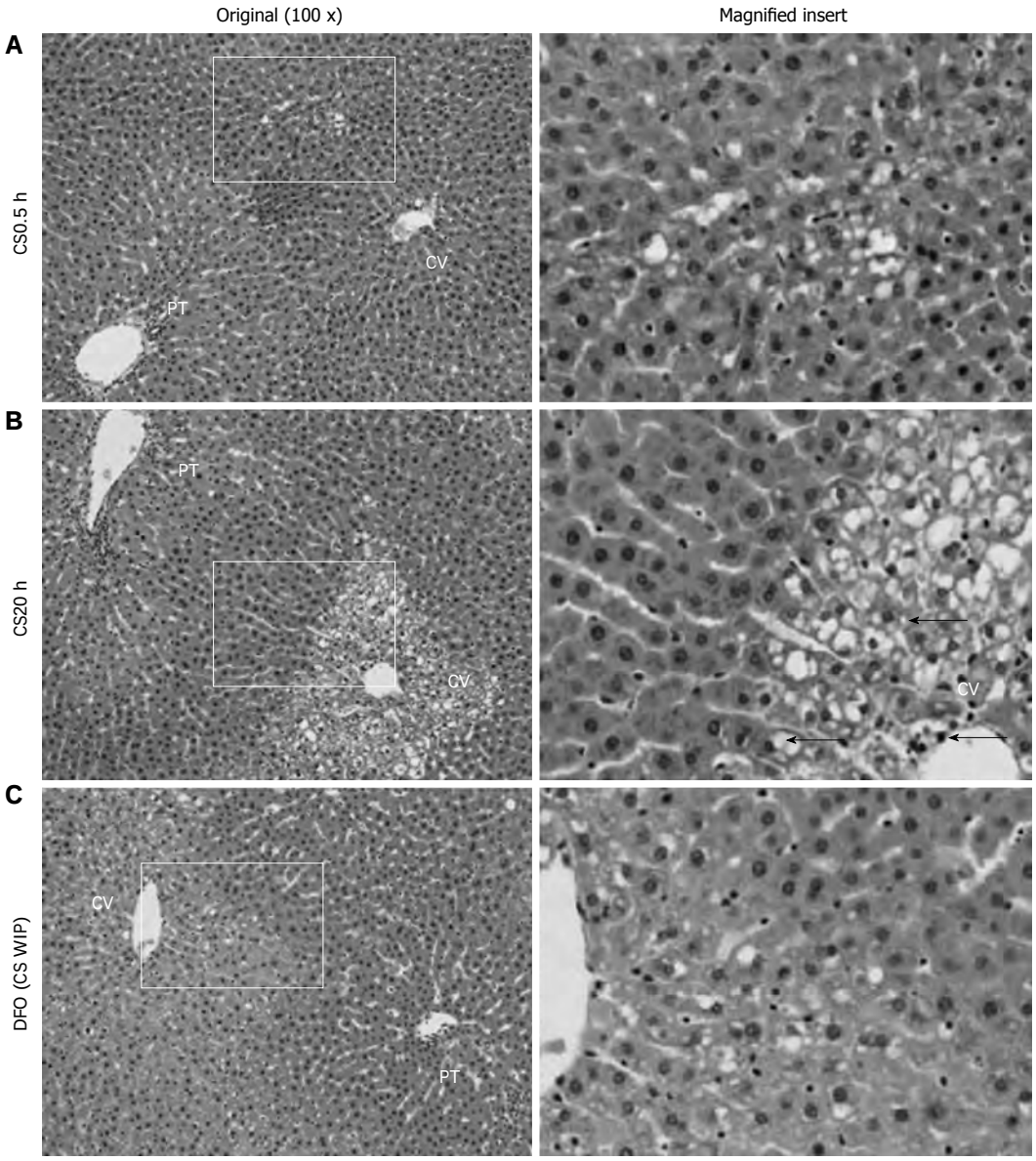


Figure 3 Histopathological appearance of livers following cold storage, warm ischemia and perfusion and the effect of desferrioxamine. A: The control liver shows some early vacuolization in zone 2 following 0.5 h of cold storage (CS0.5) (magnified insert); B: Following 20 h of cold storage (CS20), the liver shows marked vacuolization and nuclear pyknosis in zones 3 as indicated by arrows (←, magnified insert); C: Following 20 h of CS with desferrioxamine (DFO) in CS, warm ischemia and perfusion media (CS WIP), the liver shows mild vacuolization in zone 3 (magnified insert). Hematoxylin and eosin staining and the original magnification was 100 ×. PT: Portal tract; CV: Central vein.

tion as measured in our laboratory (4.43 ± 0.05 μmoL/L, n = 4) and in another study[25], so the protective effect of DFO added during CS could be a result of chelation of extracellular iron. It has also been suggested that DFO is able to reduce intracellular iron by draining cytosolic iron to the extracellular medium[26]. Consistent with this concept, we have previously shown that chelation of extracellular iron protects hepatocytes during cold incubation[7]. Therefore, during extended CS, extracellular iron could have contributed to the sensitisation of livers to subsequent WI and perfusion injury.

Intracellular redox active iron could have also contributed to the sensitization of livers to subsequent WI and perfusion injury. Intracellular redox active iron has been shown to increase during cold incubation/storage in isolated hepatocytes[9,11] and whole liver[14]. Additionally, we have linked increased intracellular redox active iron to hepatocyte cell death during extended cold incubation[7]. In previous experiments not involving CS, redox active iron has been implicated in renal ischemia and perfusion injury[12,27] and ischemia and perfusion injury in livers[28]. Therefore, an increase in the concentration of intracellular redox active iron caused by the extended CS would exacerbate an ongoing cycle of redox active iron release during WI and WP. This explanation would account for the added effectiveness of DFO during WI and WP.

The findings of this study have implications for liver transplantation. There has been a focus on protecting livers during CS by developing various cryoprotective media or improving liver function prior to transplant us-

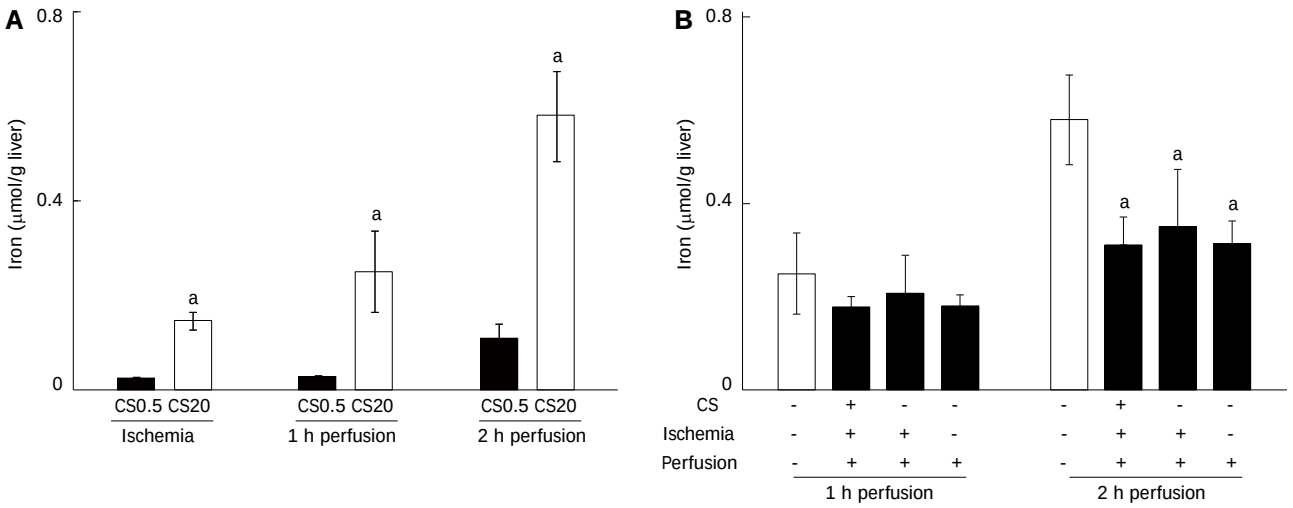


Figure 4 Iron release during warm ischemia and perfusion and effect of desferrioxamine on iron release. A: Iron release was measured in media after warm ischemia (WI) and at 1 and 2 h of perfusion from the control livers after 0.5 h of cold storage (CS0.5) and from the livers after 20 h of extended cold storage (CS20). Significantly different from the controls (mean ± SE, $n = 5$, $^{a}P < 0.05$); B: Iron release was measured in media at 1 and 2 h of perfusion from livers following 20 h of cold storage (CS). Addition of desferrioxamine (DFO) to CS, WI and perfusion media is indicated by (+). Significantly different from 20 h of CS with no DFO (mean ± SE, $n = 5$, $^{a}P < 0.05$).

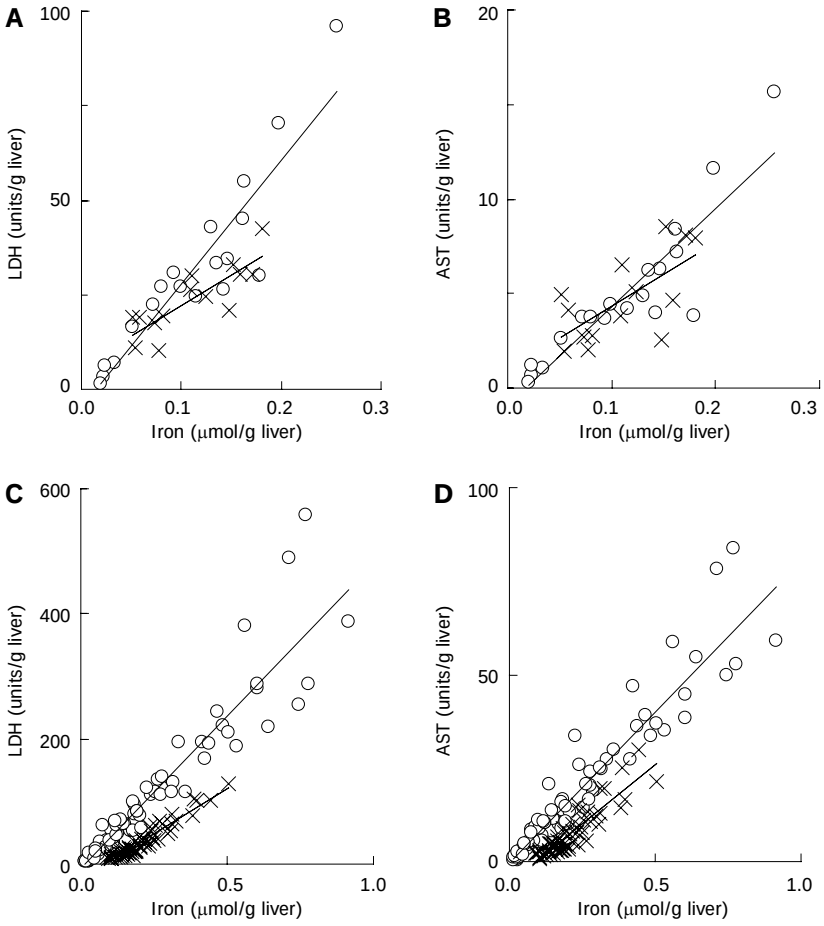


Figure 5 Relationship between presence of iron and liver damage. Following ischemia: A: The relationships between lactate dehydrogenase (LDH) and iron in the absence of desferrioxamine (DFO) (circles) and the presence of DFO (crosses) were described by y = 327x - 5 ($n = 19$, $r^2 = 0.84$) and y = 162x + 6 ($n = 14$, $r^2 = 0.69$) respectively; B: The relationships between aspartate aminotransferase (AST) and iron in the absence of DFO (circles) and the presence of DFO (crosses) were described by y = 52x - 0.9 ($n = 19$, $r^2 = 0.81$) and y = 33x + 1 ($n = 14$, $r^2 = 0.44$); C: During perfusion, samples collected at 0.5 h, 1 h, 1.5 h and 2 h were analysed. The relationships between LDH and iron in the absence of DFO (circles) and the presence of DFO (crosses) were described by y = 487x - 8.1 ($n = 76$, $r^2 = 0.88$) and y = 291x - 24.4 ($n = 56$, $r^2 = 0.91$) respectively; D: The relationships between AST and iron in the absence of DFO (circles) and the presence of DFO (crosses) were described by y = 81x - 0.5 ($n = 76$, $r^2 = 0.90$) and y = 62x - 5.6 ($n = 56$, $r^2 = 0.80$) respectively. All correlation coefficients were significant ($P < 0.05$), and all slopes for media containing iron were significantly different ($P < 0.05$) from the equivalent treatment without DFO.

ing perfusion systems. This data indicates there may be a further opportunity to enhance liver preservation during transplantation and following transplantation by chelating redox active iron. This concept will need further testing in liver transplant models as the *in vitro* model used in this study does not fully encapsulate the complexity of the recipients' response to the transplant procedure and drug intervention. From a clinical application perspective it is worth noting that DFO is already approved for a variety of clinical applications such as treating iron overload patients[29,30].

COMMENTS

Background

Cold donor organ preservation techniques were developed to reduce cellular metabolic activity and maintain cellular viability in donor organs. However, livers stored beyond about 12 h are generally considered to be unsuitable for transplantation.

Research frontiers

Extending the time of cold storage (CS) increases the susceptibility of livers to ischemia and reperfusion injury during transplantation. Preventing ischemia and reperfusion injury would permit extended times of CS.

Innovations and breakthroughs

Desferrioxamine (DFO), an iron chelator, is often included in CS media to prevent oxidative stress caused by redox active iron. The novel finding of this study was that including DFO during warm ischemia and warm perfusion could substantially decrease the vulnerability of rat livers to an extended time (20 h) of CS.

Applications

This study shows that it is possible to extend the time in which livers can be kept in CS. Extended CS times would improve the numbers of livers available for transplant.

Peer review

This is an experimental study of correct metodologia. The bibliography is sorted correctly.

REFERENCES

1 **Llarrull MS**, Pizarro MD, Scandizzi AL, Bottai H, Guibert EE, Rodriguez JV. Cold preservation of isolated hepatocytes in UW solution: experimental studies on the respiratory activity at 0 degrees C. *Cryo Letters* 2007; **28**: 313-328 [PMID: 18075701]

2 **Strasberg SM**, Howard TK, Molmenti EP, Hertl M. Selecting the donor liver: risk factors for poor function after orthotopic liver transplantation. *Hepatology* 1994; **20**: 829-838 [PMID: 7927223 DOI: 10.1002/hep.1840200410]

3 **Defamie V**, Laurens M, Patrono D, Devel L, Brault A, Saint-Paul MC, Yiotakis A, Barbry P, Gugenheim J, Crenesse D, Dive V, Huet PM, Mari B. Matrix metalloproteinase inhibition protects rat livers from prolonged cold ischemia-warm reperfusion injury. *Hepatology* 2008; **47**: 177-185 [PMID: 18008367 DOI: 10.1002/hep.21929]

4 **Li L**, Li CM, Zhang BY, Hu MD, Li XY, Ran JH, Huang M. Apoptosis of rat liver in cold preservation with custom-designed KYL solution. *Hepatobiliary Pancreat Dis Int* 2007; **6**: 497-503 [PMID: 17897913]

5 **Shimizu H**, Kataoka M, Ohtsuka M, Ito H, Kimura F, Togawa A, Yoshidome H, Kato A, Miyazaki M. Extended cold preservation of the graft liver enhances neutrophil-mediated pulmonary injury after liver transplantation. *Hepatogastroenterology* 2005; **52**: 1172-1175 [PMID: 16001655]

6 **Vajdová K**, Smreková R, Mislanová C, Kukan M, Lutterová M. Cold-preservation-induced sensitivity of rat hepatocyte function to rewarming injury and its prevention by short-term reperfusion. *Hepatology* 2000; **32**: 289-296 [PMID: 10915735 DOI: 10.1053/jhep.2000.8895]

7 **Arthur PG**, Niu X, Rigby P, Steer JH, Jeffrey GP. Oxidative stress causes a decline in lysosomal integrity during hypothermic incubation of rat hepatocytes. *Free Radic Biol Med* 2008; **44**: 24-33 [PMID: 18045544 DOI: 10.1016/j.freeradbiomed.2007.09.003]

8 **Ozaki M**, Fuchinoue S, Teraoka S, Ota K. Mobilization of low-molecular-weight iron and peroxidative damage during ischemia and reoxygenation of the rat liver. *Transplant Proc* 1994; **26**: 918-921 [PMID: 8171704]

9 **Rauen U**, Petrat F, Li T, De Groot H. Hypothermia injury/cold-induced apoptosis--evidence of an increase in chelatable iron causing oxidative injury in spite of low O2-/H2O2 formation. *FASEB J* 2000; **14**: 1953-1964 [PMID: 11023979 DOI: 10.1096/fj.00-0071com]

10 **Rauen U**, Kerkweg U, Weisheit D, Petrat F, Sustmann R, de Groot H. Cold-induced apoptosis of hepatocytes: mitochondrial permeability transition triggered by nonmitochondrial chelatable iron. *Free Radic Biol Med* 2003; **35**: 1664-1678 [PMID: 14680689 DOI: 10.1016/j.freeradbiomed.2003.09.018]

11 **Kerkweg U**, Li T, de Groot H, Rauen U. Cold-induced apoptosis of rat liver cells in University of Wisconsin solution: the central role of chelatable iron. *Hepatology* 2002; **35**: 560-567 [PMID: 11870368 DOI: 10.1053/jhep.2002.31869]

12 **Huang H**, Salahudeen AK. Cold induces catalytic iron release of cytochrome P-450 origin: a critical step in cold storage-induced renal injury. *Am J Transplant* 2002; **2**: 631-639 [PMID: 12201364 DOI: 10.1034/j.1600-6143.2002.20708.x]

13 **Pincemail J**, Sergent O, Detry O, Gaspar Y, Cheramy-Bien JP, Cillard J, Meurisse M, Defraigne JO. Intracellular free iron content of rat liver tissue after cold ischemia. *Transplant Proc* 2002; **34**: 759-761 [PMID: 12034173 DOI: 10.1016/S0041-1345(01)02904-9]

14 **Wyllie S**, Seu P, Gao FQ, Goss JA. Deregulation of iron homeostasis and cold-preservation injury to rat liver stored in University of Wisconsin solution. *Liver Transpl* 2003; **9**: 401-410 [PMID: 12682894 DOI: 10.1053/jlts.2003.50065]

15 **Emerit J**, Beaumont C, Trivin F. Iron metabolism, free radicals, and oxidative injury. *Biomed Pharmacother* 2001; **55**: 333-339 [PMID: 11478586 DOI: 10.1016/S0753-3322(01)00068-3]

16 **Vreugdenhil PK**, Rankin MA, Southard JH. Cold storage sensitizes hepatocytes to oxidative stress injury. *Transpl Int* 1997; **10**: 379-385 [PMID: 9287404 DOI: 10.1007/s001470050074]

17 **Cable H**, Lloyd JB. Cellular uptake and release of two contrasting iron chelators. *J Pharm Pharmacol* 1999; **51**: 131-134 [PMID: 10217310 DOI: 10.1211/0022357991772231]

18 **Lloyd JB**, Cable H, Rice-Evans C. Evidence that desferrioxamine cannot enter cells by passive diffusion. *Biochem Pharmacol* 1991; **41**: 1361-1363 [PMID: 2018567 DOI: 10.1016/0006-2952(91)90109-I]

19 **Passonneau JV**, Lowry OH. Chapter 7: A collection of enzyme assays. In: Enzymatic analysis: a practical guide. Totowa, New Jersey: Humana Press, 1993: 281

20 **Bessems M**, Doorschodt BM, van Vliet AK, van Gulik TM. Improved rat liver preservation by hypothermic continuous machine perfusion using polysol, a new, enriched preservation solution. *Liver Transpl* 2005; **11**: 539-546 [PMID: 15838888 DOI: 10.1002/lt.20388]

21 **The R Core Team**. R: A Language and Environment for Statistical Computing [Computer program]. Vienna: R Foundation for Statistical Computing, 2010

22 **De Mediburu F**. Agricolae: Statistical Procedures for Agricultural Research. R package version 1.09-9 [Computer program]. Vienna: R Foundation for Statistical Computing, 2010

23 **Bahde R**, Palmes D, Gemsa O, Minin E, Stratmann U, de Groot H, Rauen U, Spiegel HU. Attenuated cold stor-

age injury of rat livers using a modified HTK solution. *J Surg Res* 2008; **146**: 49-56 [PMID: 18314137 DOI: 10.1016/j.jss.2007.08.011]

24 **Huang H**, He Z, Roberts LJ, Salahudeen AK. Deferoxamine reduces cold-ischemic renal injury in a syngeneic kidney transplant model. *Am J Transplant* 2003; **3**: 1531-1537 [PMID: 14629283 DOI: 10.1046/j.1600-6135.2003.00264.x]

25 **Evans PJ**, Tredger JM, Dunne JB, Halliwell B. Catalytic metal ions and the loss of reduced glutathione from University of Wisconsin preservation solution. *Transplantation* 1996; **62**: 1046-1049 [PMID: 8900298 DOI: 10.1097/00007890-199610270-00002]

26 **Tenopoulou M**, Doulias PT, Barbouti A, Brunk U, Galaris D. Role of compartmentalized redox-active iron in hydrogen peroxide-induced DNA damage and apoptosis. *Biochem J* 2005; **387**: 703-710 [PMID: 15579135 DOI: 10.1042/BJ20041650]

27 **de Vries B**, Snoeijs MG, von Bonsdorff L, Ernest van Heurn LW, Parkkinen J, Buurman WA. Redox-active iron released during machine perfusion predicts viability of ischemically injured deceased donor kidneys. *Am J Transplant* 2006; **6**: 2686-2693 [PMID: 16889604 DOI: 10.1111/j.1600-6143.2006.01510.x]

28 **Omar R**, Nomikos I, Piccorelli G, Savino J, Agarwal N. Prevention of postischaemic lipid peroxidation and liver cell injury by iron chelation. *Gut* 1989; **30**: 510-514 [PMID: 2714683 DOI: 10.1136/gut.30.4.510]

29 **Pippard MJ**, Callender ST. The management of iron chelation therapy. *Br J Haematol* 1983; **54**: 503-507 [PMID: 6871105 DOI: 10.1111/j.1365-2141.1983.tb02128.x]

30 **Miyajima H**, Takahashi Y, Kamata T, Shimizu H, Sakai N, Gitlin JD. Use of desferrioxamine in the treatment of aceruloplasminemia. *Ann Neurol* 1997; **41**: 404-407 [PMID: 9066364 DOI: 10.1002/ana.410410318]

P- Reviewer Lopez-Andujar R **S- Editor** **L- Editor** A
E- Editor Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i5.682

World J Gastroenterol 2013 February 7; 19(5): 682-691
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

ORIGINAL ARTICLE

Interleukin-17 plays a critical role in the acute rejection of intestinal transplantation

Jian-Jun Yang, Fan Feng, Liu Hong, Li Sun, Meng-Bin Li, Ran Zhuang, Feng Pan, Ying-Mei Wang, Wei-Zhong Wang, Guo-Sheng Wu, Hong-Wei Zhang

Jian-Jun Yang, Fan Feng, Liu Hong, Li Sun, Meng-Bin Li, Wei-Zhong Wang, Guo-Sheng Wu, Hong-Wei Zhang, Department of Digestive Surgery, Xijing Hospital of Digestive Diseases, Fourth Military Medical University, Xi'an 710032, Shaanxi Province, China
Ran Zhuang, Department of Immunology, Fourth Military Medical University, Xi'an 710032, Shaanxi Province, China
Feng Pan, Department of Medical Statistics, Fourth Military Medical University, Xi'an 710032, Shaanxi Province, China
Ying-Mei Wang, Department of Pathology, Xijing Hospital, Fourth Military Medical University, Xi'an 710032, Shaanxi Province, China
Author contributions: Yang JJ, Feng F, Hong L, and Sun L contributed equally to the work; Yang JJ and Feng F designed and performed all the animal experiments, and drafted the manuscript; Hong L assisted in in vivo animal experiments; Sun L assisted in biochemical experiments; Zhuang R assisted in immunological experiments; Wang YM assisted in hematoxylin eosin and immunofluorescence staining; Pan F assisted in experiment design, and carried out data collection and data analysis; Li MB, Wang WZ and Wu GS participated in human intestinal transplantation; Zhang HW, as director of the department, coordinated its execution and design, and revised the final version of the manuscript; all authors read and approved the present version of the manuscript.
Supported by Grants from Scientific Technology Research Development Project of Shaanxi in part, No. 2011K14-05-01, No. 2008K12-01; and a Grant from the National Natural Scientific Foundation of China, No. 31100643
Correspondence to: Hong-Wei Zhang, Professor, Department of Digestive Surgery, Xijing Hospital of Digestive Diseases, Fourth Military Medical University, Xi'an 710032, Shaanxi Province, China. hongwzh@126.com
Telephone: +86-29-84771507 Fax: +86-29-82539041
Received: September 13, 2012 Revised: December 11, 2012
Accepted: December 15, 2012
Published online: February 7, 2013

Abstract

AIM: To investigate the role of interleukin (IL)-17 in small bowel allograft rejection.

METHODS: We detected the expression of helper T cell 17 (Th17) cells in biopsy specimens from 3 cases of living small bowel transplantation in our department through immunofluorescence stain. We then established a rat heterotopic small bowel transplantation model. The rats were sacrificed on the 1st, 2nd, 3rd, 5th, and 7th d after small bowel transplantation. The degrees of transplantation rejection in rat intestine graft were examined through hematoxylin eosin (HE) stain, and the expression of Th17 cells in rat intestine graft were detected through immunofluorescence stain. In addition, the recipient rats undergoing intestinal transplantation were administrated with mouse-anti-rat IL-17 monoclonal antibody (mAb), and the survival of rats was analyzed. The recipient rats which received mouse-anti-rat IL-17 mAb treatment were sacrificed on the 1st, 2nd, 3rd, 5th, and 7th d after small bowel transplantation. The degrees of transplantation rejection and the expression of Th17 cells in rat intestine graft were detected through HE and immunofluorescence stain. The expression of IL-17, IL-1β, tumor necroses factor receptor-α (TNF-α), IL-6, and IL-8 in the intestine graft or serum were also detected.

RESULTS: The expressions of Th17 cells ran parallel with the degree of acute rejection in human intestine grafts. The intestine graft rejection of rats was aggravated with prolonged duration after intestinal transplantation, and the expressions of Th17 cells were also correlated with the degree of acute rejection in rat intestine grafts. Administration of mouse-anti-rat IL-17 mAb prolonged the survival of rats after small bowel transplantation ($P < 0.001$). Furthermore, we found that the administration of mouse-anti-rat IL-17 mAb significantly decreased the intensity of $CD4^+IL\text{-}17^+$ Th17 cells in intestine grafts on the 2nd, 3rd, 5th, and the 7th d (97.22 ± 4.05 *vs* 12.45 ± 2.02 on the 7th d, $P < 0.0001$), and suppressed the severity of acute rejection. The expression of IL-17 in the intestine graft declined after mouse-anti-rat IL-17 mAb administration on the 2nd, 3rd,

5th, and the 7th d (0.88 ± 0.03 *vs* 0.35 ± 0.02 on the 7th d, $P < 0.0001$). We also detected the IL-17 serum level and found that the IL-17 level reduced from the 1st d to the 7th d (6.52 ± 0.18 ng/mL *vs* 2.04 ± 0.15 ng/mL on the 7th d, $P < 0.0001$). No significant difference in the level of IL-17 mRNA in the intestine graft was identified between the two groups. The levels of IL-1β, TNF-α, IL-6, and IL-8 mRNA in the intestine graft after the administration of mouse-anti-rat IL-17 mAb were also tested. We found that on the 3rd, 5th, and 7th d after intestinal transplantation, administration of mouse-anti-rat IL-17 mAb significantly inhibited the levels of IL-1β (12.11 ± 1.16 *vs* 1.27 ± 0.15 on the 7th d, $P < 0.001$), TNF-α (27.37 ± 2.60 *vs* 1.06 ± 0.26 on the 7th d, $P < 0.001$), IL-6 (21.43 ± 1.79 *vs* 1.90 ± 0.32 on the 7th d, $P < 0.001$), and IL-8 (20.44 ± 1.44 *vs* 1.34 ± 0.20 on the 7th d, $P < 0.001$) mRNA in the intestine graft.

CONCLUSION: IL-17 may act as a promising and potent target for inhibiting acute rejection after small bowel transplantation.

© 2013 Baishideng. All rights reserved.

Key words: Interleukin-17; Helper T cell 17; Small bowel transplantation; Acute rejection; Monoclonal antibody

Yang JJ, Feng F, Hong L, Sun L, Li MB, Zhuang R, Pan F, Wang YM, Wang WZ, Wu GS, Zhang HW. Interleukin-17 plays a critical role in the acute rejection of intestinal transplantation. *World J Gastroenterol* 2013; 19(5): 682-691 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i5/682.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i5.682

INTRODUCTION

Small bowel transplantation is a prevailing therapy for short bowel syndrome[1-3]. However, compared with liver[4,5], kidney[6,7], and heart transplantation[8,9], small bowel transplantation has less satisfying effects. The small bowel and mesentery involve redundant lymphoid tissue, which are organs with high immunogenicity prone to inducing severe transplantation rejection. FK506 (Tacrolimus) may inhibit the activation of T cells through suppressing the production of rejection related cytokines, calcium-dependent phosphatase calcineurin, and JNK/p38 pathways[10,11]. FK506 can prevent the aggregation of lymphocytes in the early rejection and chemotaxis of inflammatory cells. But the usage of FK506 after clinical small bowel transplantation may result in severe side effects, such as renal toxicity and neurotoxicity[12].

Recently, the helper T cell (Th)1/Th2 paradigm has been expanded, following the discovery of a third subset of effecter T helper cells that produce interleukin (IL)-17 (Th17) and exhibit effecter functions[13-15]. On the basis of these studies, investigators proposed that IL-17-producing T cells serve as a distinct T helper cell subset, which are called Th17 cells[16-18]. The primary function of Th17 cells appears to be the clearance of pathogens that are not adequately handled by Th1 or Th2 cells[19]. Th17 cells, as potent inducers of tissue inflammation, have a proven association with the pathogenesis of many experimental autoimmune diseases and human inflammatory conditions[20,21].

In the present study, we reveal that Th17 cells and IL-17 cytokine are expressed in the intestine graft after small bowel transplantation. Furthermore, we found that the level of Th17 cells ran parallel with the degree of rejection. The data clearly indicates that Th17 cells and IL-17 cytokine may play an important role in transplant rejection.

MATERIALS AND METHODS

Patients and specimens

This study was approved by the Ethics Committee of the Fourth Military Medical University. Biopsy specimens embedded in paraffin from 3 cases of living-related small bowel transplantation were collected from Xijing Hospital of Digestive Diseases, Fourth Military Medical University (FMMU) from 1999 to 2003. All clinical information was available. The sections were stained for pathological examination. The brief clinical characteristics of these patients are listed as follows: Patient No. 1 was an 18-year-old boy with a 40 cm intestine who received a 150 cm segment of distal ileum from his father; Patient No. 2 was a 17-year-old boy with a 8 cm intestine who received a 170-cm graft of distal ileum from his father and; Patient No. 3 was a 15-year-old boy with a 10 cm intestine who received a 160-cm graft of distal ileum from his mother. All the recipients underwent different degrees of graft rejection after operation. The first two patients survived, while the third died from acute graft rejection and severe infection.

Mice

Forty inbred male F344/NCrl BR and forty LEW/Crl rats (age: 8 to 12 wk old, weight: 180 to 230 g) were purchased from Vital River Lab Animal Technology Co., Ltd (Beijing, China). Animals were maintained in specific pathogen-free conditions. All animal experiments were approved by the Animal Experiment Administration Commission of FMMU.

Small bowel transplantation

Donors and recipients were intraperitoneally anesthetized with pentobarbital (5 g/L). The small intestine 5 cm distal to the ligament of Treitz was harvested from the donor rats and 20 cm of isolated jejunum, along with mesenteric blood vessels, was prepared for transplantation. The left kidney of each recipient was removed and the infrarenal abdominal aorta isolated. An end-to-side vascular anastomosis was performed between the recipient and donor's abdominal aortae. The cuffed portal vein was inserted into the left renal vein. The two ends of the small bowel graft were constructed as separate stomas through the left abdominal wall. Detailed procedures were followed as previously

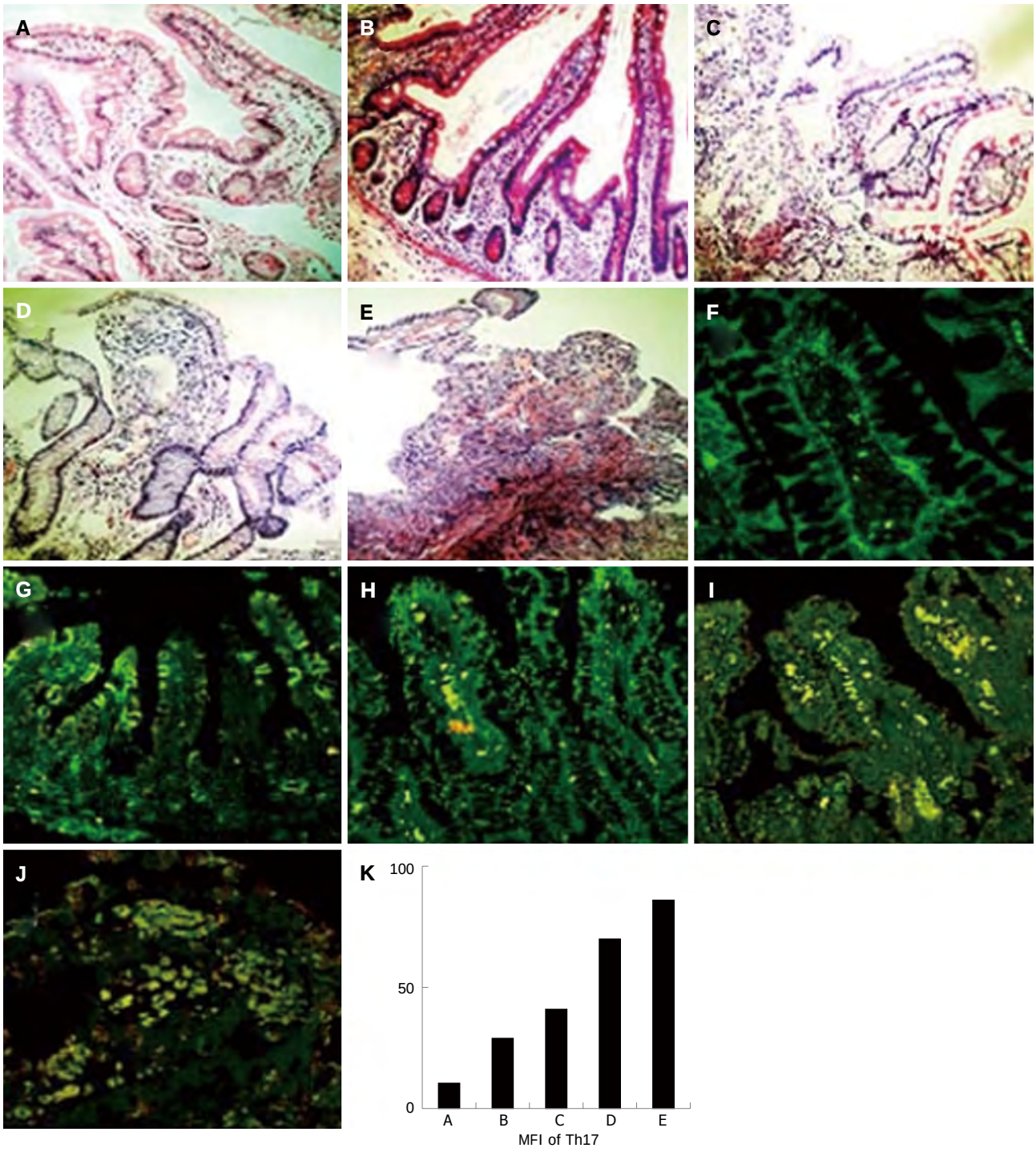


Figure 1 Expression levels of helper T cell 17 cells in intestine grafts paralleled with the degree of rejection in human recipients. The paraffin-embedded human intestine tissues after small bowel transplantation were sectioned and stained with hematoxylin eosin (HE), anti-rat-interleukin-17, and anti-rat-CD4 fluorescence antibodies (× 400). A-E: HE staining of intestine graft; F-J: Helper T cell 17 (Th17) immunofluorescence staining of intestine graft; A, F: No acute rejection; B, G: Suspicious acute rejection; C, H: Mild acute rejection; D, I: Moderate acute rejection; E, J: Severe acute rejection; K: Mean fluorescence intensity (MFI) of Th17 expression of intestine graft.

described[22]. In treatment experiments, the recipient rats in the control group were administrated with mouse-immunoglobulin G (IgG), while the recipient rats in the anti-IL-17 monoclonal antibody (mAb) group were administrated intravenously with 200 μg mouse-anti-rat IL-17 mAb[23] daily during the operation, and on the 1st, 2nd, 3rd, 5th, and 7th d afterwards.

Histology

Specimens were fixed in 4% paraformaldehyde, embedded in paraffin, sectioned at 4 μm thickness, and stained with hematoxylin and eosin, following standard methods

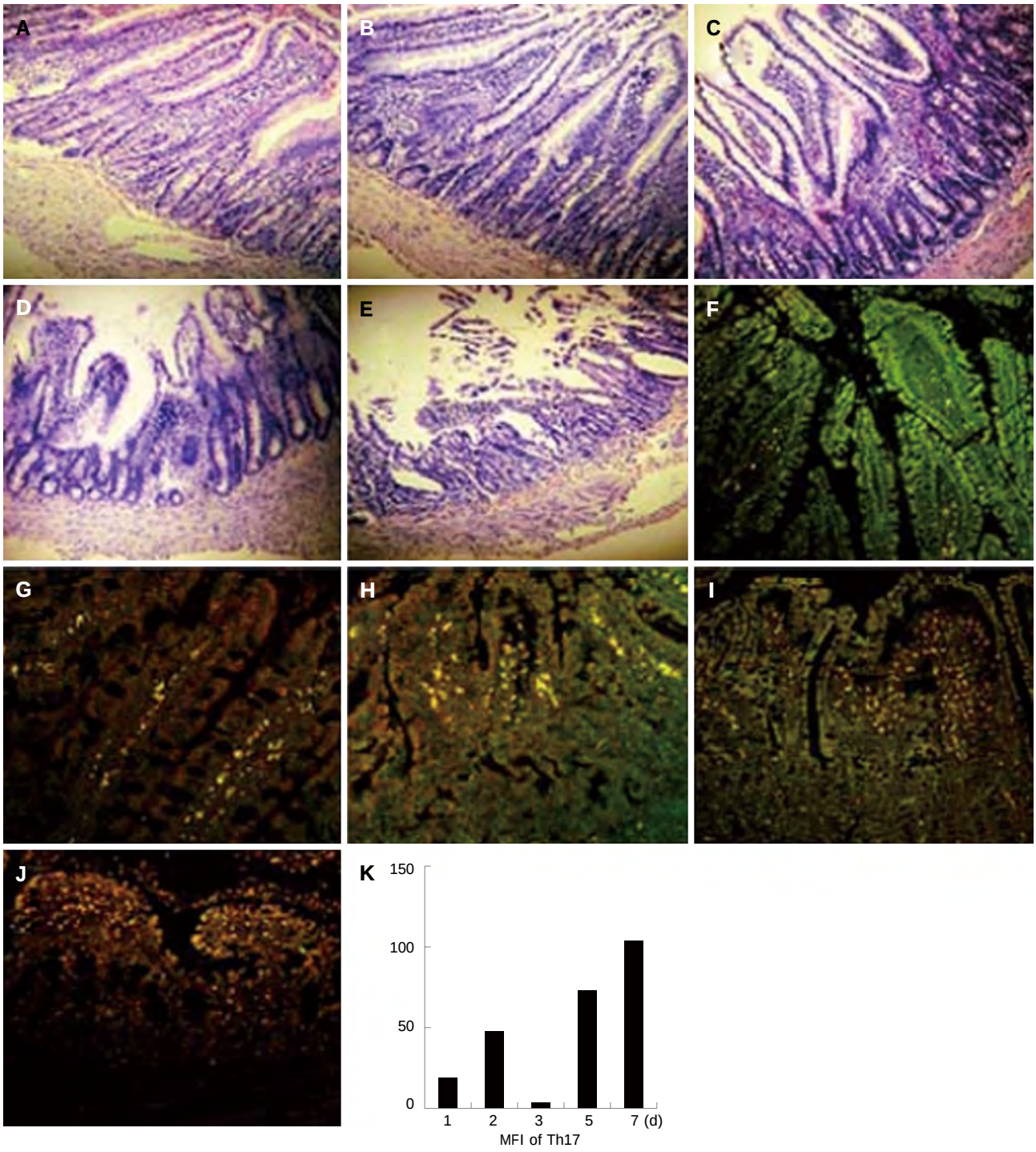


Figure 2 Expression levels of helper T cell 17 cells in intestine grafts paralleled with the degree of rejection in rat recipients. The intestines from inbred F344 were transplanted to LEW rats. The recipient rats were sacrificed on the 1st, 2nd, 3rd, 5th, and 7th d after transplantation. The grafts were removed, fixed, and embedded. The sections were stained with hematoxylin eosin (HE), anti-rat-interleukin-17, and anti-rat-CD4 fluorescence antibodies (× 400). A-E: HE staining of intestine graft; F-J: Helper T cell 17 (Th17) immunofluorescence staining of intestine graft; A, F: 1 d after transplantation; B, G: 2 d after transplantation; C, H: 3 d after transplantation; D, I: 5 d after transplantation; E, J: 7 d after transplantation; K: Mean fluorescence intensity (MFI) of Th17 expression of intestine graft.

for routine morphological analysis.

Diagnostic criteria for transplant rejection of human and rat small bowel transplantation

The biopsy specimens of human and rat intestine grafts were stained with hematoxylin eosin (HE), and analyzed by two independent pathologists. The histological degrees for acute intestine graft rejection were divided into four grades: indeterminate for acute rejection, mild acute rejection, moderate acute rejection, and severe acute rejection. The details of the diagnostic criteria for acute intestine graft rejection have been described previously[24].

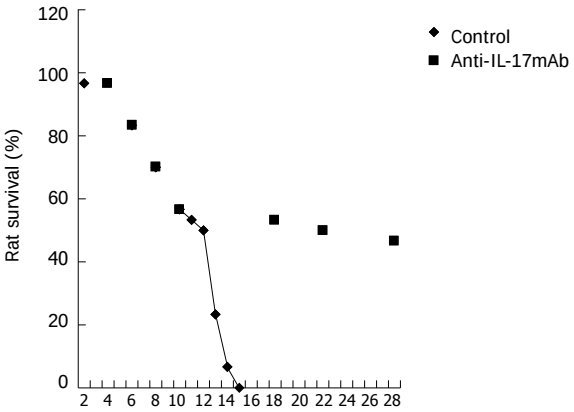


Figure 3 Administration of anti-interleukin-17 monoclonal antibody prolonged the survival of rats after small bowel transplantation. The intestines from inbred F344 were transplanted to LEW rats. The recipient rats were randomly divided into control and anti-interleukin (IL)-17 monoclonal antibody (mAb) treated groups. The survival of recipient rats was analyzed.

Immunofluorescence

The paraffin-embedded human intestine mucosa specimens were sectioned at 4 μm thickness. Immunofluorescence was performed by standard procedures, with rabbit-anti-human IL-17 polyclonal antibody (1:50 dilution; Santa Cruz Biotechnology) and mouse-anti-human CD4 mAb (1:100 dilution; Santa Cruz Biotechnology) as the primary antibodies. Goat-anti-rabbit IgG-TR (1:50 dilution; Santa Cruz Biotechnology) and goat-anti-mouse IgG-FITC (1:50 dilution; Santa Cruz Biotechnology) were taken as secondary antibodies. We did not compare the staining for IL-17 before and after transplantation, but only selected the paraffin-embedded human intestine mucosa specimens at different transplantation rejection degrees in order to detect the expressions of Th17 cells.

The negative control sections were used in our immunofluorescence method. We took phosphate buffered solution (PBS) instead of the primary antibodies as a negative control, and adopted rat cardiac allograft specimen sections as a positive control (data not shown). The methods of the immunofluorescence stain of Th17 in our present study were in accordance with our previous report[25].

Western blotting

The total proteins of the intestine graft were extracted and determined according to the manufacturer's manuals. Proteins were electrophoresed in 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis and blotted on a nitrocellulose membrane. The membrane was incubated with rabbit anti-rat IL-17 polyclonal antibody (1:500 dilutions). After three washes for 15 min in PBS-T, the membrane was incubated with the HRP-conjugated goat-anti-rabbit IgG antibody (1:2000 dilutions). Blots were developed by using an enhanced chemiluminescence system (ECL, Amersham, Little Chalfort, United Kingdom). β-actin was considered as an internal control.

Enzyme-linked immunosorbent assay

Blood from the heart was extracted when the rat was sacrificed. Serum was collected and adopted in order to detect the level of IL-17 with a rat IL-17 enzyme-linked immunosorbent assay kit (ELISA) (Rapidbio, America) by standard procedures. $A_{450\ nm}$ was recorded by a spectrophotometer, and was compared between groups.

Quantitative real time polymerase chain reaction

Total RNA was extracted from rat intestine grafts with a homogenizer by using TRIzol reagent (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Complementary DNA was prepared with a reverse-transcription kit from TOYOBO (Osaka, Japan). Real-time reverse-transcription polymerase chain reaction (RT-PCR) was performed by using a kit (SYBR Premix EX Taq, Takara) and the ABI PRISM 7500 real-time PCR system, with β-actin as an internal control. Primers used in real-time PCR were as follows, β-actin, forward: CATCCGTAAAGACCTCTATG CCAAC, reverse: ATGGAGCCACCGATCCACA; IL-17, forward: GGGAAGTTGGACCACCACAT, reverse: TTCTCCACCCGGAAAGTGAA; IL-1β, forward: CTTCAAATCTCACAGCAGCATCTCG, reverse: TCCACGGGCAAGACATAGGTAGC, tumor necroses factor receptor-α (TNF-α), forward: CTGTGCC TCAGCCTCTTCTCATTC, reverse: TTGGGA ACTTCTCCTCCTTGTTGG; IL-6, forward: GACTGATGTTGTTGACAGCCACTGC, reverse: TAGCCACTCCTTCTGT GACTCTAACT, IL-8, forward: GCCAACAC AGAAATTATTGTAAAGCTT, reverse: CCTCTGCACCCAGT TTTCCTT.

Statistical analysis

Statistical analysis was performed with the SPSS 16.0 program. Results were expressed as means ± SD. Comparisons between groups were made by the unpaired Student's *t*-test. For survival studies, Kaplan-Meier survival curves were generated and a statistical analysis was performed via the log-rank test. $P < 0.05$ was considered statistically significant.

RESULTS

Expression levels of Th17 cells in intestine graft ran parallel with the degree of rejection.

To examine the expression of Th17 cells on a human intestine graft, biopsy specimens embedded in paraffin from 4 cases of living small bowel transplantation in our department were collected and stained with HE (Figure 1A-E) and antibodies against IL-17 and CD4 (Figure 1F-J). The density of IL-17$^+$CD4$^+$ Th17 cells was calculated and analyzed by Image-Pro-Plus 5.1 software. As shown in Figure 1K, the density of IL-17$^+$CD4$^+$ Th17 cells increased when the rejection degrees aggravated.

In order to investigate whether Th17 cells exist in rat intestine grafts after transplantation, intestines from F344/NCrl BR were grafted to LEW/Crl rats; the recipi-

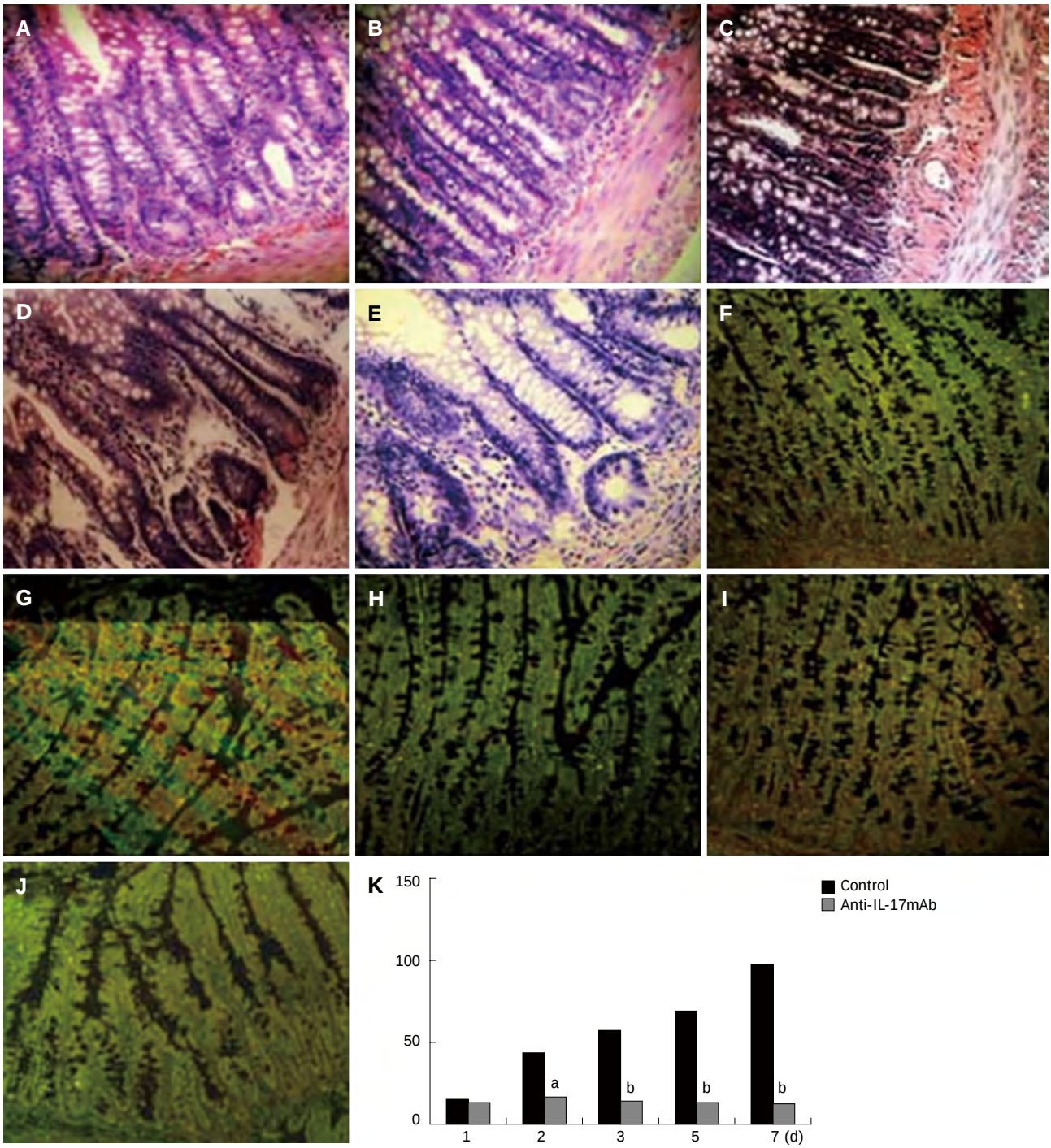


Figure 4 Administration of anti-interleukin-17 monoclonal antibody decreased the expression levels of helper T cell 17 cells in intestine grafts in rat recipients. The intestines from inbred F344 were transplanted to LEW rats. The recipient rats were randomly divided into control and anti-interleukin (IL)-17 monoclonal antibody (mAb) treated groups. The rats were sacrificed on the 1st, 2nd, 3rd, 5th, and 7th d after transplantation. The grafts were removed, fixed, and embedded. The sections were stained with hematoxylin eosin (HE), anti-rat-IL-17, and anti-rat-CD4 fluorescence antibodies (× 400). A-E: HE staining of intestine graft; F-J: Helper T cell (Th) 17 immunofluorescence staining of intestine graft; A, F: 1 d after transplantation; B, G: 2 d after transplantation; C, H: 3 d after transplantation; D, I: 5 d after transplantation; E, J: 7 d after transplantation; K: Mean fluorescence intensity of Th17 expression of intestine graft. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* control group.

ent rats were sacrificed on the 1st, 2nd, 3rd, 5th, and 7th d after transplantation, and the expression of Th17 cells in rat intestine grafts were analyzed (Figure 2A-J). In accordance with the findings in human intestine grafts, we found that Th17 cells were located in rat intestine grafts and the degree of Th17 cells corresponded to the severity of transplant rejection (Figure 2K). Taken together, Th17 cells did exist in intestine grafts, and the levels of Th17 cells might relate to the transplant rejection degrees in both human and rat recipients.

Administration of anti-IL-17 mAb prolonged the survival of rat post-transplantation

Since IL-17 may play a role in small bowel transplantation rejections, we hypothesized that IL-17 could be considered as a potential target for the treatment of graft rejec-

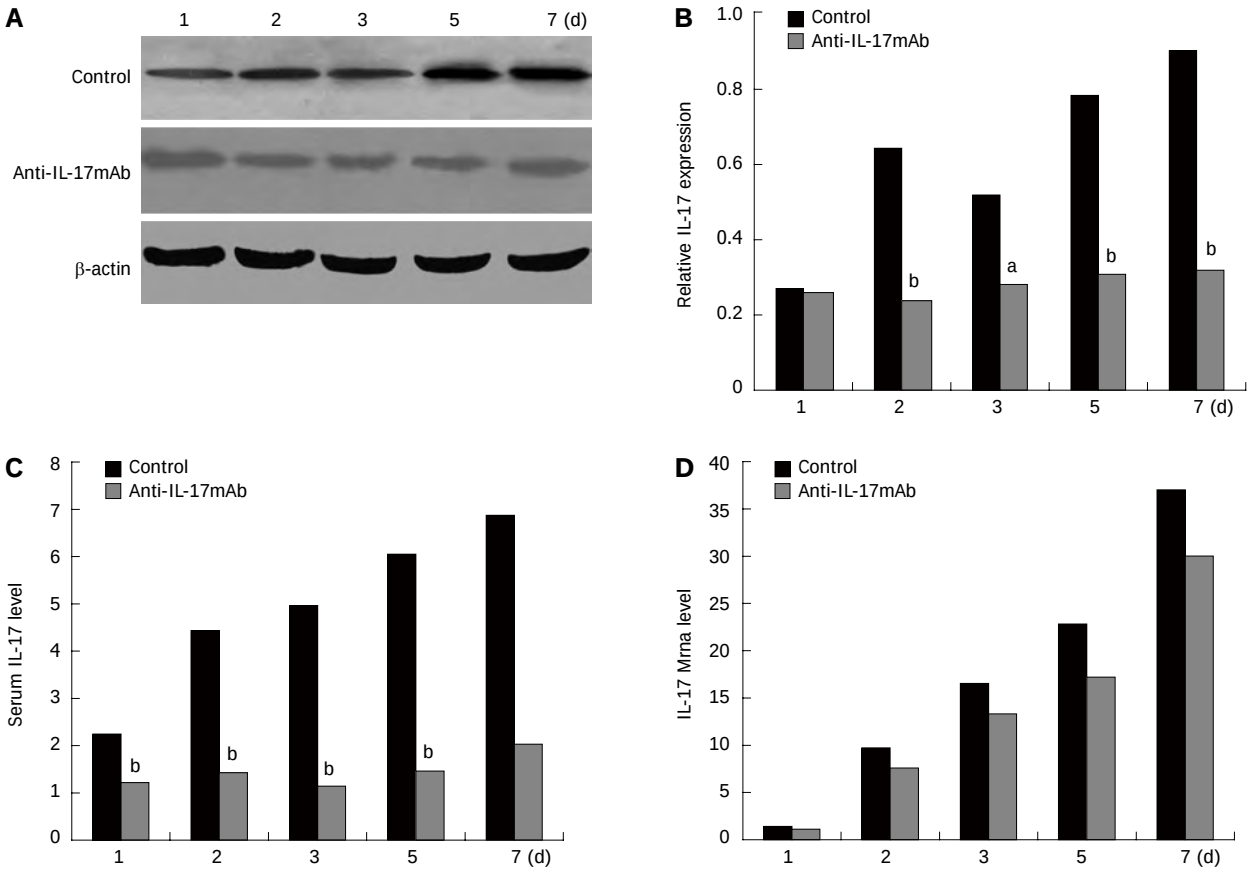


Figure 5 Expression of interleukin-17 in rat intestine and serum after transplantation. The intestines from inbred F344 were transplanted to LEW rats. The recipient rats were randomly divided into control and anti-interleukin (IL)-17 monoclonal antibody (mAb) treated groups. The rats were sacrificed on the 1st, 2nd, 3rd, 5th, and 7th d after transplantation. A: Western blot analysis of graft proteins of different groups. β-actin were considered as an internal control; B: The bands were scanned and the protein expressions were analyzed with Band Scan software; C: Blood from the heart was extracted and the serum IL-17 levels was detected by enzyme-linked immunosorbent assay; D: The mRNA of IL-17 was detected by quantitative reverse transcriptase polymerase chain reaction. β-actin was considered as an internal control. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* control group.

tion. In order to further demonstrate our hypothesis, the recipients were administrated with mouse-anti-rat IL-17 monoclonal antibody intravenously during, and after, small bowel transplantation, and the survival of recipients were analyzed. We found that administration of anti-IL-17 mAb could significantly prolong the survival of rats after small bowel transplantation (Figure 3).

Anti-IL-17 mAb administration inhibited transplant rejection of intestine graft

We further analyzed the effect of anti-IL-17 administration on graft rejections. The administration of anti-IL-17 mAb was proved to effectively inhibit transplant rejection of intestinal grafts in rats post-transplantation by HE staining (Figure 4A-E). Furthermore, we found that the expression of Th17 cells in intestine grafts also dramatically declined (Figure 4F-K), indicating that anti-IL-17 mAb could possibly prolong the survival of recipient rats by inhibiting transplant rejection.

Anti-IL-17 mAb administration decreased IL-17 expressions in rat

During and after allotransplantation, recipient rats were administrated with anti-IL-17 mAb. The recipient rats were sacrificed on the 1st, 2nd, 3rd, 5th, and 7th d after transplantation. The expressions of IL-17 in intestine grafts and serums were further detected by western blot and ELISA (Figure 5). In the control group which only received saline administration, the levels of IL-17 protein in the intestine graft and serum were found to have increased post-allotransplantation and run parallel with transplant rejection degrees. In the anti-IL-17 mAb administration group, the expression of IL-17 could also be detected, but the degrees were remarkably lower than the control group. The levels of IL-17 mRNA in the intestine graft in two groups were not significantly different. Therefore, the administration of anti-IL-17 mAb could decrease the IL-17 level in intestine grafts and serum.

Anti-IL-17 mAb administration decreased expressions of related cytokines

As shown in Figure 6, the expressions of IL-1β, IL-6, IL-8, and TNF-α mRNA of intestine grafts in the control group were found to increase post-allotransplantation and correlate to transplant rejection degrees. Administration of anti-IL-17 mAb could dramatically decrease the expression of mRNA levels of these cytokines in intestine grafts, compared with the control group.

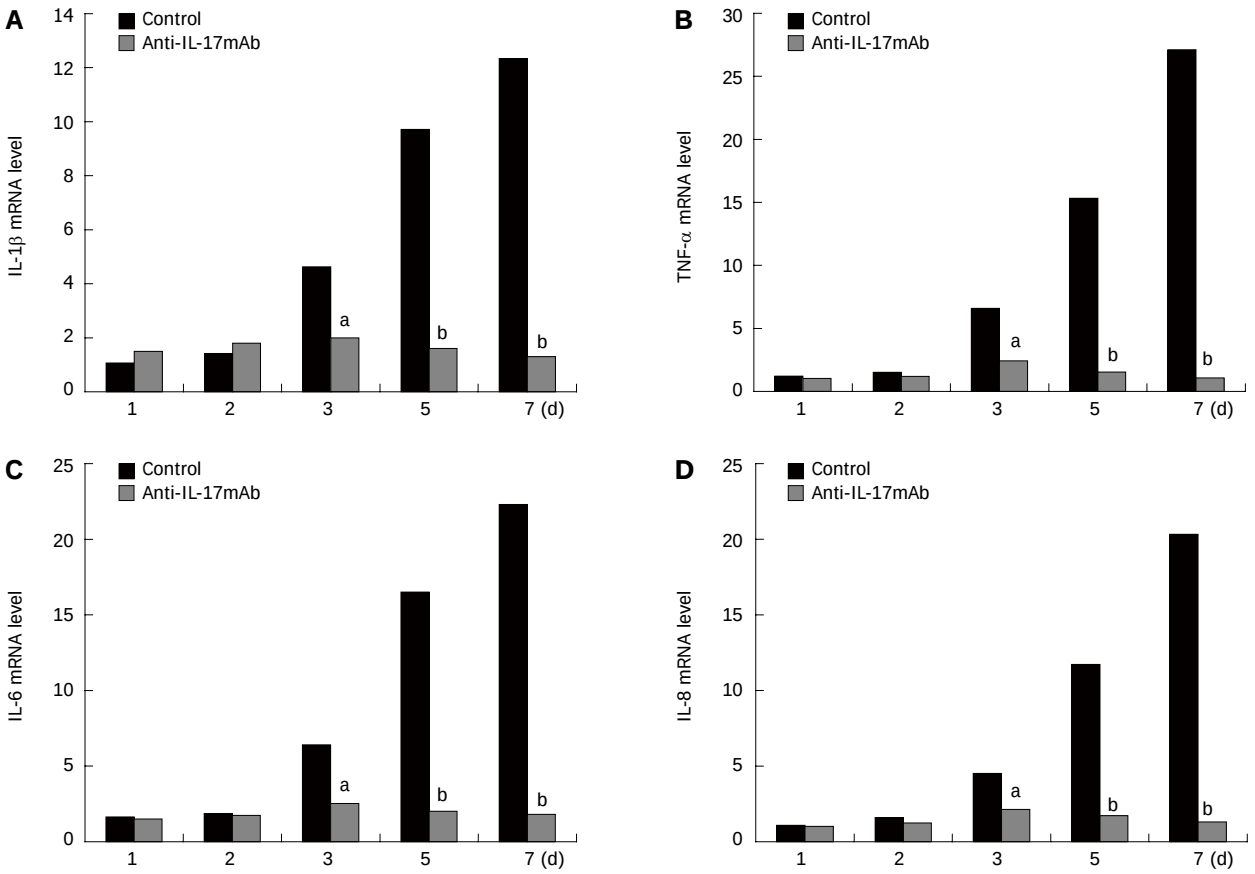


Figure 6 mRNA level of interleukin-1β (A), tumor necrosis factor-α (B), interleukin-6 (C), and interleukin-8 (D) were analyzed by reverse transcriptase polymerase chain reaction. The intestines from inbred F344 were transplanted to LEW rats. The recipient rats were randomly divided into control and anti-interleukin (IL)-17 mAb treated groups. The rats were sacrificed on the 1st, 2nd, 3rd, 5th, and 7th d after transplantation. The mRNA was extracted and quantitative reverse transcriptase polymerase chain reaction was performed. β-actin was considered as an internal control. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* control group.

DISCUSSION

Th17, known as a distinct lineage of helper T cells from Th1 and Th2, has both a defense role against microbe infection and a pathogenic role in several autoimmune diseases[26]. Th17 cells have been shown to be implicated in allograft rejection of solid organs, such as lung and cardiac transplantation[25,27,28]. Vanaudenaerde *et al*[27] observed that IL-17 expression increased in bronchoalveolar lavages in patients with acute lung transplantation rejection. The disease-promoting role of Th17 in cardiac allograft rejection was also confirmed, especially in the absence of a Th1 response[25]. Th17 cells not only function in host-versus-graft disease, but also participate in graft-versus-host disease[29-31]. So far, the relationship between Th17 cells and small bowel transplantation has been unclear. In this study, we demonstrated that Th17 cells participated in the development of human and rat small bowel transplantation rejection.

Here we found that Th17 cells existed in intestine grafts with different degrees of acute rejection. Furthermore, we indicated that the density of Th17 cells increased when the rejection degree aggravated, suggesting that it might be related to the transplant rejection degrees in human recipients. Th17 cells were also found to be located in rat intestine grafts, and the degree of Th17 cells correlated with transplant rejection degrees, which was in accordance with the findings in human intestine grafts. These demonstrate that Th17/IL-17 may participate and play a critical role in the graft rejections of small bowel transplantation.

IL-17, secreted by Th17 cells, is a highly inflammatory cytokine with robust effects on stromal cells in many tissues[32,33]. Hsieh *et al*[34] reported that IL-17 could serve as a predictive parameter for borderline subclinical renal allograft rejection in the future. Itoh *et al*[35] found that IL-17-deficient recipient mice had decreased allograft inflammatory cell recruitment, and demonstrated that IL-17 contributed to the pathogenesis of chronic allograft rejection. In order to demonstrate the key role of IL-17 in the graft rejection of small bowel transplantation, we utilized mouse-anti-rat IL-17 mAb to treat recipient rats undergoing small bowel transplantation for the first time. Surprisingly, after administration of anti-IL-17 mAb, the acute rejection degrees of recipient rats significantly decreased compared to the control group. The levels of Th17 cells that had infiltrated the intestine graft during anti-IL-17 mAb administration also fell greatly. Administration with anti-IL-17 mAb could extend the survival time of rats undergoing small bowel transplantation. To sum up, IL-17 cytokine could probably be taken as a potent target to treat acute rejection in small bowel

transplantation.

The differentiation factors (transforming growth factor beta plus IL-6 or IL-21), the growth and stabilization factor (IL-23), and the transcription factors (signal transducer and activator of transcription 3, related orphan receptor-γt (RORγt), and RORα) were involved in the development of Th17 cells. Mice reconstituted with the bone marrow of RORγt deficient mice showed an impaired Th17 differentiation. The combinations of IL-1β plus IL-6[36] or IL-1β plus IL-23[37] were proposed to be the differentiation factors for human Th17 cells. We found that the expression of IL-1β and IL-6 was significantly decreased after anti-IL-17 mAb administration. Therefore, anti-IL-17 mAb might suppress the expression of IL-1β and IL-6 in rat intestine grafts, and then inhibit the development and activation of Th17 cells. TNF is also induced by IL-17 cytokine. Its expression was found to be reduced after anti-IL-17 mAb administration. The migration and infiltration of inflammatory cells into intestine grafts requires the expression of chemokines. Chemokine (C-X-C motif) ligand 8 (IL-8), a target of IL-17, is involved in transplant rejections. Compared to the control group, the expression of IL-8 was found to be obviously decreased in intestine grafts treated with anti-IL-17 mAb. This suggests that anti-IL-17 mAb administration might suppress the migration and infiltration of Th17 cells into intestine grafts by decreasing the expression of chemokines.

In conclusion, we have illustrated that Th17 cells might play an important role in human and rat small bowel acute transplantation rejection. The administration of anti-IL-17 mAb could significantly suppress the acute rejection degree of rat intestine grafts and prolong the survival time of recipient rats. IL-17 could be considered a promising and potent target for inhibiting acute rejection after small bowel transplantation.

COMMENTS

Background

Small bowel transplantation is a widespread therapy for short bowel syndrome. However, the efficacy of small bowel transplantation is not satisfactory due to severe transplantation rejection. Although administration of FK506 may inhibit the activation of T cells, prevent the aggregation of lymphocytes in early rejection, and restrain the chemotaxis of inflammatory cells, its administration after human small bowel transplantation may cause severe side effects such as renal toxicity and neurotoxicity.

Research frontiers

The helper T cell (Th) 17 cell, as a distinct lineage of helper T cells from Th1 and Th2, has a proven implication in the allograft rejection of solid organs, such as lung and cardiac transplantation. Interleukin (IL)-17 expression was increased in bronchoalveolar lavages in patients with acute lung transplantation rejection. The disease-promoting role of Th17 in cardiac allograft rejection was also confirmed. Th17 cells are involved not only in host-versus-graft disease, but graft-versus-host disease as well.

Innovations and breakthroughs

So far, the relationship between Th17 cells and small bowel transplantation has been unclear. Authors demonstrated the presence of Th17 cells in the intestine grafts of humans and rats, and further found that the expression levels of Th17 cells in intestine grafts correlated with the degree of rejection. The authors hypothesized that Th17/IL-17 might play a critical role in small bowel transplantation rejection and could be regarded as a potential target for the treatment of graft rejection. They then treated recipient rats with mouse-anti-rat IL-17 monoclonal antibody (mAb) and found that its administration could significantly prolong the survival of rats after small bowel transplantation. Furthermore, the authors found that the expression of Th17 cells in intestine grafts also dramatically declined, indicating that mouse-anti-rat IL-17 mAb may prolong the survival of recipient rats by inhibiting transplant rejections. In the present study, the authors demonstrated that Th17 cells participated in the development of human and rat small bowel transplantation rejection.

Applications

The administration of mouse-anti-rat IL-17 mAb could significantly suppress the acute rejection degree of rat intestine grafts and prolong the survival time of recipient rats. IL-17 could be considered as a promising and potent target for inhibiting acute rejection after small bowel transplantation.

Terminology

Small bowel transplantation: an operation to replace a diseased or shortened small bowel with a healthy bowel from a donor and a valuable therapy for short bowel syndrome. Transplant rejection: immune system attacks between the transplant recipient and the transplanted organ or tissue, which include host-versus-graft disease and graft-versus-host disease. Th17 cell: known as a distinct lineage of helper T cells from Th1 and Th2 that has both a defense role against microbe infection and a pathogenic role in several autoimmune diseases.

Peer review

This is an investigation of the role of IL-17 in acute intestinal transplantation rejection and the administration of anti-IL-17 monoclonal antibodies for the suppression of IL-17 production by Th17 cells in the intestine graft and, as a result, suppression of acute rejection of the intestine graft in both human and rat models. It is a very good work and I hope it is applicable in human subjects undergoing intestinal transplantation.

REFERENCES

1 **Donohoe CL**, Reynolds JV. Short bowel syndrome. *Surgeon* 2010; **8**: 270-279 [PMID: 20709285 DOI: 10.1016/j.surge.2010.06.004]

2 **Tzvetanov IG**, Oberholzer J, Benedetti E. Current status of living donor small bowel transplantation. *Curr Opin Organ Transplant* 2010; **15**: 346-348 [PMID: 20445448 DOI: 10.1097/MOT.0b013e3283398fa4]

3 **Li M**, Ji G, Feng F, Song W, Ling R, Chen D, Liu X, Li J, Shi H, Wang W, Zhang H. Living-related small bowel transplantation for three patients with short gut syndrome. *Transplant Proc* 2008; **40**: 3629-3633 [PMID: 19100455 DOI: 10.1016/j.transproceed.2008.06.064]

4 **Kamath BM**, Olthoff KM. Liver transplantation in children: update 2010. *Pediatr Clin North Am* 2010; **57**: 401-414, table of contents [PMID: 20371044 DOI: 10.1016/j.pcl.2010.01.012]

5 **Starzl TE**, Fung JJ. Themes of liver transplantation. *Hepatology* 2010; **51**: 1869-1884 [PMID: 20235333 DOI: 10.1002/hep.23595]

6 **Freitas MC**. Kidney transplantation in the US: an analysis of the OPTN/UNOS registry. *Clin Transpl* 2011; 1-16 [PMID: 22755397]

7 **Huang E**, Segev DL, Rabb H. Kidney transplantation in the elderly. *Semin Nephrol* 2009; **29**: 621-635 [PMID: 20006794 DOI: 10.1016/j.semnephrol.2009.07.011]

8 **Everly MJ**. Cardiac transplantation in the United States: an analysis of the UNOS registry. *Clin Transpl* 2008; 35-43 [PMID: 19708444]

9 **Kruse CD**, Helvind M, Jensen T, Gustafsson F, Mortensen SA, Andersen HO. Good long-term survival after paediatric heart transplantation. *Dan Med J* 2012; **59**: A4367 [PMID: 22239842]

10 **Thomson AW**, Bonham CA, Zeevi A. Mode of action of tacrolimus (FK506): molecular and cellular mechanisms. *Ther Drug Monit* 1995; **17**: 584-591 [PMID: 8588225]

11 **Kochel I**, Strzadała L. [FK506 - binding proteins in the regulation of transcription factors activity in T cells]. *Postepy Hig Med Dosw (Online)* 2004; **58**: 118-127 [PMID: 15077060]

12 **Zhu Y**, Wei W, Li Y. FK506 treatment in a Long-term chronic rejection rat model of small bowel transplantation. *Clin Invest Med* 2010; **33**: E168-E173 [PMID: 20519095]

13 **Bettelli E**, Carrier Y, Gao W, Korn T, Strom TB, Oukka M, Weiner HL, Kuchroo VK. Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 2006; **441**: 235-238 [PMID: 16648838 DOI: 10.1038/nature04753]

14 **Mangan PR**, Harrington LE, O'Quinn DB, Helms WS, Bullard DC, Elson CO, Hatton RD, Wahl SM, Schoeb TR, Weaver CT. Transforming growth factor-beta induces development of the T(H)17 lineage. *Nature* 2006; **441**: 231-234 [PMID: 16648837 DOI: 10.1038/nature04754]

15 **Zhou L**, Ivanov II, Spolski R, Min R, Shenderov K, Egawa T, Levy DE, Leonard WJ, Littman DR. IL-6 programs T(H)-17 cell differentiation by promoting sequential engagement of the IL-21 and IL-23 pathways. *Nat Immunol* 2007; **8**: 967-974 [PMID: 17581537 DOI: 10.1038/ni1488]

16 **Langrish CL**, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, McClanahan T, Kastelein RA, Cua DJ. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med* 2005; **201**: 233-240 [PMID: 15657292 DOI: 10.1084/jem.20041257]

17 **Park H**, Li Z, Yang XO, Chang SH, Nurieva R, Wang YH, Wang Y, Hood L, Zhu Z, Tian Q, Dong C. A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol* 2005; **6**: 1133-1141 [PMID: 16200068 DOI: 10.1038/ni1261]

18 **Wynn TA**. T(H)-17: a giant step from T(H)1 and T(H)2. *Nat Immunol* 2005; **6**: 1069-1070 [PMID: 16239919 DOI: 10.1038/ni1105-1069]

19 **Oukka M**. Th17 cells in immunity and autoimmunity. *Ann Rheum Dis* 2008; **67** Suppl 3: iii26-iii29 [PMID: 19022809 DOI: 10.1136/ard.2008.098004]

20 **Korn T**, Bettelli E, Oukka M, Kuchroo VK. IL-17 and Th17 Cells. *Annu Rev Immunol* 2009; **27**: 485-517 [PMID: 19132915 DOI: 10.1146/annurev.immunol.021908.132710]

21 **Miossec P**. Diseases that may benefit from manipulating the Th17 pathway. *Eur J Immunol* 2009; **39**: 667-669 [PMID: 19283719 DOI: 10.1002/eji.200839088]

22 **Yang J**, Li M, Zhang H, Hong L, Feng F, Pan F, Wang Y, Wang W. Application of a self-made swivel intravenous transfusion device in constructing allogenic small bowel transplantation rejection model in rats. *Transplant Proc* 2009; **41**: 4397-4400 [PMID: 20005407 DOI: 10.1016/j.transproceed.2009.09.085]

23 **Yang J**, Hong L, Wang W, Zhang H. A novel monoclonal antibody specific to mouse IL-17. *Hybridoma (Larchmt)* 2011; **30**: 287-289 [PMID: 21707364 DOI: 10.1089/hyb.2011.0005.]

24 **Wu T**, Abu-Elmagd K, Bond G, Nalesnik MA, Randhawa P, Demetris AJ. A schema for histologic grading of small intestine allograft acute rejection. *Transplantation* 2003; **75**: 1241-1248 [PMID: 12717210]

25 **Yuan X**, Paez-Cortez J, Schmitt-Knosalla I, D'Addio F, Mfarrej B, Donnarumma M, Habicht A, Clarkson MR, Iacomini J, Glimcher LH, Sayegh MH, Ansari MJ. A novel role of CD4 Th17 cells in mediating cardiac allograft rejection and vasculopathy. *J Exp Med* 2008; **205**: 3133-3144 [PMID: 19047438 DOI: 10.1084/jem.20081937]

26 **Weaver CT**, Hatton RD, Mangan PR, Harrington LE. IL-17 family cytokines and the expanding diversity of effector T cell lineages. *Annu Rev Immunol* 2007; **25**: 821-852 [PMID: 17201677 DOI: 10.1146/annurev.immunol.25.022106.141557]

27 **Vanaudenaerde BM**, Dupont LJ, Wuyts WA, Verbeken EK, Meyts I, Bullens DM, Dilissen E, Luyts L, Van Raemdonck DE, Verleden GM. The role of interleukin-17 during acute rejection after lung transplantation. *Eur Respir J* 2006; **27**: 779-787 [PMID: 16585086 DOI: 10.1183/09031936.06.00019405]

28 **Shilling RA**, Wilkes DS. Role of Th17 cells and IL-17 in lung transplant rejection. *Semin Immunopathol* 2011; **33**: 129-134 [PMID: 21279808 DOI: 10.1007/s00281-011-0257-9]

29 **Carlson MJ**, West ML, Coghill JM, Panoskaltsis-Mortari A, Blazar BR, Serody JS. In vitro-differentiated TH17 cells mediate lethal acute graft-versus-host disease with severe cutaneous and pulmonary pathologic manifestations. *Blood* 2009; **113**: 1365-1374 [PMID: 18957685 DOI: 10.1182/blood-2008-06-162420]

30 **Mauermann N**, Burian J, von Garnier C, Dirnhofer S, Germano D, Schuett C, Tamm M, Bingisser R, Eriksson U, Hunziker L. Interferon-gamma regulates idiopathic pneumonia syndrome, a Th17+CD4+ T-cell-mediated graft-versus-host disease. *Am J Respir Crit Care Med* 2008; **178**: 379-388 [PMID: 18511701 DOI: 10.1164/rccm.200711-1648OC]

31 **Chen H**, Wang W, Xie H, Xu X, Wu J, Jiang Z, Zhang M, Zhou L, Zheng S. A pathogenic role of IL- 17 at the early stage of corneal allograft rejection. *Transpl Immunol* 2009; **21**: 155-161 [PMID: 19358887 DOI: 10.1016/j.trim.2009.03.006]

32 **Tesmer LA**, Lundy SK, Sarkar S, Fox DA. Th17 cells in human disease. *Immunol Rev* 2008; **223**: 87-113 [PMID: 18613831 DOI: 10.1111/j.1600-065X.2008.00628.x]

33 **Miossec P**. IL-17 and Th17 cells in human inflammatory diseases. *Microbes Infect* 2009; **11**: 625-630 [PMID: 19371791 DOI: 10.1016/j.micinf.2009.04.003]

34 **Hsieh HG**, Loong CC, Lui WY, Chen A, Lin CY. IL-17 expression as a possible predictive parameter for subclinical renal allograft rejection. *Transpl Int* 2001; **14**: 287-298 [PMID: 11692212 DOI: 10.1111/j.1432-2277.2001.tb00062.x]

35 **Itoh S**, Nakae S, Axtell RC, Velotta JB, Kimura N, Kajiwara N, Iwakura Y, Saito H, Adachi H, Steinman L, Robbins RC, Fischbein MP. IL-17 contributes to the development of chronic rejection in a murine heart transplant model. *J Clin Immunol* 2010; **30**: 235-240 [PMID: 20130970 DOI: 10.1007/s10875-009-9366-9]

36 **Acosta-Rodriguez EV**, Napolitani G, Lanzavecchia A, Sallusto F. Interleukins 1beta and 6 but not transforming growth factor-beta are essential for the differentiation of interleukin 17-producing human T helper cells. *Nat Immunol* 2007; **8**: 942-949 [PMID: 17676045 DOI: 10.1038/ni1496]

37 **Wilson NJ**, Boniface K, Chan JR, McKenzie BS, Blumenschein WM, Mattson JD, Basham B, Smith K, Chen T, Morel F, Lecron JC, Kastelein RA, Cua DJ, McClanahan TK, Bowman EP, de Waal Malefyt R. Development, cytokine profile and function of human interleukin 17-producing helper T cells. *Nat Immunol* 2007; **8**: 950-957 [PMID: 17676044 DOI: 10.1038/ni1497]

P- Reviewers Vorobjova T, Eghtesad B **S- Editor** Gou SX
L- Editor Rutherford A **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i5.692

World J Gastroenterol 2013 February 7; 19(5): 692-705
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

ORIGINAL ARTICLE

Comparison of different methods of intestinal obstruction in a rat model

Meng-Lang Yuan, Zheng Yang, Yu-Cheng Li, Lan-Lan Shi, Jia-Ling Guo, Yu-Qin Huang, Xia Kang, Jing-Jing Cheng, Yang Chen, Ting Yu, De-Qi Cao, Huan Pang, Xiao Zhang

Meng-Lang Yuan, Yu-Cheng Li, Jia-Ling Guo, Yu-Qin Huang, Xia Kang, Jing-Jing Cheng, Huan Pang, Department of Clinical Medicine, Chengdu Medical College, Chengdu 610081, Sichuan Province, China
Zheng Yang, Xiao Zhang, Lan-Lan Shi, Department of Experimental Technology, Chengdu Medical College, Chengdu 610081, Sichuan Province, China
Yang Chen, Ting Yu, Department of Pharmaceutics, Chengdu Medical College, Chengdu 610081, Sichuan Province, China
De-Qi Cao, Department of Laboratory Medicine, Chengdu Medical College, Chengdu 610081, Sichuan Province, China
Author contributions: Yuan ML and Yang Z contributed equally to this work; Yang Z designed the study; Yuan ML, Guo JL, Huang YQ, Kang X and Cheng JJ performed the majority of experiments; Chen Y, Yu T, Li YC and Cao DQ collected and analyzed the data; Yuan ML and Yang Z wrote the manuscript; Yang Z, Shi LL, Pang H and Zhang X reviewed the paper.
Supported by Higher Education Quality Project of Sichuan Province: Innovative Scientific Experiment Project of Sichuan Province, Grant No. SJCX201110; Chengdu Medical College Innovative Scientific Experiment Project, Grant No. CX201220 and CX201115
Correspondence to: Zheng Yang, Experimentalist, Department of Experimental Technology, Chengdu Medical College, Chengdu 610081, Sichuan Province, China. yz3191021@yahoo.com.cn
Telephone: +86-28-68289174 Fax: +86-28-68289152
Received: September 25, 2012 Revised: December 24, 2012
Accepted: January 5, 2013
Published online: February 7, 2013

Abstract

AIM: To investigate different methods of creating incomplete intestinal obstruction in a rat model and to compare their electrophysiologic, morphologic and histologic characteristics.

METHODS: Rat ileum was partially obstructed by the respective application of: braided silk (penetrated the mesentery and surrounded intestine); half ligation (penetrated directly and ligated 1/2 cross-section of the intestine); wide pipe (6 mm in width, surrounded the intestine); narrow pipe (2 mm in width, surrounded the intestine). A control was also included (no obstruction). Various behavioral and electrophysiologic variables, as well as morphologic and immunohistochemical observations were recorded by blinded investigators at different time points (12, 24, 48, 72 h), including daily general condition, ileal wet weight and circumference, macromorphous and micromorphous intestine, bowel movement capability *in vivo* and *in vitro*, slow wave and neural electrical activity, and the number of c-Kit positive interstitial cells of Cajal (ICC).

RESULTS: Despite being of a similar general condition, these methods resulted in different levels of obstruction in each group compared with the control at different time points (12, 24, 48, 72 h). However, these fields of the wide pipe rat showed significantly differences when compared with the other three obstructed groups at 12 to 72 h, including macroscopic and histological presentation, intestinal transit ratio and contractility, circumference and wet weight, amplitude and frequency of nerve electrical discharge and slow wave, and ICC numbers (all $P < 0.01$).

CONCLUSION: The wide pipe rat method is significantly more reliable and stable than the other methods of obstruction, demonstrating that use of the wide pipe method can be a useful model of incomplete intestinal obstruction.

© 2013 Baishideng. All rights reserved.

Key words: Intestinal obstruction; Model; Comparative study; Electrophysiology; Morphology; Interstitial cells of Cajal

Yuan ML, Yang Z, Li YC, Shi LL, Guo JL, Huang YQ, Kang X, Cheng JJ, Chen Y, Yu T, Cao DQ, Pang H, Zhang X. Comparison of different methods of intestinal obstruction in a rat model. *World J Gastroenterol* 2013; 19(5): 692-705 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i5/692.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i5.692

INTRODUCTION

Intestinal obstruction (IO) is one of the most common diseases in abdominal surgery. It can slowly lead to changes in intestinal structure and function, and in extreme cases it can be life-threatening[1,2]. Incomplete intestinal obstruction is the initial phase of intestinal obstruction and is the clinical pathological basis for other types of intestinal obstruction. In addition, when considering the impairment associated with incomplete intestinal obstruction, exploring its diagnosis and treatment has important clinical significance.

Animal models of incomplete intestinal obstruction are important in the investigation of the pathogenic mechanisms involved in human intestinal obstruction, and play an important role in identifying and testing novel interventions. To date, animal models of incomplete intestinal obstruction have included mice, rats, rabbits, pigs and approximately six other types of animals[3-5]. No particular model parallels the complex nature of human intestinal obstruction. The drawback of using animal models is that the basis for the high rate of incomplete intestinal obstruction is not known.

Therefore, it is necessary to establish a reliable animal model to study the pathogenesis of intestinal obstruction, and to have a good experimental model to allow the exploration, research and ultimate treatment of intestinal obstruction. Different types of materials to induce obstruction are currently available. The prosthetic valve has been used since 1996[5]. This was followed by half ligation using braided silk, which was developed in 2004 and has been available since 2008. One report describes the changes elicited by ligating the intestines outside the body[4]. A 24-Fr latex T-tube, cut to form a pipe, was used in China to establish a model of intestinal obstruction[6].

With regard to species selection, the anatomy and physiology of primates are similar to humans, but due to ethical and economic limitations this model is not widely used. Whichever method or species is selected, the ideal animal model should be similar and comparable to the human disease, demonstrate repeatability, be economic and easy to use[7]. In this study, rats were selected and three different materials were used to establish intestinal obstruction.

Based on the above factors, the purpose of the present experimental study was to compare the characteristics of four methods of incomplete intestinal obstruction in the rat model. In addition, we also obtained electrophysiologic, morphologic and histologic information on each method which may have clinical relevance.

MATERIALS AND METHODS

Animals

Healthy adult female and male Sprague-Dawley rats, weighing between 180 and 280 g, were provided by the Laboratory Animal Center [Licence No. SCXK (CHUAN) 2006-10; Sichuan University, Chengdu, China], and were individually housed at 22 ℃ in a 12-h light/dark cycle, with free access to tap water and a standard pellet diet (Laboratory Animal Center, Sichuan University, Chengdu, China) for at least 1 wk before the start of the experiments.

Surgical procedure

One hundred and fifty rats were randomly divided into five groups of thirty in each group. 12 h prior to each study, the animals were fasted but had free access to water, and were anesthetized with 1.5% pentobarbital sodium (30 mg/kg body weight, intraperitoneal; Beijing Chemical Reagent, batch number, 090205; Beijing, China). In this study, a loop of intestine was exposed, and partially obstructed using the respective material. Three different materials were used to obstruct the intestine. A round needle with braided silk No. 0 (Pudong Jinhuan Medical Products, batch number, 11L02028; Shanghai, China) penetrated the mesentery, a polyethylene pipe (2 mm exterior diameter) was placed at the side of the intestine and a ligature was formed with the braided silk, the pipe was then pulled out smoothly from the ligation. This group was known as the braided silk group. Braided silk No. 3-0 (Pudong Jinhuan Medical Products, batch number, 11L02028; Shanghai, China) was used to directly penetrated the intestine and ligate 1/2 cross-section of the intestine. This group was known as the half ligation group. A 24-Fr latex T-tube (Nantong Angel Medical Instruments, batch number, 20110304; Jiangsu, China) was cut to form a pipe 10 mm in length and 6 mm in width which surrounded the intestine. This group was known as the wide pipe group. In another group of rats, a narrow pipe 10 mm in length and 2 mm in width was used to obstruct the intestine. This group was known as the narrow pipe group. The rings were located in the ileum 20 mm oral to the ileocecal sphincter, and this was followed by closure of the abdomen. All materials were placed with the cut side between the lateral extensions of the mesenteric vascular bed to avoid vascular injury[3]. The control group underwent a similar procedure, but the rings used in these animals were 10 mm in length and did not result in occlusion of the ileum.

Postoperative care

After surgery, the animals were treated with an intraperitoneal injection of a 2 mL mixture of glucose (9 mg/mL; Shuanghe Pharmaceutical Industry, batch number,

Table 1 Criteria for scoring macromorphous intestinal damage

Feature	Description	Score
Adhesions	None	0
	Slight, ileum can be separated from other tissues with little effort	1
	Involved in a number of intestinal loops	2
Stenosis	None	0
	Slight	2
	Severe, proximal intestinal distention	3
Ulceration	None	0
	Damage extended to < 1 cm along the length at one site	1
	Damage extended to < 1 cm along the length at two sites	2
	Damage extended to > 1 cm along the length at one siteor multiple lesions	3
Bowel wall thickness	< 1 mm	0
	1-3 mm	1
	> 3 mm	2
Total score		10

Table 2 Criteria for scoring micromorphous intestinal damage

Feature	Description	Score
Ulceration	None	0
	Ulceration < 3 mm, no hyperemia or focal hyperemia, no ulceration	1
	Ulceration < 3 mm, with focal hyperemia or ulceration > 3 mm, without hyperemia	2
	Ulceration > 3 mm or hyperemia	3
Inflammation	None	0
	Slight	1
	Moderate	2
	Severe	3
Fibrogenesis	None	0
	Slight	1
	Severe	2
Extent of involved bowel wall	None	0
	Submucosa	1
	Muscular layer and even serosa	2
Total score		10

100829 6K; Anhui, China) and sodium chloride (50 mg/mL; Shuanghe Pharmaceutical Industry, batch number, 100825 1E; Anhui, China) in a ratio of 1:1 (vol/vol), and 0.1 mL potassium chloride (100 mg/mL; Jiaozuo Pharmaceutical Industry, batch number, 11031641; Tianjin, China). The animals were then allowed to recover on a heated blanket. The rats were allowed to fast 2 h before the last administration, and were rapidly euthanized with pentobarbital sodium at 12, 24, 48, and 72 h, respectively. The abdomen was then cut open and the test was started.

Daily general condition

After surgery, the rats were housed in individual cages. Each day at approximately 9 AM, body weight and food intake were recorded, and feces were collected from each cage. The number of feces pellets was counted and color was observed. Feces from each rat were then lyophilized and weighed to determine fecal dry weight. The total number and dry weight of the fecal pellets were calculated. The mental status and death of rats were recorded.

Measurement of ileum circumference and wet weight

Following sacrifice of the rats, a 3 cm fresh intestinal segment was obtained, the intestinal contents were removed using Tyrode's solution, the segment was then dried with filter paper, and ileal weight was recorded. The ileal wet weight index was calculated using the fasting body weight (kg) divided by the weight (g) of the 3 cm intestinal segment. A bowel segment (10 cm) cut longitudinally along the opposite side of the mesenteric edge was obtained, and its circumference was measured at three sites, and the average was recorded.

Morphological studies

The ileum was opened by an incision along the mesenteric border and laid flat. Tissue damage was assessed and was scored on the 3 cm intestinal segment which was taken 5 cm oral to the ileus, using the criteria outlined in Tables 1 and 2 adapted from Butzner *et al*[8]. Each section was then graded by two blinded observers. Scoring was based on a number of features which were scored from 0 to 10 depending on the presence and severity of visible ileal damage, which consisted of the extent and severity of adhesions, stenosis, ulceration, and bowel wall thickness. A score of 0 to 10 was used to describe the total scores of ulceration, inflammation, fibrogenesis and extent of bowel wall involved. Finally, the extent of histological intestinal mucosal damage was evaluated according to the grading system of Chiu *et al*[9].

Hematoxylin and eosin staining

Histological analysis of intestinal damage was carried out on pieces of muscle taken the same distance from the site of occlusion adjacent to the tissue regions used for electrophysiology. The intestinal tissues in Tyrode's solution were pinned. Connective tissues were removed, cut and pinned into a rectangular shape (1 cm × 2 cm, width and length). The ileal tissues were then fixed in 4% paraformaldehyde for 6 h at room temperature (RT). Two longitudinal sections, 1 cm in length and 2 mm thick, were taken from each fixed tissue segment. These sections were rinsed for 2 h in running water, dehydrated through a graded series of alcohols and embedded in paraffin using the Intelligent Program-controlled Automatic Hydroextractor of Biological Organization (Xiaogan Taiwei Science and Technology Industry, Hubei, China) and a tissue embedding console system (Jinhua Kedi Instrumental Equipment, Zhejiang, China). All paraffin-embedded tissue blocks were sectioned at 5 μm thickness using a microtome (Microsystems Nussloch; Leica Instruments, Shanghai, China), and slides were then prepared. The paraffin was removed from the slides by treatment with xylene. Sections were rehydrated with an ethanol series and stained with hematoxylin and eosin

and examined by light microscopy.

Bowel movement capability analysis

After the rats had fasted for 4 h, all groups received 3% activated charcoal suspension (Sigma, Aldrich, United States) in saline (1.5 mL/rat, *po*) as a charcoal meal. One hour after administration of the marker, the animals were anesthetized with an intraperitoneal injection (2 mL/kg) of pentobarbital sodium, and the small intestine from the pylorus sphincter to the ileocecal sphincter was removed. The intestinal transit ratio was calculated using the percentage of the small intestine length divided by the length the charcoal had travelled.

After the animals had been killed, the small intestine was exposed by a mid-line abdominal incision, and a 3 cm tubular segment of intestine was obtained 1 cm oral to the ileus. The bowel was opened along the mesenteric border and the intestinal contents were rinsed with Tyrode's solution. Segments from a 2 cm piece of bowel were cut and the mucosa was removed by sharp dissection. Strips of muscle (20 mm × 5 mm) were pinned to the hook and contractions were measured isometrically under a resting tension of 0.5-1 g in a 20 mL tissue chamber (Chengdu Technology and Market, Sichuan, China) containing Tyrode's solution for at least 30 min. A force transducer coupled to the BL-420F Biological Function Experimental System (Chengdu Technology and Market, Sichuan, China) was used to record the contractility of the ileum. The settings for the tension pattern was: scanning speed, 2.56 s/div, power gain, 2000, time constant, 5 s, and high-frequency filtering, 3 Hz. The sustained record time was up to 30 min, followed by 30 s of recorded data. Frequency and amplitude were recorded and calculated.

Measurement of neural electrical activity

Along the central line of the neck, a longitudinal cut open to the skin, layer by layer separating the muscles, fascia, and exposing the carotid sheath was made. After separating the vagus nerve, the nerve was immersed in 37 ℃ warm paraffin (Jiangcheng Lipid Chemical Industry, batch number, 100101; Jilin, China) and insulated with the surrounding tissue to prevent the nerve drying during recording. The recording electrodes were hooked to the vagus nerve, the reference electrode was placed on the skin fold, and the electrode input was connected to the BL-420F Biological Function Experimental System. The settings for the neural discharge pattern were: scanning speed, 80 ms/div, power gain, 10 000, time constant, 0.1 s, and high-frequency filtering, 1 KHz. As mentioned above, both frequency and amplitude were recorded.

Determination of slow wave

Ileal slow waves were measured using electrophysiology. A pair of self-made silver electrodes (5 mm in length, 0.1 mm in diameter) was implanted into the seromuscular layer of the intestine. The distance between the electrodes was about 5 mm and they were 10-20 mm away from the ring. The reference electrode was placed on the skin fold, the terminal was wired and connected to the BL-420F Biological Function Experimental System. A layer of paraffin gauze was used to cover the exposed ileum. The settings for the slow electrical signals pattern were: scanning speed, 1.28 s/div, power gain, 2000, time constant, 5 s, and high frequency filtering, 30 Hz. The sustained record time was up to 30 min, followed by 30 s of recorded data. Ten periods were then randomly intercepted using the modified Tomita method[10], and both frequency and amplitude were recorded, calculated and compared (as described earlier).

Immunohistochemistry

The expression of c-Kit in ileal tissue was assessed by immunohistochemistry. Specimens were fixed in 4% paraformaldehyde, dehydrated, and embedded in paraffin, as described previously. A mixed liquid suspension containing 5 mL garlic juice and fresh egg white was added to 100 mL glycerol, which was then used to coat a glass slide. The tissues were then deparaffinized in xylene and rehydrated in a descending ethanol series. After dewaxing and rehydration, antigen retrieval was performed using high pressure for 2 min, and then blocked by incubation in 3% H_2O_2 for 15 min at 37 ℃ to ablate endogenous peroxidase. The sections were washed in phosphate-buffered saline (PBS; 0.01 mol/L, pH 7.4), and then incubated with goat anti-c-Kit specific polyclonal antibody (sc-1494, 1:100, Santa Cruz, Biotechnology, United States) for 1 h at 37 ℃ in a wormwall incubator (Huyueming Instrument, Guangzhou, China). The slides were washed in PBS (5 × 3 min), and then incubated with a horseradish-peroxidase-conjugated rabbit anti-goat IgG(H+L) (Zhongshan Golden Bridge Biotechnology, Beijing, China) for 40 min at 37 ℃, rinsed (5 × 3 min) in PBS, and the sections were colored with 3,3-diaminobenzidine, kept at RT in the dark for 30 s. Following that, the slides were counterstained with hematoxylin, differentiated with 5 g/L hydrochloric ethanol, 1:400 ammonia recurrented blue, sequential ethanol dehydration, and then clearing and mounting with neutral resin. In the negative control the same steps as previously described were carried out, but the primary antibody was replaced by PBS. The positive expression of c-Kit in interstitial cells of Cajal (ICC) was shown by dotted dark brown cells with a nucleus or individual processes of dark brown rods.

Statistical analysis

All data were presented as means ± SE. P values < 0.01 were considered statistically significant. Each intestinal segment was taken from individual animals. The electrical parameters of each segment were analyzed: (1) frequency; (2) amplitude; and (3) contraction capability (contraction frequency multiplied by amplitude). A one-way ANOVA

test was used to compare parameters and the Kruskal Wallis test was used to compare parameters displaying abnormal distribution among the groups. Tukey's Honestly Significant Difference (HSD) test was used to detect the group causing variation when comparing qualitative data, and the Mann Whitney *U* test was used to detect the group causing variation. χ^2 or Fisher's exact test was used to compare qualitative data. All analyses were performed using SPSS 13.0 for Windows (SPSS Inc, United States). Adobe Photoshop 8.0 (Adobe, Mountain View, CA, United States) was used to construct and display the figures made from digital photos.

RESULTS

Clinical

Body weight, food intake, feces, mental status as well as mortality were recorded every 12 h. Of the average values in the control group, body weight, food intake, feces, mental status and death showed a wider band change from 12 to 24 h, this index showed a narrower band change from 24 to 72 h. Mean body weight, food intake, feces, mental status and death in the four different obstructed groups showed significant changes with persistent loss or gain ($P < 0.01$). The general condition of the rats is shown in Table 3.

Macroscopic and histological evaluation

The rings were placed on the ileum 20 mm oral to the ileocecal sphincter, which resulted in visible intestinal damage 12 to 72 h after surgery. In the control rats, no marked gross morphological changes in the intestine were observed (Figure 1A). However, morphological observation of the intestines revealed adhesions, stenosis, ulceration and bowel wall thickness of the rings to varying degrees in the rats with obstruction. In addition, flushing, tumefaction of intestinal mucosa, hyperemia and hemorrhage of bowel wall and stasis of bowel contents from the proximal region to the site of the ring were noted (Figure 1B-E). Figure 1F shows analytical data of the total scores of intestinal damage following macroscopic evaluation in obstructed and control rats.

The histological findings in ileal tissues are presented in Figure 2. No marked changes were observed in the control rats (Figure 2A). Histological observation of the intestines showed some features (including ulceration, inflammation, fibrogenesis and extent of involved bowel wall) of obstruction to varying degrees. As shown in Figure 2B-E, ulceration in the mucosa and submucosa, mucosal edema and inflammation, vascular congestion with focal hemorrhage from the mucosa to the muscular layer and even the serosa, occasional fibrogenesis, and infiltration of polymorphonuclear cells, plasma cells and neutrophils were observed. Figure 2F shows analytical data of the total scores of intestinal damage following histological evaluation and the scores of histological mucosal damage in obstructed and control rats. In the obstructed intestine, there was a marked increase in the thickness of the muscle layers. However, no apparent inflammatory changes, such as marked leucocyte infiltration of the tunica muscularis, were observed.

Circumference and wet weight of the intestine

After removing the intestine, 3 cm dilated intestinal segments were blotted with filter paper and weighed on an analytical balance. The sections were cut longitudinally and the circumference was measured at three sites. The average values for the ileal wet weight (Figure 3A) and ileal circumference (Figure 3B) in the dilated portion of the obstruction rat tissue were significantly larger ($P < 0.01$) than those in the corresponding portion of the controls.

Ileal contractility

We examined the contractile response of the obstructed and control rats, and the study was evaluated in terms of absolute force (mN per mg tissue wet weight). We also examined spontaneous contractile activity of the intestinal segments using isometric force measurements. Figure 4A-B shows the typical spontaneous contractile activity of the dilated intestine isolated from obstructed and control rats, which demonstrated that the slow form of the intestinal segments from control rats was regular; in contrast, the slow form of the intestinal segments from the obstructed rats at 12 to 72 h was attenuated and irregular to varying degrees. Analytical data of the intestinal transit ratio were obtained at four different time points and are shown in Figure 4C.

Autonomic nerve electrical discharge

Typical autonomic electrical activity of the vagus nerve was determined in obstructed and control rats *in vivo*, which indicated that the slow form of the vagus nerve autonomic electrical discharge in control rats was normal (Figure 5A); however, the slow form in obstructed rats at 12 to 72 h was attenuated and irregular to varying degrees (Figure 5B-C). At various time points, the obstructed group showed significant differences in nerve electrical discharge frequency and amplitude compared with the control group ($P < 0.01$). When the four groups were compared, the differences in the wide pipe group appeared to be most obvious, followed by the braided silk group, the half ligation and the narrow pipe group.

Ileum myoelectricity

The myoelectric changes in the ileum of each rat were determined (Figure 6A). The intestinal segments from the control rats did not show significant differences at the various time points, and the slow form was regular. In contrast, the obstructed rats showed significant reductions in vagus nerve electrical discharge frequency and amplitude ($P < 0.01$), the reduction in the wide pipe group was most obvious, followed by the braided silk group, the half ligation and the narrow pipe group. The

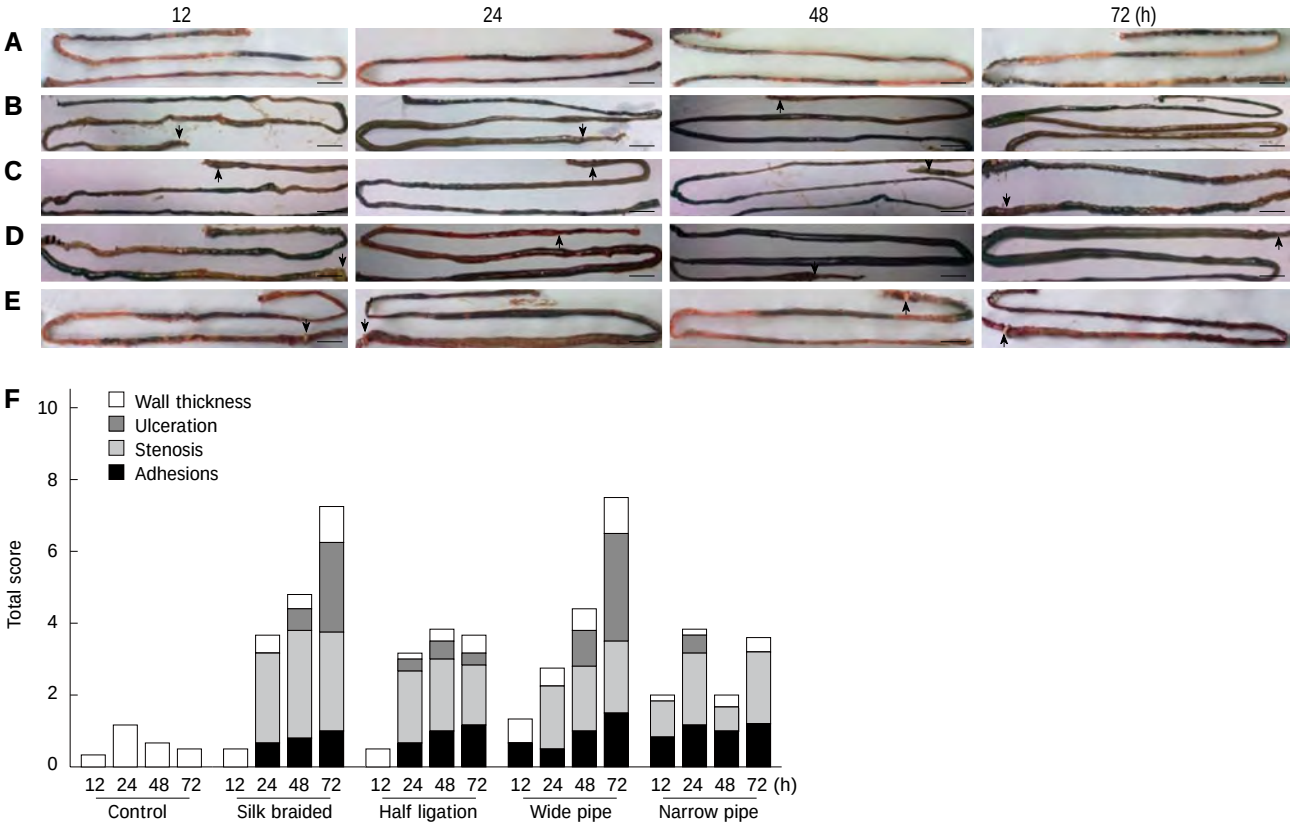


Figure 1 Dissection of the entire intestine from the pyloric antrum to the ileocecal valve in the obstructed and control rats. A: Control; B: Braided silk; C: Half ligation; D: Wide pipe; E: Narrow pipe. Rats were killed at different time points after surgery; F: The morphological score of changes in the extent and severity of adhesions, stenosis, ulceration and bowel wall thickness. The arrow indicates the portion of obstructed intestine bearing a ring (scale bar: 1 cm).

waveform of the dilated intestinal segment at 12 to 72 h was irregular to varying degrees and the appearance of a notching wave resembled a sine curve. The amplitude of the slow waves in the different groups was compared and is shown in Figure 6C. Amplitude and frequency values of the slow waves were significantly different in the obstructed group compared with the control group (both $P < 0.01$). Slow-wave amplitude was significantly reduced ($P < 0.01$) at all sites tested after surgery compared with the control rats. Frequency was reduced only at 72 h at the obstructed sites (Figure 6B).

ICC networks in the intestine

ICC networks are widely distributed within the submucosal (ICC-SM), intramuscular (ICC-IM, ICC-DMP) and inter-muscular layers (ICC-MY)[11,12]. Interestingly, the ICC-MY play an important role in the myenteric plexus and are characteristic of intestinal ICC. The control rats exhibited a dense network in the ICC-MY region of intestinal tissue from 12 to 72 h (Figure 7A), whereas the ICC-MY appeared to be greatly disrupted or absent in the obstructed rat tissue (Figure 7B-E). Analysis of c-Kit-like immunopositive cells in the obstructed rats showed that the immunopositive cells were significantly decreased compared with control animals.

DISCUSSION

An animal model is a reliable method of studying disease mechanisms and to test the effectiveness of a variety of therapeutic measures. Although the pathogenesis of intestinal obstruction is known, complete simulation of the pathology to establish an animal model of incomplete intestinal obstruction is difficult. In recent years, a number of studies have been published, such as that conducted by Chang *et al*[3], where the authors established incomplete intestinal obstruction in a mouse model by placing a polyethylene clip onto the intestine. The morphological and electrophysiological changes which occurred proximal to the site of the clip were investigated. Won *et al*[13] used a silicon tube to partially obstruct the ileum, and observed changes in intestinal contractility associated with the immunologically activated components.

In the present study, all rings were successfully placed to produce incomplete obstruction of the ileum. 12 to 72 h after obstruction, daily behavior did not significantly change in the control group, however, different daily behaviors were seen in the rats with intestinal obstruction (Table 3). In the obstructed rats, a significant reduction in body weight was observed ($P < 0.01$), which was most obvious in the narrow pipe group followed by the

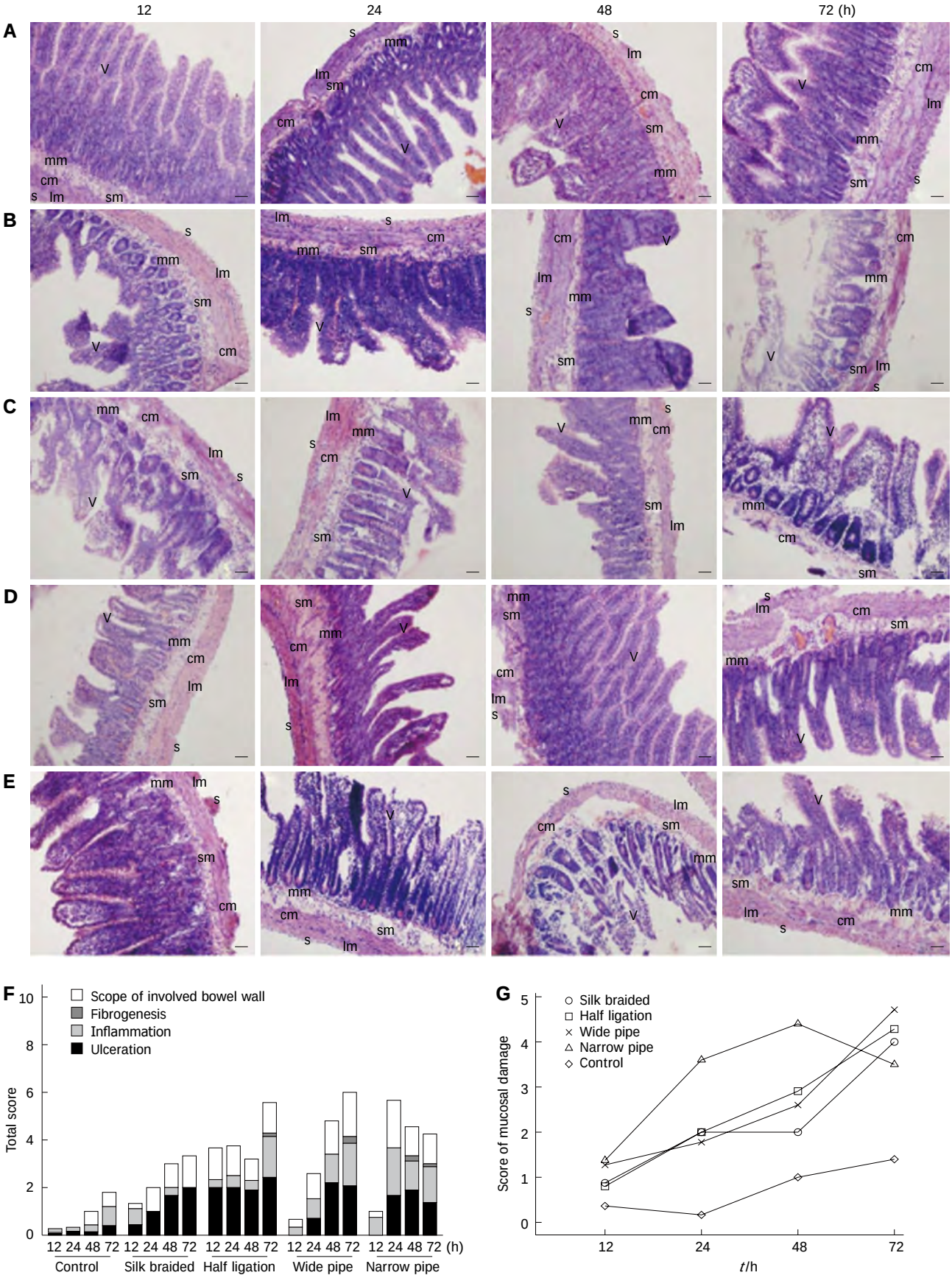


Figure 2 Cross-section of the ileum in obstructed and control rat tissues stained with hematoxylin-eosin. A: Control; B: Braided silk; C: Half ligation; D: Wide pipe; E: Narrow pipe. Rats were killed at different time points after surgery; F: The histopathological score of changes in the extent and severity of ulceration, inflammation, fibrogenesis and scope of involved bowel wall; G: Analytical data of the total scores of intestinal damage following histological mucosal damage in obstructed and control rats. (scale bar: 20 μm).

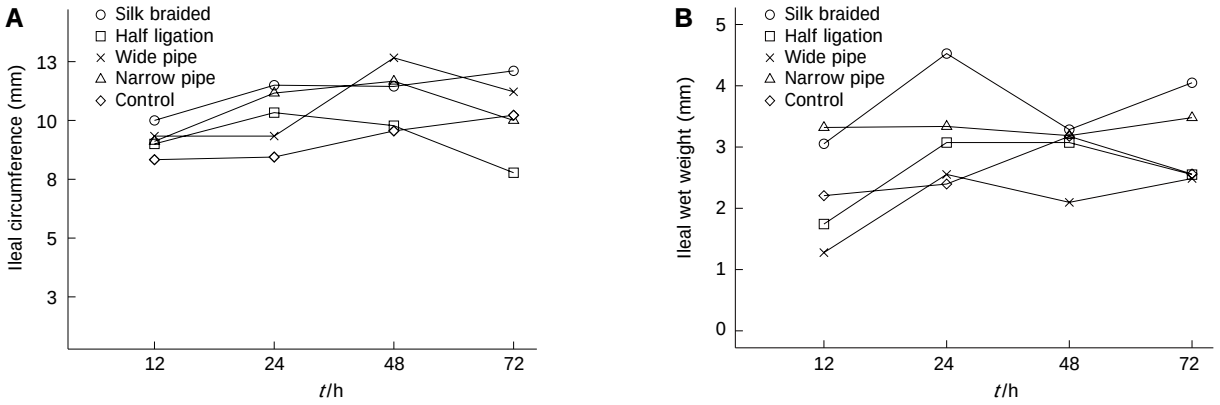


Figure 3 Average ileal wet weight and circumference in the obstructed and control rats. A: The average values for the ileal circumference in the dilated portion of the obstruction rat tissue were significantly larger than those in the corresponding portion of the controls ($P < 0.01$); B: In the obstructed intestine, there was a marked increase in the wet weight due to thickening of the muscle layers. When the groups were compared, these increases were most obvious in the braided silk group, followed by the wide pipe, the narrow pipe group and the half ligation group.

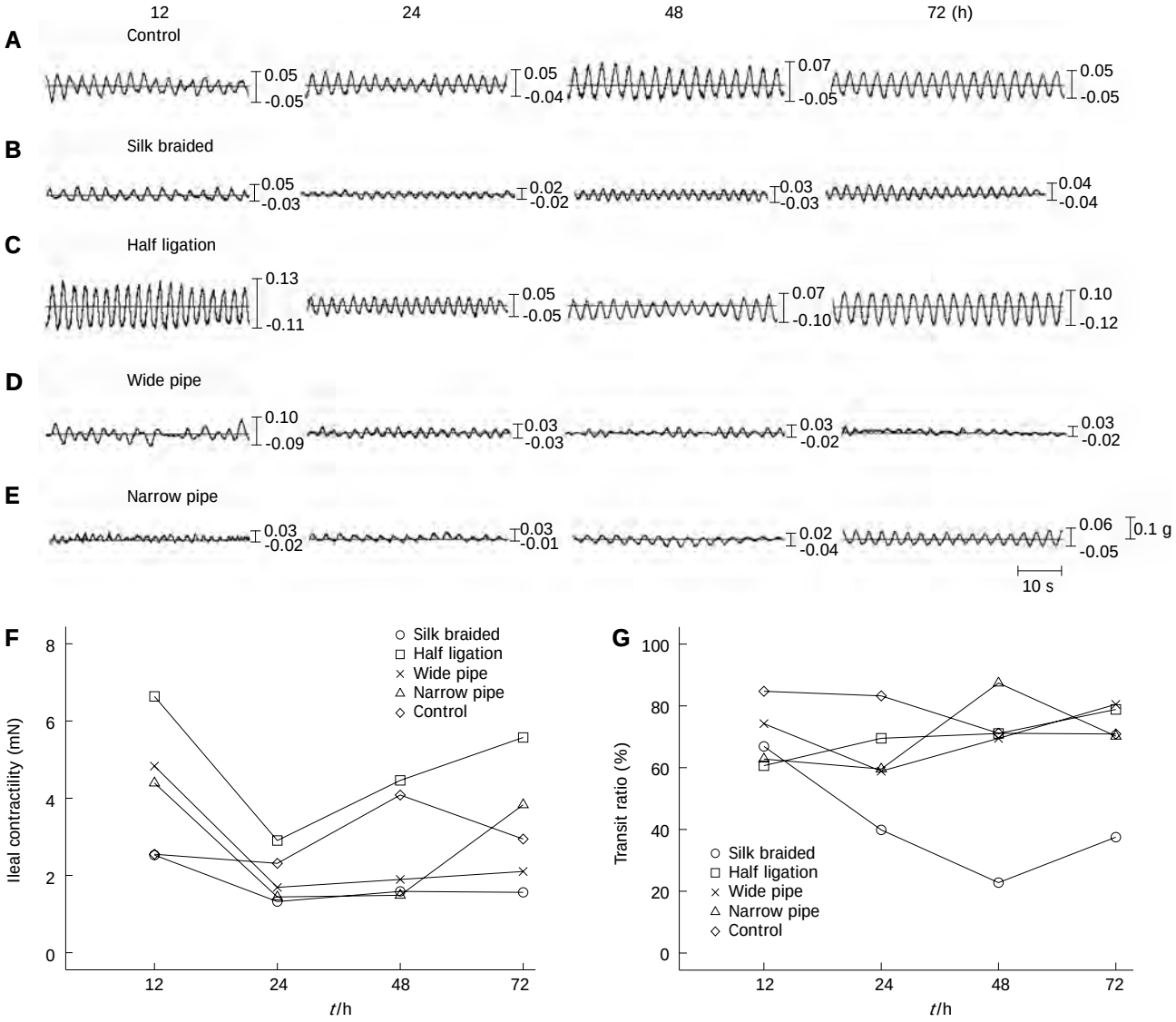


Figure 4 Changes in spontaneous rhythmic contractions. Original traces of rhythmic contractions in obstructed and control ileum. A: Control; B: Braided silk; C: Half ligation; D: Wide pipe; E: Narrow pipe. The data illustrate typical traces of spontaneous contractions at different time points; F: Data showing ileal contractility of spontaneous rhythmic motility; G: Data of intestinal transit ratio showing movement capability *in vivo*.

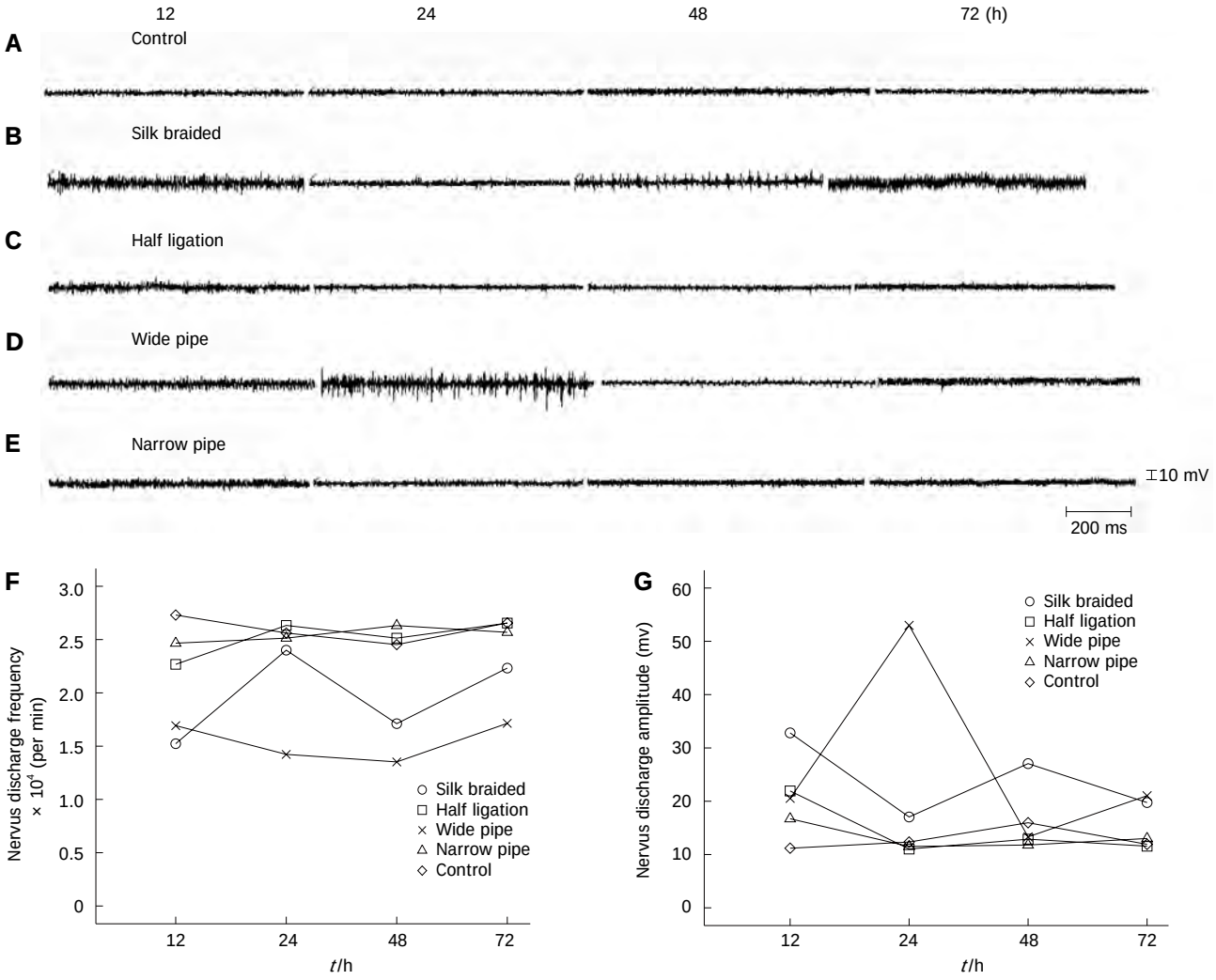


Figure 5 Changes in autonomic nerve electrical discharge. Original traces of the vagus nerve in the obstructed and control ileum. A: Control; B: Braided silk; C: Half ligation; D: Wide pipe; E: Narrow pipe. The data illustrate typical traces of autonomic electrical discharge at different time points; F: Data of frequency of vagus electrical discharge showing autonomic nerve electrical activity; G: Data of amplitude of vagus electrical discharge showing autonomic nerve electrical activity.

half ligation group, the braided silk group and then the wide pipe group which showed a slight decrease at 12 h, but gradually increased from 24 to 72 h. With regard to food intake and feces, these increased in the narrow pipe and half ligation groups compared with the controls. In contrast, the braided silk and wide pipe groups showed significant changes with varying degrees of weight loss or gain ($P < 0.01$). The mental status of the controls, narrow pipe and half ligation groups was good or moderate, however, the braided silk and wide pipe groups displayed the opposite status. Although not statistically significant, the number of deaths was lowest in the control rats. Deaths due to obstruction were higher in the controls, followed by the narrow pipe group the braided silk group, the half ligation group and the wide pipe group. These findings show that the braided silk and wide pipe established the incomplete intestinal obstruction model more successfully, with fewer deaths, than the other models.

Consistent with the findings in other reports[3-6,13-15], the changes in the extent and severity of adhesions, stenosis, ulceration and bowel wall thickness in the obstructed and control rats were assessed using the total scores of intestinal damage following macroscopic evaluation (Figure 1F). The controls (Figure 1A) showed slight intestinal wall thickness and no evidence of intestinal damage. In contrast, obstructed rats showed flushing, tumefaction of intestinal mucosa, hyperemia and hemorrhage of bowel wall and stasis of bowel contents to varying degrees from the proximal region to the site of the ring compared with control rats (Figure 1B-E). Statistically significant differences in the scores of intestinal damage were observed in obstructed rats compared with the controls ($P < 0.01$). Intestinal damage due to the braided silk and narrow pipe was serious as time passed, especially stenosis and ulceration. The half ligation and wide pipe groups showed medium damage at 12 to 72 h.

Some studies reported that intestinal wet weight and circumference were able to reflect the degree of dilation and increased weight of the intestine[15-16], therefore, we

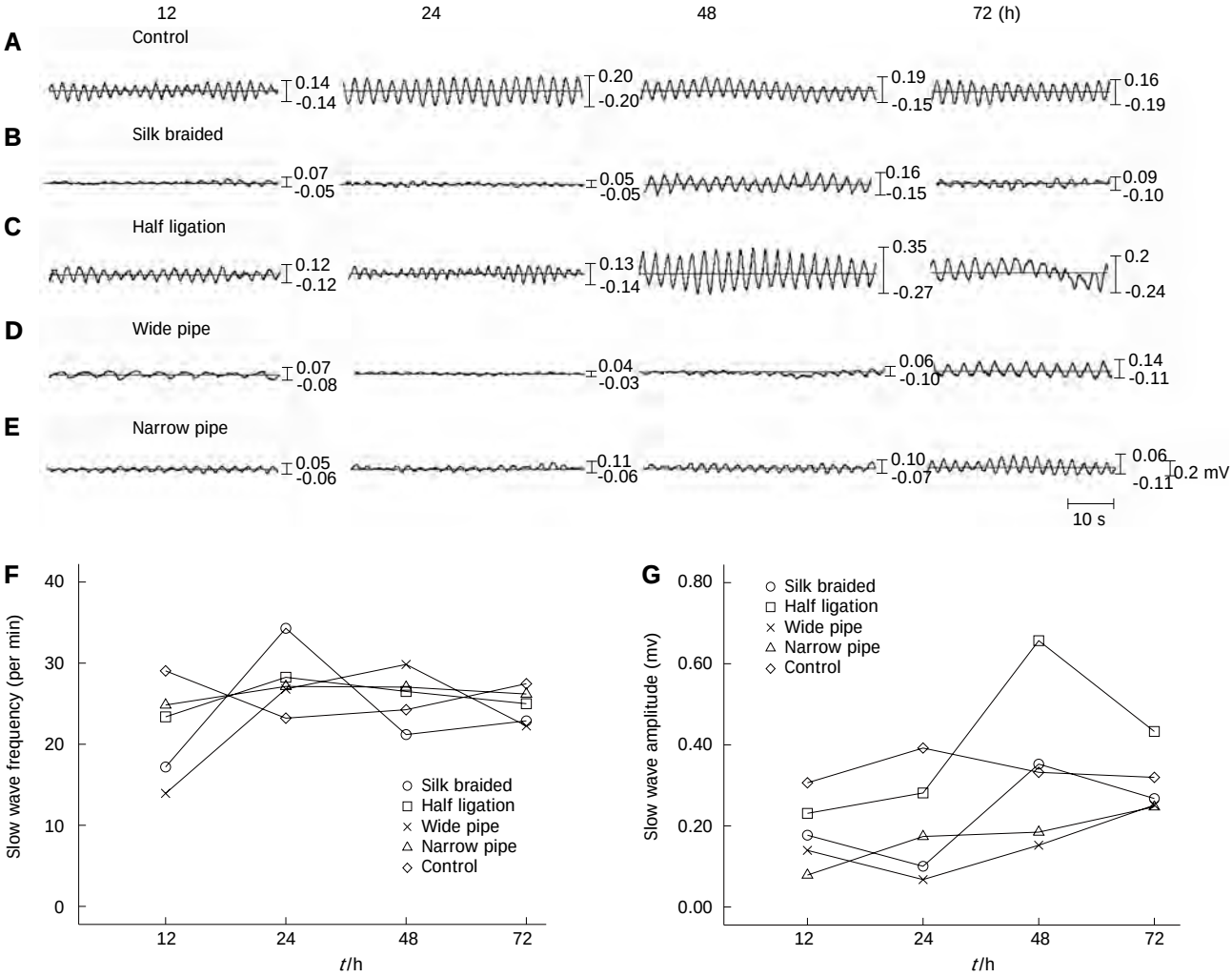


Figure 6 Change in pacemaker activity in the ileum. Typical intracellular electrical activity (slow waves) recorded in the small intestine of control rats and at 12-72 h at the site of intestinal obstruction. A: Control; B: Braided silk; C: Half ligation; D: Wide pipe; E: Narrow pipe; F: Data on slow wave frequency in normal functioning tissue away from the obstruction site; G: Slow-wave amplitude was markedly reduced near the site of obstruction and increased in amplitude in normal functioning tissue away from the obstruction site.

measured the wet weight and circumference of ileum 3 cm distal to the obstruction. In the *in vivo* study, ileal wet weights of the braided silk tissue in the dilated portion were significantly greater ($P < 0.01$) when compared with the controls (Figure 3B). The magnitude of this increase was reduced in the narrow pipe group. In contrast, other obstructed rats showed a decrease compared with those in the corresponding portion of the controls. The ileal circumference showed a negligible increase in both the obstructed and control groups (Figure 3A).

In accordance with the above findings, we speculated that the braided silk and wide pipe models would be useful in the clinic. Therefore, we evaluated the photomicrographs of tissues stained with hematoxylin and eosin (Figure 2), and found that all rats with intestinal obstruction showed ulceration of the mucosa and submucosa, and vascular congestion with focal hemorrhage in the entire tissue layer. In addition, mucosal edema and inflammation were found in the wide pipe and narrow pipe groups, and occasional fibrogenesis in the half ligation, wide pipe and narrow pipe groups at later time periods. Compared with the control rats (Figure 2A), the dilated intestinal region of obstructed rats (Figure 2B-E) showed infiltration of polymorphonuclear cells, plasma cells and neutrophils. A comparison of these scores in the obstructed rats showed that they were significantly different from each other ($P < 0.01$), and the scores were increased in obstructed rats compared with control rats (Figure 2F). Therefore, the braided silk and wide pipe models may better simulate intestinal obstruction found in the clinic.

In the *in vitro* experiments, the braided silk and wide pipe groups demonstrated reduced ileal contractility compared with the controls, the narrow pipe group demonstrated a moderate reduction, and the half ligation group demonstrated an increase in ileal contractility (Figure 4A-B). We observed that the basic spontaneous mechanical contraction activities of dilated intestine *in vivo* and *in vitro* were significantly reduced ($P < 0.01$). The transit ratio in obstructed rats was less than that in con-

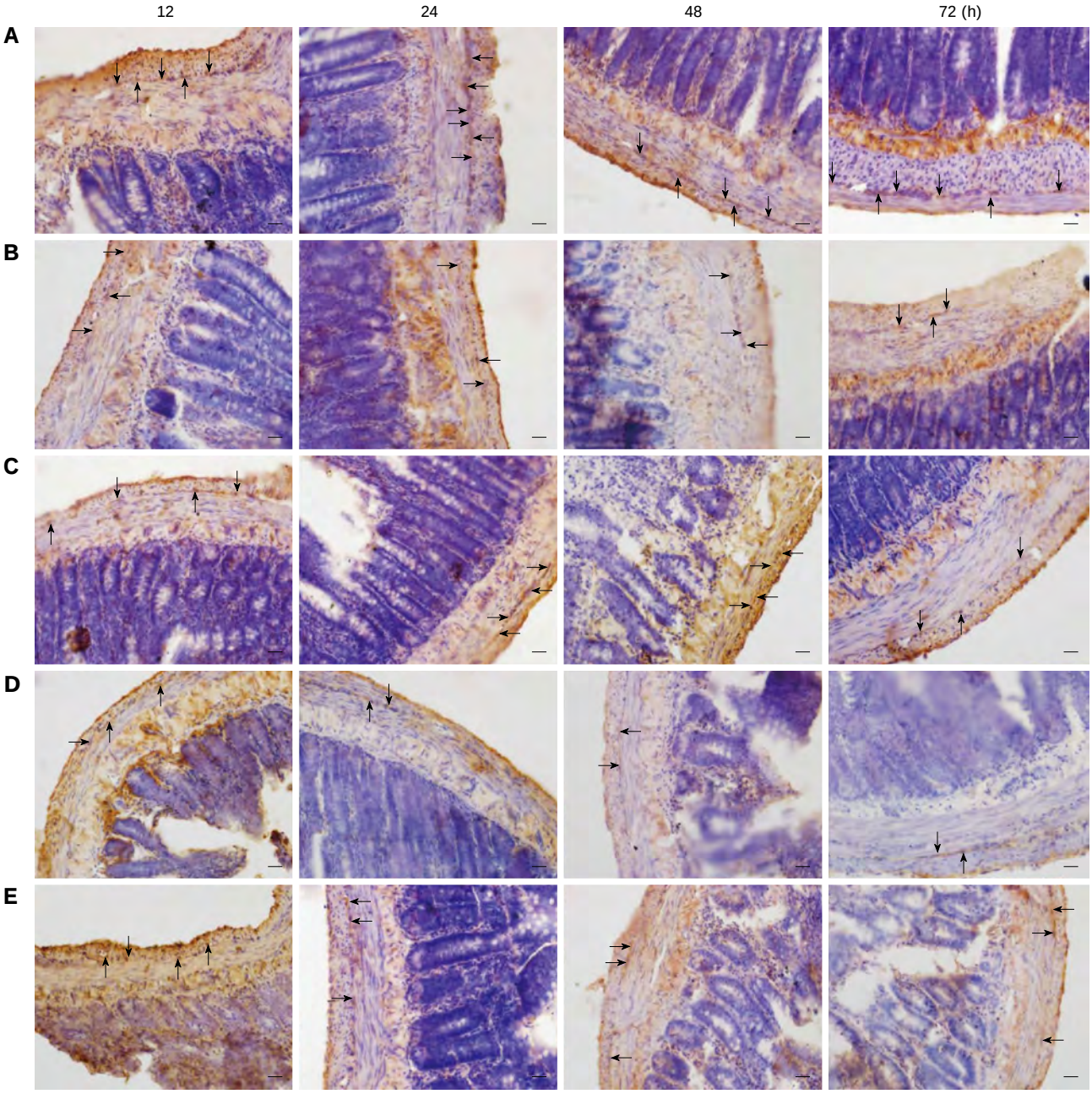


Figure 7 Immunohistochemistry of c-Kit positive interstitial cells of Cajal (arrow) in whole mount preparations of ileum in the obstructed and control rats. A: Control; B: Braided silk; C: Half ligation; D: Wide pipe; E: Narrow pipe. Rats were killed at different time points after surgery. Each panel shows c-Kit positive interstitial cells of Cajal at different times (scale bar: 20 μm).

trols, especially in the braided silk group, and the other obstructed groups showed a smaller reduction in the transit ratio. In contrast to the *in vitro* approach, these *in vivo* studies showed that the incomplete intestinal obstruction models using braided silk and wide pipe were better than the other models, particularly with regard to influence on intestinal movement capability (Figure 4C). These findings are similar to those of Huizinga *et al*[17] who demonstrated that there was a close relationship between intestinal transit and myogenic mechanisms, and indicated that electrical and mechanical activities can influence each other.

Cheng *et al*[18] reported that intestinal motility is an autonomic rhythmic activity under normal circumstances, which is regulated by nerves, hormones and other factors. The autonomic nervous system plays an important role in the regulation of intestinal motility rhythm. External innervation of the intestinal tract is mainly by the vagus nerve, and the sympathetic nerves play a role in balance and coordination[19,20]. In the present study, we compared nerve electrophysiological activity in different models of intestinal obstruction in rats (Figure 5A), to determine the vagus nerve autonomic electrical discharge which represents changes in the complex enteric nervous system. The frequency and amplitude of vagus nerve autonomic electrical discharge in control rats were

Table 3 Summary of daily general condition

General condition	Control				Braided silk				Half ligation				Wide pipe				Narrow pipe			
	12 h	24 h	48 h	72 h	12 h	24 h	48 h	72 h	12 h	24 h	48 h	72 h	12 h	24 h	48 h	72 h	12 h	24 h	48 h	72 h
Body weight (E = 10)	--	→	-		-	→	+		-				-	→	+ +		--			
Food intake (B = 5.0, E = 2.5)	±	→	+		±	→	--		+	→	±		+ +	→	±		-			
Feces																				
Dry weight (B = 1.0, E = 0.50)	-	→	+ +		--				-	→	+ +		-	→	--		-	→	+	
Color	Yellow and black → Yellow				Yellow and black → Black				Yellow and black				Yellow and black				Yellow and black → Yellow			
Pill (B =15, E =10)	±	→	+		--	→	-		±	→	+ +		-	→	--		-	→	+	
Score (B = 20, E = 10)	-	→	+		--	→	-		--	→	+ +		--				+	→	+ +	
Death	+	±	±	±	+	±	++	+	++	±	±	+	+	±	±	+	±	±	++	++

B: Base value; E: Extent of increase and reduction; + +: Wider band increase (> E); +: Narrower band increase (< E); ±: No increase or reduction; -: Narrower band reduction (< E); --: Wider band reduction (> E). The values of the score are equal to pill multiplied by color. The color is defined by Yellow = 1, Yellow and black = 1.5, Black = 2.

normal and rhythmic. Those in the wide pipe group were irregular, moderate in the braided silk group, and were normal at each time point in the half ligation and narrow pipe groups (Figure 5B-C). These results demonstrated that the wide pipe can be used successfully to establish a model of incomplete intestinal obstruction in rats.

Considering the possible link between spontaneous mechanical contraction activities and the slow wave of dilated intestine *in vivo* and *in vitro*[17,21], we investigated the waveform and reflected frequency and amplitude of the slow wave in different rats (Figure 6A). We found that the waveform in the wide pipe group resembled a sine curve and showed a notching wave which was irregular at 12 to 72 h. The half ligation group showed no obvious change in the waveform compared with the controls. Peak amplitudes in the braided silk and narrow pipe groups demonstrated different degrees of weakening. In this study, we also found a close relationship between frequency (Figure 6B) and peak amplitudes (Figure 6C) in the vagus nerve electrical discharge at baseline and intestinal motility. This suggested that the wide pipe model may be similar to human incomplete intestinal obstruction.

It is well known that slow waves are relatively regular periodic electrical activities and the basis of intestinal smooth muscle action potentials. In earlier research, we found that the frequency and amplitude of slow wave propagation were reduced in ileac muscles[6]. Other studies[3,22] showed that myoelectrical rhythm is irregular and the slow waves decrease. In this study, the waveform and reflected frequency and amplitude of the slow wave in control rats were normal. Those in the wide pipe group were irregular, were moderate in the braided silk group, and were normal in the half ligation and narrow pipe groups at 12 to 72 h (Figure 6). These results suggest that slow wave abnormalities are responsible for intestinal motility dysfunction.

In our previous intestinal studies, we observed that the enteric nervous system (ENS) - ICC - smooth muscle cell (SMC) network constitute the basic functional unit of gastrointestinal motility and has an important relationship with spontaneous rhythmic activity[12,14,17-18,23]. We supposed that the wide pipe group abolished the propulsive peristaltic waves in the dilated intestine compared with the other obstructed groups, which caused a significant disturbance in spontaneous rhythmic contractions of intestinal smooth muscle. In smooth muscle, there are several factors which increase the frequency of contraction, such as increased pacemaker activity and shortened action potential[24].

It has been previously shown that ICC are regarded as the pacemaker cells of the gastrointestinal (GI) tract[25-28], which suggests that ICC may play a role in GI motility. In order to compare the differences in obstructed rats, and to identify the mutual influence between the ENS-ICC-SMC networks, we investigated the distribution of ICC in intestinal tissue using light microscopy.

Another interesting observation from this study was that, in the wide pipe group the increase in intestinal contractility was closely related to a reduction in the number of ICC and slow wave, which was different in the other obstructed groups. In addition, it was reported that a reduction in the frequency of rhythmic contractions was connected to the disruption of ICC in the dilated intestine of obstructed rats[29]. These findings confirmed those in our study (Figure 7). Therefore, it is likely that ICC lost their original cell phenotype, resulting in an increase in the number of smooth muscle cell-like cells, thereby improving contractility of the ileum. At the present time, we do not know the precise mechanisms underlying such alterations. Further study will be needed to clarify this point.

In conclusion, the results of the present study suggest that there are significant differences *in vivo* and *in vitro* among the four animal models of incomplete intestinal obstruction. The pathogenic mechanism and clinical features in the different models have essential distinctions. Despite being of a similar general condition, the rats in each group showed differences including macroscopic and histological presentation, intestinal transit ratio and contractility, circumference and wet

weight, amplitude and frequency of nerve electrical discharge and slow wave, and ICC numbers. These findings may allow use of the wide pipe to establish a model that is approximate to human incomplete intestinal obstruction. However, a detailed analysis of this is necessary based on the characteristics of the different models used in this study which can be used as a reference.

ACKNOWLEDGMENTS

We greatly thank Zhang Xiao of our department for their technical assistance with the present study.

COMMENTS

Background

Considering the impairment associated with incomplete intestinal obstruction, the use of animal models is now a focus in this research field, however, to date, no particular model parallels the complex nature of human intestinal obstruction.

Research frontiers

Animal have been used to establish incomplete intestinal obstruction models. However, animal models are not been widely used and research comparing the characteristics in different rat models is rare. In this study, the authors successfully established a reliable animal model and demonstrated that the wide pipe rat model is similar to human incomplete intestinal obstruction.

Innovations and breakthroughs

Recent reports have highlighted the need to establish a reliable animal model of intestinal obstruction. Different types of materials are currently available. This is the first experimental study to compare rat models of incomplete intestinal obstruction. Furthermore, the authors obtained electrophysiologic, morphologic and histologic information which may have some clinical relevance.

Applications

These findings may provide a valuable theoretical basis for selecting incomplete intestinal obstruction models with better feasibility and reproducibility.

Peer review

This is an animal model study which comprehensively demonstrates the results. Overall, it is suitable for publication.

REFERENCES

1 **Kulaylat MN**, Doerr RJ. Small bowel obstruction. In: Holzheimer RG, Mannick JA. Surgical Treatment: Evidence-Based and Problem-Oriented. Munich: Zuckschwerdt, 2001, cited 2012-05-21. Available from: URL: http//www.ncbi.nlm.nih.gov/ books/NBK6873/.

2 **Kibayashi K**, Sumida T, Shojo H, Tokunaga O. Unexpected death due to intestinal obstruction by a duplication cyst in an infant. *Forensic Sci Int* 2007; **173**: 175-177 [PMID: 17236734 DOI: 10.1016/j.forsciint.2006.12.010]

3 **Chang IY**, Glasgow NJ, Takayama I, Horiguchi K, Sanders KM, Ward SM. Loss of interstitial cells of Cajal and development of electrical dysfunction in murine small bowel obstruction. *J Physiol* 2001; **536**: 555-568 [PMID: 11600689 DOI: 10.1111/j.1469-7793.2001.0555c.xd]

4 **Yang S**, Shen L, Jin Y, Liu J, Gao J, Li D. Effect of Dachengqi decoction on NF-kappaB p65 expression in lung of rats with partial intestinal obstruction and the underlying mechanism. *J Huazhong Univ Sci Technolog Med Sci* 2010; **30**: 217-221 [PMID: 20407877]

5 **Collins J**, Vicente Y, Georgeson K, Kelly D. Partial intestinal obstruction induces substantial mucosal proliferation in the pig. *J Pediatr Surg* 1996; **31**: 415-419 [PMID: 8708915 DOI: 10.1016/S0022-3468(96)90750-2]

6 **Yuan ML**, Yang Z, Qiu YB, Guo JL, Huang YQ, Kang X, Cheng JJ, Xi L, Zhou Y, Zhang Y, Chen Y, Gong D, Zhang X. Effects of Raphanus Sativus Extract on Intestine Slow Waves of Incomplete Intestinal Obstruction in Rats. *Lishizhen Med and Materia Med Res* 2012; **23**: 952-954

7 **Institute for Laboratory Animal Research**. Guide for the care and use of laboratory animals. 8th ed. Washington DC: National Academies Press, 2011: 168-173

8 **Butzner JD**, Parmar R, Bell CJ, Dalal V. Butyrate enema therapy stimulates mucosal repair in experimental colitis in the rat. *Gut* 1996; **38**: 568-573 [PMID: 8707089 DOI: 10.1136/gut.38.4.568]

9 **Chiu CJ**, McArdle AH, Brown R, Scott HJ, Gurd FN. Intestinal mucosal lesion in low-flow states. I. A morphological, hemodynamic, and metabolic reappraisal. *Arch Surg* 1970; **101**: 478-483 [PMID: 5457245 DOI: 10.1001/archsurg.1970.01340280030009]

10 **Tomita R**. Regulation of the peptidergic nerves (substance P and vasoactive intestinal peptide) in the colon of women patients with slow transit constipation: an in vitro study. *Hepatogastroenterology* 2008; **55**: 500-507 [PMID: 18613396]

11 **Sanders KM**, Ordög T, Koh SD, Torihashi S, Ward SM. Development and plasticity of interstitial cells of Cajal. *Neurogastroenterol Motil* 1999; **11**: 311-338 [PMID: 10520164 DOI: 10.1046/j.1365-2982.1999.00164.x]

12 **Takaki M**. Gut pacemaker cells: the interstitial cells of Cajal (ICC). *J Smooth Muscle Res* 2003; **39**: 137-161 [PMID: 14695026 DOI: 10.1540/jsmr.39.137]

13 **Won KJ**, Suzuki T, Hori M, Ozaki H. Motility disorder in experimentally obstructed intestine: relationship between muscularis inflammation and disruption of the ICC network. *Neurogastroenterol Motil* 2006; **18**: 53-61 [PMID: 16371083]

14 **Ward SM**, Sanders KM. Physiology and pathophysiology of the interstitial cell of Cajal: from bench to bedside. I. Functional development and plasticity of interstitial cells of Cajal networks. *Am J Physiol Gastrointest Liver Physiol* 2001; **281**: G602-G611 [PMID: 11518672]

15 **Shroyer NF**, Helmrath MA, Wang VY, Antalffy B, Henning SJ, Zoghbi HY. Intestine-specific ablation of mouse atonal homolog 1 (Math1) reveals a role in cellular homeostasis. *Gastroenterology* 2007; **132**: 2478-2488 [PMID: 17570220 DOI: 10.1053/j.gastro.2007.03.047]

16 **Dou Y**, Fan Y, Zhao J, Gregersen H. Longitudinal residual strain and stress-strain relationship in rat small intestine. *Biomed Eng Online* 2006; **5**: 37 [PMID: 16759387]

17 **Huizinga JD**, Ambrous K, Der-Silaphet T. Co-operation between neural and myogenic mechanisms in the control of distension-induced peristalsis in the mouse small intestine. *J Physiol* 1998; **506** (Pt 3): 843-856 [PMID: 9503342 DOI: 10.1111/j.1469-7793.1998.843bv.x]

18 **Cheng W**, Lui VC, Chen QM, Tam PK. Enteric nervous system, interstitial cells of cajal, and smooth muscle vacuolization in segmental dilatation of jejunum. *J Pediatr Surg* 2001; **36**: 930-935 [PMID: 11381429 DOI: 10.1053/jpsu.2001.23979]

19 **Gao Z**, Müller MH, Karpitschka M, Mittler S, Kasparek MS, Renz B, Sibaev A, Glatzle J, Li Y, Kreis ME. Role of the vagus nerve on the development of postoperative ileus. *Langenbecks Arch Surg* 2010; **395**: 407-411 [PMID: 20333399 DOI: 10.1007/s00423-010-0594-5]

20 **Balemba OB**, Hay-Schmidt A, Assey RJ, Kahwa CK, Semuguruka WD, Dantzer V. An immunohistochemical study of the organization of ganglia and nerve fibres in the mucosa of the porcine intestine. *Anat Histol Embryol* 2002; **31**: 237-246 [PMID: 12196267 DOI: 10.1046/j.1439-0264.2002.00403.x]

21 **Huizinga JD**. Physiology and pathophysiology of the interstitial cell of Cajal: from bench to bedside. II. Gastric motility: lessons from mutant mice on slow waves and in-

nervation. *Am J Physiol Gastrointest Liver Physiol* 2001; **281**: G1129-G1134 [PMID: 11668020]
22 **Shafik A**, El-Sibai O, Shafik AA, Ahmed I. Electric activity of the colon in irritable bowel syndrome: the 'tachyarrhythmic' electric pattern. *J Gastroenterol Hepatol* 2004; **19**: 205-210 [PMID: 14731132 DOI: 10.1111/j.1440-1746.2004.03279.x]
23 **Camilleri M**, Lee JS, Viramontes B, Bharucha AE, Tangalos EG. Insights into the pathophysiology and mechanisms of constipation, irritable bowel syndrome, and diverticulosis in older people. *J Am Geriatr Soc* 2000; **48**: 1142-1150 [PMID: 10983917]
24 **Wray S**. Uterine contraction and physiological mechanisms of modulation. *Am J Physiol* 1993; **264**: C1-18 [PMID: 8430759]
25 **Huizinga JD**, Thuneberg L, Klüppel M, Malysz J, Mikkelsen HB, Bernstein A. W/kit gene required for interstitial cells of Cajal and for intestinal pacemaker activity. *Nature* 1995; **373**: 347-349 [PMID: 7530333]
26 **Sanders KM**. A case for interstitial cells of Cajal as pacemakers and mediators of neurotransmission in the gastrointestinal tract. *Gastroenterology* 1996; **111**: 492-515 [PMID: 8690216 DOI: 10.1053/gast.1996.v111.pm8690216]
27 **Sanders KM**, Ordög T, Ward SM. Physiology and pathophysiology of the interstitial cells of Cajal: from bench to bedside. IV. Genetic and animal models of GI motility disorders caused by loss of interstitial cells of Cajal. *Am J Physiol Gastrointest Liver Physiol* 2002; **282**: G747-G756 [PMID: 11960771]
28 **Ward SM**, Sanders KM, Hirst GD. Role of interstitial cells of Cajal in neural control of gastrointestinal smooth muscles. *Neurogastroenterol Motil* 2004; **16** Suppl 1: 112-117 [PMID: 15066015 DOI: 10.1111/j.1743-3150.2004.00485.x]
29 **Lou Z**, Li JS. Interstitial cells of Cajal in abdominal sepsis and hypertension-induced ileus in rats. *Eur Surg Res* 2009; **43**: 47-52 [PMID: 19439971 DOI: 10.1159/000218102]

P- Reviewers Losanoff JE, Kate V **S- Editor** Wen LL
L- Editor A **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i5.706

World J Gastroenterol 2013 February 7; 19(5): 706-714
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

ORIGINAL ARTICLE

Comparison of modified percutaneous transhepatic variceal embolization and endoscopic cyanoacrylate injection for gastric variceal rebleeding

Jing Wang, Xiang-Guo Tian, Yan Li, Chun-Qing Zhang, Fu-Li Liu, Yi Cui, Ji-Yong Liu

Jing Wang, Xiang-Guo Tian, Yan Li, Chun-Qing Zhang, Fu-Li Liu, Yi Cui, Ji-Yong Liu, Department of Gastroenterology, Provincial Hospital Affiliated to Shandong University, Jinan 250021, Shandong Province, China

Author contributions: Wang J and Tian XG wrote the paper; Zhang CQ designed the research; Zhang CQ, Liu FL and Cui Y performed the procedures; Li Y was responsible for the statistical work; and Liu JY provided clinical advice.

Correspondence to: Chun-Qing Zhang, MD, Department of Gastroenterology, Provincial Hospital Affiliated to Shandong University, 324 Jingwu Weiqi Road, Jinan 250021, Shandong Province, China. zhchqing@medmail.com.cn

Telephone: +86-531-86701339 Fax: +86-531-87987938
Received: May 14, 2012 Revised: November 19, 2012
Accepted: November 24, 2012
Published online: February 7, 2013

Abstract

AIM: To compare the efficacy of modified percutaneous transhepatic variceal embolization (PTVE) with 2-octyl-cyanoacrylate (2-OCA) and endoscopic variceal obturation (EVO) with an injection of 2-OCA for prophylaxis of gastric variceal rebleeding.

METHODS: In this retrospective study, the medical records of liver cirrhosis patients with gastric variceal bleeding who underwent either endoscopic 2-OCA (EVO) or modified PTVE using 2-OCA at Shandong Provincial Hospital from January 2006 to December 2008 were reviewed. Patient demographics, rebleeding rate, survival rate, and complications were compared between the two groups (PTVE and EVO). All results were expressed as mean ± SD, or as a percentage. Quantitative variables were compared by two sample Student *t* tests, and qualitative variables were compared by the Fisher exact test or the χ^2 test (with Yates correction) where appropriate. A *P* value less than 0.05 was considered significant. Statistical computation was performed using SPSS 13.0 software.

RESULTS: A total of 77 patients were included; 45 patients who underwent EVO and 32 patients who received PTVE. During the follow-up (19.78 ± 7.70 mo in the EVO group, *vs* 21.53 ± 8.56 mo in the PTVE group) rebleeding occurred in 17 patients in the EVO group and in 4 patients in the PTVE group (37.78% *vs* 12.5%, *P* = 0.028). The cumulative rebleeding-free rate was 75%, 59%, and 49% in 1, 2, and 3 years respectively for EVO, and 93%, 84%, and 84% for PTVE (*P* = 0.011). Cox analysis was used to identify independent factors that predicted rebleeding after treatment. Variables including age, gender, cause, Child-Pugh classification, size of gastric varices (GV), location of GV, and treatment methods were analyzed. It was revealed that Child-Pugh classification [risk ratio (RR) 2.10, 95%CI: 1.03-4.28, *P* = 0.040], choice of treatment (RR 0.25, 95%CI: 0.08-0.80, *P* = 0.019), and size of GV (RR 2.14, 95%CI: 1.07-4.28, *P* = 0.032) were the independent factors for predicting rebleeding. Follow-up computed tomography revealed that cyanoacrylate was retained in the varices and in the feeding veins of PTVE patients. During the follow-up, eight patients in the EVO group and four patients in the PTVE group died. The cumulative survival rates at 1, 2, and 3 years were 93%, 84%, and 67% respectively in the EVO group, and 97%, 88%, and 74% respectively in the PTVE group. The survival rates were not significantly different between the two groups (*P* = 0.432). Cox analysis showed that the Child-Pugh classification was the most significant prognostic factor of survival (RR 2.77, 95%CI: 1.12-6.80, *P* = 0.027). The incidence of complications was similar in both groups.

CONCLUSION: With extensive and permanent obliteration of gastric varices and its feeding veins, PTVE with 2-OCA is superior to endoscopic 2-OCA injection for preventing gastric variceal rebleeding.

© 2013 Baishideng. All rights reserved.

Key words: Gastric varices; Endoscopic variceal obturation; Percutaneous transhepatic variceal embolization; 2-octyl-cyanoacrylate; Bleeding

Wang J, Tian XG, Li Y, Zhang CQ, Liu FL, Cui Y, Liu JY. Comparison of modified percutaneous transhepatic variceal embolization and endoscopic cyanoacrylate injection for gastric variceal rebleeding. *World J Gastroenterol* 2013; 19(5): 706-714 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i5/706.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i5.706

INTRODUCTION

Although the incidence of bleeding from gastric varices (GV) is lower than from esophageal varices (EV), once it occurs the outcome is worse, and with higher mortality[1-3]. After an episode of acute variceal bleeding, patients are at high risk for recurrent bleeding and death. Thus, prevention of recurrent bleeding is essential[4,5]. However, rupture from gastric varices, especially varices located in the gastric fundus, poses particular therapeutic challenges because of the location and rapid blood flow. The current treatment methods for gastric varices are far from ideal.

Endoscopic variceal obturation (EVO) with the injection of cyanoacrylate has been widely adopted and proved to be effective in the emergency hemostasis of bleeding from gastric varices since it was proposed in 1986[6]. At present, this method is the first-line treatment for gastric variceal bleeding recommended by Baveno IV consensus and AASLD guidelines[7,8]. However, the long-term rebleeding rate after endoscopic cyanoacrylate injection is still high[9-12]. Additionally, there is also a potential risk of systemic embolism in patients with underlying gastrorenal shunts, and other serious complications such as sepsis, fistula, and pericarditis[13-16].

Balloon-occluded retrograde transvenous obliteration (BRTO) has become the standard treatment for gastric varices in Japan. However, patients without catheterizable gastrorenal shunts cannot be treated by BRTO[17,18]. Therefore for these patients, percutaneous transhepatic variceal embolization (PTVE) with N-butyl-2-cyanoacrylate was introduced and showed satisfactory results[19,20] in a small series of patients.

Based on our previous reports of modified PTVE with 2-octyl cyanoacrylate (2-OCA) for bleeding EV[21,22], we have performed modified PTVE with 2-OCA to prevent gastric variceal rebleeding in recent years.

In this retrospective study, 77 patients with prior bleeding from gastric varices who underwent EVO or modified PTVE for prevention of rebleeding were analyzed. Rebleeding rate, survival, and complications were compared between these two procedures. To our knowledge, at the time of writing, no other report in the literature has compared these two procedures in the prevention of gastric variceal rebleeding.

MATERIALS AND METHODS

Patients

The medical records of liver cirrhosis patients with gastric variceal bleeding who underwent either endoscopic 2-OCA injection or modified PTVE using 2-OCA in our hospital from January 2006 to December 2008 were reviewed. Local ethics committee approval was obtained for the chart review.

The inclusion criteria: (1) diagnosis of liver cirrhosis by biopsy or clinical examination and imaging, including ultrasound, computed tomography (CT), or magnetic resonance imaging; (2) patient suffered bleeding within 6 mo before being admitted or acute bleeding with achieved hemostasis by pharmacological treatment; (3) endoscopically-confirmed bleeding from gastric varices: active spurting or oozing of blood from gastric varices during endoscopy, blood clot coating on gastric varices or the presence of erosive spots on gastric varices, with no other potential source of bleeding; and (4) patient aged between 20-65 years.

Exclusion criteria: (1) hepatocellular carcinoma or other malignancies; (2) a history of transjugular intrahepatic portosystemic shunt (TIPS), surgery, or endoscopic therapy for esophagogastric variceal bleeding; (3) portal vein thrombosis; or (4) infection.

Choice of treatment method was based on the patients' intentions after being given a sufficient explanation of the two treatment methods. Informed written consent was obtained from each patient.

Endoscopic injection procedure

Endoscopic intra variceal injection of 2-octyl cyanoacrylate (Baiyun Medical Adhesive Corporation, Guangzhou, China) was performed using a video endoscope (XQ230, Olympus Optical, Tokyo, Japan) and a 23-gauge disposable injection needle (Medwork Medical Products and Services GmbH). Each injection contained a 0.5-2.0 mL mixture of 2-OCA and Lipiodol (1:1). The injection was aimed at the varices that were either bleeding, possessed red color signs, or were the most prominent. Obliteration was assessed by blunt palpation with a catheter, and presence of hardness indicated a complete obliteration. If the varices remained soft, additional injections were done until all gastric varices became hardened. If necessary, additional injections of cyanoacrylate were performed 2-3 wk after the initial session.

PTVE procedure

PTVE was performed alone, or combined with left renal vein obstruction with a balloon catheter if a large gastrorenal shunt was present (Figure 1). Shortly after a percutaneous transhepatic puncture of the intrahepatic branch of the portal vein, a 5F cobra catheter (Cook,

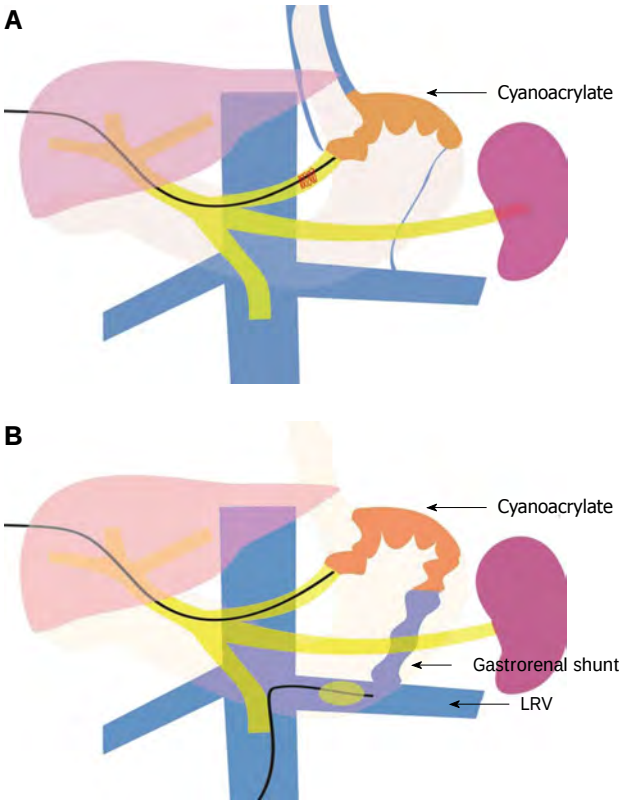


Figure 1 Illustrations of the two types of percutaneous transhepatic variceal embolization. A: Cyanoacrylate was directly injected into the gastric varices by percutaneous transhepatic variceal embolization (PTVE) alone in patients without large gastrorenal shunts; B: Cyanoacrylate injection with PTVE combined with a balloon catheter inserted into the left renal vein (LRV) in patients with large gastrorenal shunt.

Bloomington, IN) was inserted into the splenic vein and a splenoportography was performed to evaluate the gastric varices, the feeding veins, and draining veins. The main gastric feeding vein (*e.g.*, the left gastric vein, short gastric vein, or posterior gastric vein) was then selected with the 5F catheter, and a venography was performed to assess blood flow velocity and the size of varices (the gastrorenal shunt's size being of particular interest).

Based on these data, the embolization of 2-OCA was carried out in the following ways: (1) PTVE alone: In patients without large gastrorenal shunts, the blood flow in gastric varices was slow, and the contrast material could stay in the varices for more than 5 s after injection. In these patients, the cyanoacrylate was directly injected into the gastric varices; and (2) PTVE combined with left renal vein obstruction with a balloon catheter: In patients with large gastrorenal shunts, there was rapid blood flow in the varices and the contrast material disappeared within 3-5 s after injection. For these patients, a 6F balloon catheter (Cook, Bloomington, IN) with a diameter of 15 to 20 mm was inserted into the left renal vein via the right femoral vein to reduce the blood flow of the gastrorenal shunt and varices, and then the cyanoacrylate was injected. In these two ways, the cyanoacrylate could obliterate the entire varices and feeding veins, while cyanoacrylate migration to the systemic circulation could be avoided.

When the cyanoacrylate was flowing into all the gastric varices, the catheter was immediately withdrawn. Splenoportography was again performed to assess the obliteration of the varices. If other feeding veins (such as the short or posterior gastric veins) were present, the procedure would be repeated until the gastric varices and feeding veins were completely filled with cyanoacrylate. Finally, the 5F sheath system was withdrawn, and the puncture tract was embolized with microcoils. Low molecular heparin (100 IU/kg body weight, daily) was subcutaneously administered 24 h after the procedure for 5 to 7 d to prevent portal venous thrombosis.

Follow-up

Follow-up endoscopy was performed for the two groups at intervals of 1, 3, and 6 mo after the procedures, and then every 6-12 mo or when it was considered clinically necessary. In patients with rebleeding, endoscopy was performed to identify the cause of the bleeding. Portal venography was performed at 1 mo after the procedure, and every 6 mo thereafter with 3-dimensional multidetector row CT (GE Medical systems, Milwaukee, WI) to observe the formation of portal vein thrombosis, the location and extent of cyanoacrylate glue, variceal recanalization, and the occurrence of collateral vessels. Rebleeding, survival, and complications were recorded.

Recurrent bleeding was defined as the presence of hematemesis or melena, with the bleeding source being endoscopically proven to originate from gastric or EV, or other resources after the index treatment. Bleeding from gastric varices was distinguished from that of EV on the basis of whether active bleeding or erosive spots were present on the gastric varices themselves.

Complications were defined as any untoward events that required active treatment or prolonged hospitalization.

Statistical analysis

All results were expressed as mean ± SD, or as a percentage. Quantitative variables were compared by two sample Student *t* tests, and qualitative variables were compared by the Fisher exact test or the chi-squared test (with Yates correction) where appropriate. The Kaplan-Meier estimation was used to examine recurrence and rebleeding of gastric varices and rate of survival. Comparisons were performed using the log-rank test. A Cox's analysis was performed to detect possible independent predictors for variceal rebleeding and death. A P value less than 0.05 was considered significant. Statistical computation was performed using SPSS 13.0 software.

RESULTS

Demographic characteristics of patients

From January 2006 to December 2008, EVO or PTVE was performed in a total of 92 cirrhotic patients with a history of gastric variceal bleeding in Shandong Pro-

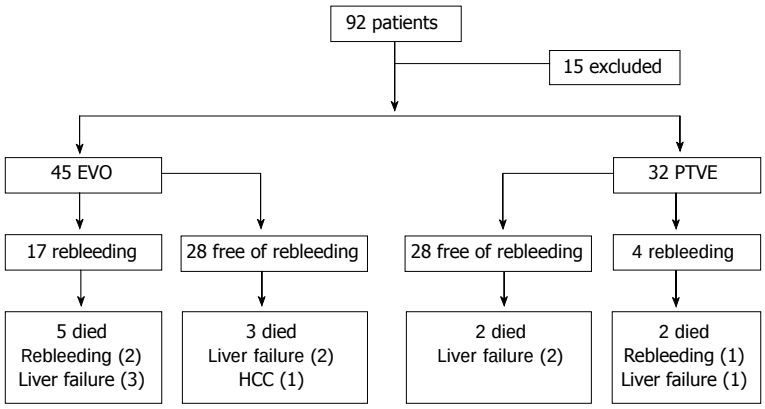


Figure 2 Flow diagram of rebleeding and death after procedures. EVO: Endoscopic variceal obturation; PTVE: Percutaneous transhepatic variceal embolization; HCC: Hepatocellular carcinoma.

Table 1 Clinical characteristics of patients

	EVO (n = 45)	PTVE (n = 32)	P value
Sex (M/F)	33/12	22/10	0.661
Age (mean ± SD, yr)	52.69 ± 8.99	50.65 ± 7.23	0.293
Etiology of cirrhosis			0.999
Hepatitis B	25	18	
Hepatitis C	9	6	
Alcohol	8	6	
Others	3	2	
Child-Pugh class (A/B/C)	14/21/10	9/17/6	0.851
Bleeding onset			1.000
Recent variceal bleeding	40	28	
Acute variceal bleeding	5	4	
Blood transfusion (unit)	4.50±3.32	5.33±3.60	0.542
Form of GV (F1/F2/F3)[1]	12/23/10	7/13/12	0.343
Location of GV[2]			0.467
GOV2	29	18	
IGV1	16	14	
Ascites	26	20	0.857
Duration of follow-up (mo)	19.78 ± 7.70	21.53 ± 8.56	0.350

[1]Forms of gastric varices (GV) were graded by the classification described by Hashizume *et al*[24]: F1, tortuous winding varices; F2, nodular-shaped varices; and F3, tumorous huge varices; [2]Locations of GV were based on the criteria proposed by Sarin *et al*[1]. M/F: Male/female; GOV2: Gastric varices extended from the esophageal varices toward the gastric fundus; IGV1: Isolated gastric varices located in the fundus; PTVE: Percutaneous transhepatic variceal embolization; EVO: Endoscopic variceal obturation.

Table 2 Procedures in endoscopic variceal obturation and percutaneous transhepatic variceal embolization groups

	EVO (n = 45)	PTVE (n = 32)	P value
Status of GV			0.290
Disappeared	17/45	16/32	
Collapsed	16/45	12/32	
Remained	12/45	4/32	
Amount of cyanoacrylate (mL)	3.03 ± 1.04	6.69 ± 2.92	0.000
Rebleeding	17/45	4/32	0.028
Rebleeding from GV	13/45	2/32	0.029
Rebleeding from other sources	4/45	2/32	1.000
EV bleeding	2	1	1.000
PHG	1	1	1.000
Unknown	1	0	1.000
Death during follow-up	8/45	4/32	0.751
Causes of death			
Progressive Liver failure	5/45	3/32	1.000
Rebleeding	2/45	1/32	1.000
HCC	1/45	0/32	1.000

EV: Esophageal varices; GV: Gastric varices; EVO: Endoscopic variceal obturation; PHG: Portal hypertensive gastropathy; HCC: Hepatocellular carcinoma; PTVE: Percutaneous transhepatic variceal embolization.

vincial Hospital. Of the 92 patients, six had hepatocellular carcinoma, and nine had previously received TIPS or shunt surgery; these 15 patients were excluded. Of the remaining 77 patients, EVO was performed in 45, and PTVE was performed in 32 (Figure 2). The clinical characteristics of the 77 patients in the two groups were retrospectively reviewed from a computerized database of our hospital. Gastric varices were subdivided by Sarin classification[4], the form of gastric varices was classified according to Hashizume classification[23], and liver function was estimated based on the Child-Pugh classification[24]. The follow-up records were carefully reviewed. The median follow-up period was 19.78 ± 7.70 mo (range, 3 to 41 mo) in the EVO group and 21.53 ± 8.56 mo (range, 6 to 44 mo) in the PTVE group (P = 0.350). Table 1 shows the patient characteristics in the two groups, for which there was no significant difference.

Technique outcomes

The outcomes of the procedures are shown in Table 2. In the EVO group, 35 patients (77.78%) achieved complete obliteration of gastric varices. Of the 35 patients, one session of EVO was needed to achieve complete obliteration in 23 patients, and two or three sessions were required in 12 patients. The total volume of cyanoacrylate used in the initial session of each EVO group patient was 3.03 ± 1.04 mL (range, 1.5-5.5 mL). In the PTVE group, 18 patients underwent gastric variceal embolization by PTVE alone, while the other 14 patients underwent combined PTVE with left renal vein balloon obstruction. Thirty patients (93.75%) achieved complete obliteration, which was confirmed by a splenoportography after the PTVE procedure. The volume of cyanoacrylate used in

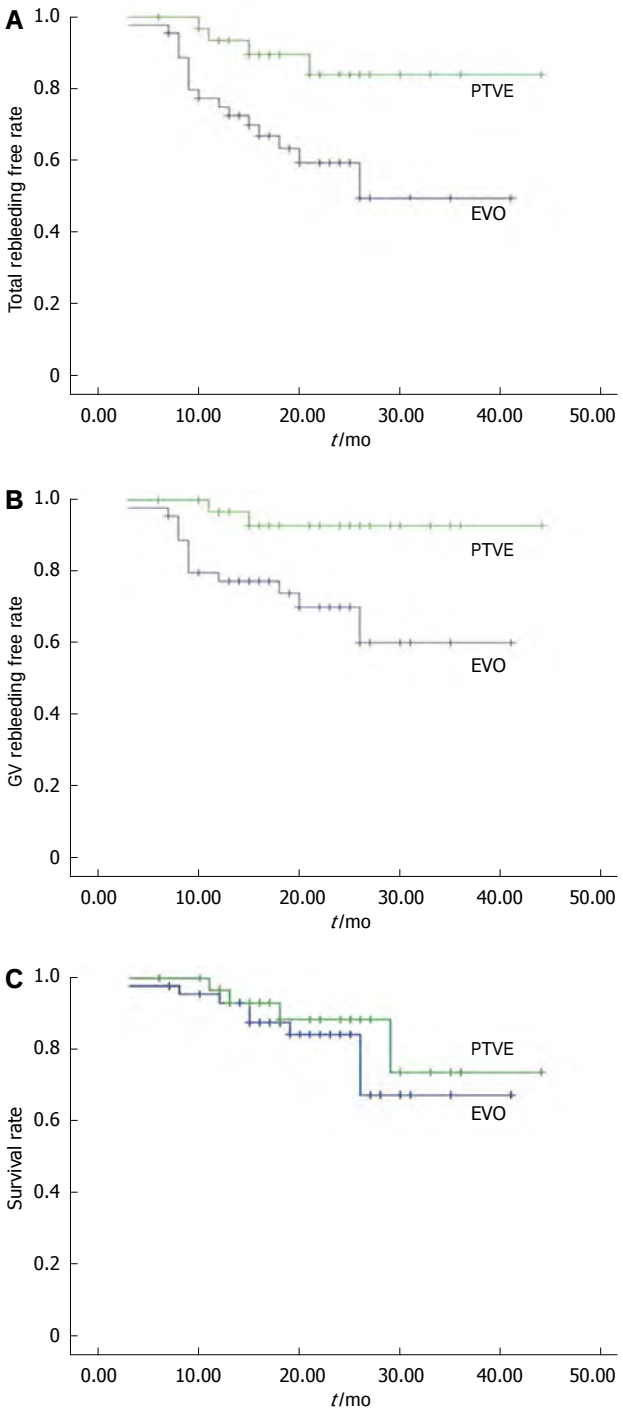


Figure 3 Cumulative survival. A: The cumulative rebleeding free rate was higher in the percutaneous transhepatic variceal embolization (PTVE) group than in the endoscopic variceal obturation (EVO) group, $P = 0.011$, log-rank test; B: The cumulative free gastric varix (GV) rebleeding rate was higher in the PTVE group than in the EVO group, $P = 0.012$, log-rank test; C: The cumulative survival rate was not different between the two groups. $P = 0.432$, Log-rank test.

the PTVE group was 6.69 ± 2.92 mL (range, 3-16 mL); more than in the EVO group ($P = 0.000$).

Endoscopic surveillance

An endoscopic follow-up was performed in all patients in both groups. Generally, 1-3 mo after the injection, the cyanoacrylate plug began to slough off the submucosa in some patients, and the varices disappeared or collapsed after complete or partial expulsion of the glue within one year in the PTVE group. In patients who underwent EVO, the endoscopic findings were similar to that in the PTVE group. During the endoscopic follow-up, the gastric varices disappeared in 16 patients or shrunk in 12 patients in the PTVE group, and disappeared in 17 patients or shrunk in 16 patient in the EVO group ($P = 0.290$).

CT and portal venography follow-up

Twenty patients in the EVO group and fifteen patients in the PTVE group underwent contrast-enhanced CT and portal venography during follow-up. In the PTVE group, it was confirmed that the gastric varices, the perforating veins in the fundus, the perifundus veins, and all the feeding veins were filled with cyanoacrylate 1 mo after the procedure. 3-6 mo after PTVE, the amount of cyanoacrylate in the submucosal varices was reduced compared to previously. Twelve months later, the cyanoacrylate in the submucosal varices almost completely gone, but the cyanoacrylate in the perifundus varices and feeding veins remained the same as at the start. Portal venography showed that there was no blood flow in the eradicated varices with sufficient amount of cyanoacrylate after one year. In the EVO group, CT revealed that the cyanoacrylate was only scattered in the gastric varices and the perifundus varices, which was not as extensive and complete as in the PTVE group.

Rebleeding

During the follow-up, rebleeding occurred in 17 patients (37.78%) in the EVO group and four patients (12.5%) in the PTVE group. The rebleeding rate was significantly lower in the PTVE group compared to the EVO group ($P = 0.028$). The cumulative rebleeding-free rate was 75%, 59%, and 49% in 1, 2, and 3 years respectively for the EVO group, and 93%, 84%, and 84% for the PTVE group ($P = 0.011$, Figure 3A).

The causes of rebleeding included: gastric variceal rebleeding (13 in EVO and 2 in PTVE), aggravated esophageal variceal bleeding (2 in EVO and 1 in PTVE), portal hypertensive gastropathy (1 in EVO and 1 in PTVE), and unknown reasons (1 in EVO and nil in PTVE). The cumulative rate free of GV rebleeding at 1, 2, and 3 years was 77%, 70%, and 60% respectively for the EVO group, and 97%, 93%, and 93% for the PTVE group ($P = 0.012$, Figure 3B). Of the 15 patients with rebleeding from GV, three died of uncontrolled bleeding, four received repeated EVO, three received TIPS, and five received surgery. No patient died of bleeding from EV or other sources.

Cox analysis was used to identify the independent factors that predicted rebleeding after treatment. Variables including age, gender, cause, Child-Pugh classification, size of GV, location of GV, and treatment methods were analyzed. It was revealed that the Child-Pugh classification (RR 2.10, 95%CI: 1.03-4.28, $P = 0.040$), choice of treatment (RR 0.25, 95%CI: 0.08-0.80, $P = 0.019$), and size of GV (RR 2.14, 95%CI: 1.07-4.28, $P = 0.032$) were

Table 3 Procedural complications

	EVO (n = 45)	PTVE (n = 32)	P value
Total complications (%)	29	22	0.881
Bacteremia	4	1	0.395
Fever	19	12	0.857
Abdominal pain	12	15	0.112
Ulcer	3	1	0.637
SBP	2	3	0.644
Ascites	3	6	0.152
Portal vein thrombosis	0	2	0.170
Pulmonary embolism	1	0	1.000

SBP: Spontaneous bacterial peritonitis; EVO: Endoscopic variceal obturation; PTVE: Percutaneous transhepatic variceal embolization.

the independent predicting factors for rebleeding.

Survival

During the follow-up, eight patients in the EVO group and four patients in the PTVE group died. The cumulative survival rates at 1, 2, and 3 years were 93%, 84%, and 67% respectively in the EVO group, and 97%, 88%, and 74% respectively in the PTVE group. The survival rates were not significantly different between the two groups (P = 0.432, Figure 3C). In the EVO group, five patients died of progression of hepatic failure, compared with three patients in the PTVE group. Two patients in the EVO group and one in the PTVE group died of uncontrolled rebleeding. The remaining patient in the EVO group died of hepatocellular carcinoma, which occurred after the procedure.

Seven variables (sex, age, the Child Pugh classification, etiology, choice of modality, form of GV, and location of GV) were taken into consideration in the multivariate analysis using the Cox regression model. Based on the Cox analysis, the Child-Pugh classification was the most significant prognostic factor of survival (RR 2.77, 95%CI: 1.12-6.80, P = 0.027).

Complications

Complications of the procedures are shown in Table 3. Twenty-nine patients experienced complications in the EVO group, compared to twenty-two patients in the PTVE group (P = 0.881). There was no significant difference between the groups. The common complications after treatment were fever and abdominal pain. 19 patients in the EVO group and 12 patients in the PTVE group suffered from fever (P = 0.857). Abdominal pain was encountered in 12 patients in the EVO group and 15 patients in the PTVE group (P = 0.112). The patients were treated with conventional medicinal therapy, with fever and abdominal pain usually being alleviated within 1-2 wk. One patient in the EVO group encountered pulmonary embolism, while no patient encountered systemic embolization in the modified PTVE group (P=1.000). Mild to moderate ascites appeared in 3 patients in the EVO group and 6 in the PTVE group (P = 0.152), and all of them were controlled by medication. Partial portal vein thrombosis appeared in two patients in the PTVE group, but the portal vein was patent under Doppler ultrasound. No patient died of complications in either group.

DISCUSSION

Although gastric varices tend to bleed less than EV, the mortality associated with gastric variceal hemorrhage is substantial[1-3] (Kim, 1997 #5; Sarin, 1992 #4; Ryan, 2004 #6) (Kim, 1997 #5; Sarin, 1992 #4; Ryan, 2004 #6). Furthermore, with the successful management of bleeding EV, as well as successful prophylaxis of first bleeding from EV, "secondary" gastric varices develop in 9% to 15% of patients, which have a higher frequency of bleeding compared with primary gastric varices[1,23]. Unlike EV, gastric varices pose particular therapeutic challenges because of their size and location. Standard endoscopic therapies used for EV, such as sclerotherapy and band ligation, are less effective for gastric varices and have been shown to be associated with high complication rates[6,7]. This leads to the need for more aggressive and costly interventions, such as TIPS and BRTO, however each has its limitations. The current methods for the treatment of gastric varices are far from ideal.

EVO with the injection of agents such as N-buyt1-2-cyanoacrylate for the treatment of GV has been widely adopted, and both the immediate and long-term efficacies have been confirmed in the treatment of GV[25-28]. However, the long-term rebleeding rate after EVO was still high[9-12]. Incomplete obliteration and recurrence of varices are considered to be important causes of rebleeding[29]. However, the unobliterated components of the GV are sometimes small, and a further intraluminal injection might be difficult due to the previously injected polymers. In such circumstances, GV obliteration might not be complete. Over time, the residual GV can become larger, and rebleeding can occur. Incomplete obliteration and recurrence of varices are considered to be important causes of rebleeding[9]. Additionally, although rare, fatal complications do occur[13-16].

PTVE with cyanoacrylate is a modified procedure of the classical percutaneous transhepatic obliteration[30-32]. Though this novel technique was used only in a small number of patients with gastric varices and without gastrorenal shunts who were not candidate for BRTO[19,20], it started a new treatment method for gastric varices. In recent years, based on the good results of modified PTVE with 2-OCA in the treatment of EV reported in our previous study[21,22], we introduced 2-OCA in modified PTVE to treat gastric varices. In this retrospective study, we compared modified PTVE with an endoscopic injection using 2-OCA in the management of gastric varices. Despite the retrospective nature of the study, there were no significant differences in age, sex, cause of disease, or severity of liver disease between the studied patients.

In our study, the rebleeding rate of EVO was 37.8%, while it was only 12.5% in patients who underwent

PTVE with cyanoacrylate. This showed that PTVE with 2-OCA was superior to endoscopic 2-OCA injection with respect to preventing rebleeding. Regarding the risk factors of variceal rebleeding, it was revealed that liver function, size of GV, and treatment methods were independent factors that predicted the rebleeding risk of GV. Although the rebleeding rate of GV was lower in patients who underwent PTVE, this did not yield a decreased mortality compared with patients in the EVO group. Cox analysis showed the liver function in the Child-Pugh classification was the most significant factor.

There are two technical superiorities of PTVE over EVO, which may contribute to the lower variceal rebleeding rate of PTVE. The first is that PTVE can achieve a more extensive obliteration area than EVO. In the present study, with regards to the PTVE procedure, 2-OCA was injected into entire gastric varices, perforating veins in the fundus and perigastric veins, including all the feeding veins in most of the patients (30/32, 93.75%). In the EVO procedure, although we tried to achieve complete obliteration in one session, some patients needed repeated injections, and even then, the obliteration rate was only 77.7%. Contrast CT follow-up showed that the PTVE could obliterate all collaterals in the vicinity of the gastric fundus over a wider area and in deeper layers compared with EVO. Adequate 2-OCA injection is important for complete variceal obliteration. In our study, more cyanoacrylate was used in the PTVE procedure than in EVO, which also indicates a wider obliteration range in the PTVE group. The second technical superiority of PTVE over EVO is that 2-OCA can be permanently embolized in perforating veins in the fundus and perigastric veins, including all its feeding veins. In our study, follow-up endoscopy and CT scans showed that the cyanoacrylate in the submucosa could be released with time, and lead to the eradication of the varices after PTVE. However, CT scans revealed that the 2-OCA still stayed in the perifundus varices and perforating veins in the gastric fundus and all the afferent veins during follow-up. This might explain why PTVE achieved long-term obliteration, and prevented recanalization and rebleeding of gastric varices.

Complications were similar in patients who underwent EVO or modified PTVE. Fever and abdominal pain were the two common complications in both groups. They were given conventional treatment and the fever and abdominal pain were usually alleviated within 1-2 wk. Six patients in the PTVE group and three in the EVO group experienced mild to moderate ascites. They were given diuretics and or albumin transfusion, and no patients experienced refractory ascites. Partial portal vein thrombosis presented in two patients in PTVE group, but the portal vein was patent under Doppler ultrasound without serious consequences. In the present study, one patient in the EVO group encountered pulmonary embolism. However, no patient encountered systemic embolization in the modified PTVE group. The modification of PTVE contributed to this satisfactory result. Firstly, in patients without a large gastrorenal shunt, the blood flow in the gastric varices was slow, so there was enough time for 2-OCA to obliterate the varices, and it was unlikely that the tissue glue would migrate into the systemic circulation. Therefore, the 2-OCA could be injected into the gastric varices with percutaneous transhepatic variceal embolization alone. Secondly, in patients with a large gastrorenal shunt and rapid blood flow in the gastric varices, percutaneous transhepatic embolization combined with left renal vein balloon catheter obstruction was performed to prevent systemic embolization.

Some authors tried to obliterate the afferent vein of the gastric varices using TIPS combined with embolotherapy[33]. However, TIPS for gastric varices is not as effective as that for EV; moreover, stent failure and hepatic encephalopathy also limit its application[34-36]. BRTO embolized the gastric varices through the outflow vein (the gastrorenal shunt), and it is recognized as a safe and effective treatment for gastric varices with a gastrorenal shunt. However, patients without a catheterizable gastrorenal shunt are not suitable for BRTO. In our study, even in patients with a large gastrorenal shunt, the embolization was still performed by PTVE, in which the gastric varices were embolized through the inflow veins, such as the left gastric vein, short gastric vein and/or posterior gastric vein. We suppose that PTVE could achieve a more extensive obliteration range than BRTO. As antegrade transcatheter embolization, PTVE with 2-OCA could embolized the entire gastric varices and all its inflow veins (left gastric vein, short gastric vein and/or posterior gastric vein). But BRTO variceal embolization is performed by gastrorenal shunt (outflow vein), in which the inflow veins perhaps could not be completely embolized.

In conclusion, with extensive and permanent obliteration of both gastric varices and its feeding veins, PTVE with 2-OCA is a prospective modality for the treatment of gastric varices. It is superior to endoscopic 2-OCA injection in terms of preventing rebleeding. However, our study is a retrospective single-center study. A future prospective, randomized, and controlled trial is required.

ACKNOWLEDGMENTS

We would like to thank Dr. Vijay Shah from Mayo Clinic for his critical review of the manuscript. Thanks to Dr. Edward C Mignot, Shandong University, for linguistic advice.

COMMENTS

Background

Although gastric varices tend to bleed less than esophageal varices (EV), the mortality associated with gastric variceal hemorrhage is substantial. Unlike EV, gastric varices pose particular therapeutic challenges because of their size and location. The current methods for the treatment of gastric varices are far from ideal.

Research frontiers

At present, endoscopic variceal obturation with the injection of agents such as N-butyl-2-cyanoacrylate is the first-line treatment for gastric variceal bleeding. However, the unobliterated components of the gastric varices (GV) are sometimes small, and a further intraluminal injection might be difficult due to the previously injected polymers. In such circumstances, GV obliteration might

not be complete. Over time, the residual GV can become larger, and rebleeding can occur. Incomplete obliteration and recurrence of varices are considered to be important causes of rebleeding.

Innovations and breakthroughs

Percutaneous transhepatic variceal embolization (PTVE) with cyanoacrylate is a modified procedure of the classical percutaneous transhepatic obliteration. With GV, the perifundus veins and all the feeding veins in the vicinity of the gastric fundus are sufficiently obliterated with cyanoacrylate. PTVE with 2-octyl-cyanoacrylate (2-OCA) can improve long-term efficacy by preventing gastric varices rebleeding. In this retrospective study, the authors compared modified PTVE with endoscopic injection using 2-OCA in the management of gastric varices.

Applications

With extensive and permanent obliteration of both GV and its feeding veins, PTVE with 2-OCA is a prospective modality for the treatment of GV. It is superior to endoscopic 2-OCA injection in terms of preventing rebleeding.

Peer review

The authors report their results of a retrospective control study: modified PTVE with 2-OCA and endoscopic variceal obturation with the injection of 2-OCA for prophylaxis of gastric variceal rebleeding. Important data including the rebleeding rate, survival rate, complications, and prognostic predictors were reported. This study indicates that PTVE with 2-OCA can be a better option for secondary prophylaxis of the gastric variceal hemorrhage. However, given the small sample size and retrospective nature of this study, their results may not be representative of the broader population of patients with gastric variceal and a future prospective, randomized, and controlled trial is required.

REFERENCES

1 **Sarin SK**, Lahoti D, Saxena SP, Murthy NS, Makwana UK. Prevalence, classification and natural history of gastric varices: a long-term follow-up study in 568 portal hypertension patients. *Hepatology* 1992; **16**: 1343-1349 [PMID: 1446890]

2 **Kim T**, Shijo H, Kokawa H, Tokumitsu H, Kubara K, Ota K, Akiyoshi N, Iida T, Yokoyama M, Okumura M. Risk factors for hemorrhage from gastric fundal varices. *Hepatology* 1997; **25**: 307-312 [PMID: 9021939]

3 **Ryan BM**, Stockbrugger RW, Ryan JM. A pathophysiologic, gastroenterologic, and radiologic approach to the management of gastric varices. *Gastroenterology* 2004; **126**: 1175-1189 [PMID: 15057756]

4 **D'Amico G**, Pagliaro L, Bosch J. Pharmacological treatment of portal hypertension: an evidence-based approach. *Semin Liver Dis* 1999; **19**: 475-505 [PMID: 10643630 DOI: 10.1055/s-2007-1007133.]

5 **Bosch J**, García-Pagán JC. Prevention of variceal rebleeding. *Lancet* 2003; **361**: 952-954 [PMID: 12648985 DOI: 10.1016/S0140-6736(03)12778-X]

6 **Soehendra N**, Nam VC, Grimm H, Kempeneers I. Endoscopic obliteration of large esophagogastric varices with bucrylate. *Endoscopy* 1986; **18**: 25-26 [PMID: 3512261 DOI: 10.1055/s-2007-1013014.]

7 **de Franchis R**. Evolving consensus in portal hypertension. Report of the Baveno IV consensus workshop on methodology of diagnosis and therapy in portal hypertension. *J Hepatol* 2005; **43**: 167-176 [PMID: 15925423 DOI: 10.1016/j.jhep.2005.05.009.]

8 **Garcia-Tsao G**, Sanyal AJ, Grace ND, Carey W. Prevention and management of gastroesophageal varices and variceal hemorrhage in cirrhosis. *Hepatology* 2007; **46**: 922-938 [PMID: 17879356 DOI: 10.1002/hep.21907.]

9 **Huang YH**, Yeh HZ, Chen GH, Chang CS, Wu CY, Poon SK, Lien HC, Yang SS. Endoscopic treatment of bleeding gastric varices by N-butyl-2-cyanoacrylate (Histoacryl) injection: long-term efficacy and safety. *Gastrointest Endosc* 2000; **52**: 160-167 [PMID: 10922085]

10 **Akahoshi T**, Hashizume M, Shimabukuro R, Tanoue K, Tomikawa M, Okita K, Gotoh N, Konishi K, Tsutsumi N, Sugimachi K. Long-term results of endoscopic Histoacryl injection sclerotherapy for gastric variceal bleeding: a 10-year experience. *Surgery* 2002; **131**: S176-S181 [PMID: 11821807]

11 **Lo GH**, Liang HL, Chen WC, Chen MH, Lai KH, Hsu PI, Lin CK, Chan HH, Pan HB. A prospective, randomized controlled trial of transjugular intrahepatic portosystemic shunt versus cyanoacrylate injection in the prevention of gastric variceal rebleeding. *Endoscopy* 2007; **39**: 679-685 [PMID: 17661241 DOI: 10.1053/jhep.2001.24116]

12 **Hou MC**, Lin HC, Lee HS, Liao WC, Lee FY, Lee SD. A randomized trial of endoscopic cyanoacrylate injection for acute gastric variceal bleeding: 0.5 mL versus 1.0 mL. *Gastrointest Endosc* 2009; **70**: 668-675 [PMID: 19559427 DOI: 10.1016/j.gie.2009.02.005.]

13 **Greenwald BD**, Caldwell SH, Hespenheide EE, Patrie JT, Williams J, Binmoeller KF, Woodall L, Haluszka O. N-2-butyl-cyanoacrylate for bleeding gastric varices: a United States pilot study and cost analysis. *Am J Gastroenterol* 2003; **98**: 1982-1988 [PMID: 14499775 DOI: 10.1111/j.1572-0241.2003.07637.x]

14 **Tan YM**, Goh KL, Kamarulzaman A, Tan PS, Ranjeev P, Salem O, Vasudevan AE, Rosaida MS, Rosmawati M, Tan LH. Multiple systemic embolisms with septicemia after gastric variceal obliteration with cyanoacrylate. *Gastrointest Endosc* 2002; **55**: 276-278 [PMID: 11818941 DOI: 10.1067/mge.2001.118651.]

15 **Battaglia G**, Morbin T, Patarnello E, Merkel C, Corona MC, Ancona E. Visceral fistula as a complication of endoscopic treatment of esophageal and gastric varices using isobutyl-2-cyanoacrylate: report of two cases. *Gastrointest Endosc* 2000; **52**: 267-270 [PMID: 10922108 DOI: 10.1067/mge.2000.105080.]

16 **Chen YY**, Shen TC, Soon MS, Lai JH. Life-threatening pericarditis after N-butyl-2-cyanoacrylate injection for esophageal variceal bleeding: Case report. *Gastrointest Endosc* 2005; **61**: 487-489 [PMID: 15758933]

17 **Cho SK**, Shin SW, Lee IH, Do YS, Choo SW, Park KB, Yoo BC. Balloon-occluded retrograde transvenous obliteration of gastric varices: outcomes and complications in 49 patients. *AJR Am J Roentgenol* 2007; **189**: W365-W372 [PMID: 18029851 DOI: 10.2214/AJR.07.2266.]

18 **Ninoi T**, Nishida N, Kaminou T, Sakai Y, Kitayama T, Hamuro M, Yamada R, Nakamura K, Arakawa T, Inoue Y. Balloon-occluded retrograde transvenous obliteration of gastric varices with gastrorenal shunt: long-term follow-up in 78 patients. *AJR Am J Roentgenol* 2005; **184**: 1340-1346 [PMID: 15788621]

19 **Kwak HS**, Han YM. Percutaneous transportal sclerotherapy with N-butyl-2-cyanoacrylate for gastric varices: technique and clinical efficacy. *Korean J Radiol* 2008; **9**: 526-533 [PMID: 19039269 DOI: 10.3348/kjr.2008.9.6.526]

20 **Kiyosue H**, Matsumoto S, Yamada Y, Hori Y, Okino Y, Okahara M, Mori H. Transportal intravariceal sclerotherapy with N-butyl-2-cyanoacrylate for gastric varices. *J Vasc Interv Radiol* 2004; **15**: 505-509 [PMID: 15126663]

21 **Zhang CQ**, Liu FL, Liang B, Sun ZQ, Xu HW, Xu L, Feng K, Liu ZC. A modified percutaneous transhepatic variceal embolization with 2-octyl cyanoacrylate versus endoscopic ligation in esophageal variceal bleeding management: randomized controlled trial. *Dig Dis Sci* 2008; **53**: 2258-2267 [PMID: 18038208 DOI: 10.1007/s10620-007-0106-9]

22 **Zhang CQ**, Liu FL, Liang B, Xu HW, Xu L, Feng K, Liu ZC. A modified percutaneous transhepatic varices embolization with 2-octyl cyanoacrylate in the treatment of bleeding esophageal varices. *J Clin Gastroenterol* 2009; **43**: 463-469 [PMID: 19142166 DOI: 10.1097/MCG.0b013e31817ff90f]

23 **Hashizume M**, Kitano S, Yamaga H, Koyanagi N, Sugimachi K. Endoscopic classification of gastric varices. *Gastrointest Endosc* 1990; **36**: 276-280 [PMID: 2365213]

24 **Pugh RN**, Murray-Lyon IM, Dawson JL, Pietroni MC, Williams R. Transection of the oesophagus for bleeding oesophageal varices. *Br J Surg* 1973; **60**: 646-649 [PMID: 4541913]

25 **Lo GH**, Lai KH, Cheng JS, Chen MH, Chiang HT. A prospective, randomized trial of butyl cyanoacrylate injection versus band ligation in the management of bleeding gastric varices. *Hepatology* 2001; **33**: 1060-1064 [PMID: 11343232 DOI: 10.1053/jhep.2001.24116.]

26 **Tan PC**, Hou MC, Lin HC, Liu TT, Lee FY, Chang FY, Lee SD. A randomized trial of endoscopic treatment of acute gastric variceal hemorrhage: N-butyl-2-cyanoacrylate injection versus band ligation. *Hepatology* 2006; **43**: 690-697 [PMID: 16557539 DOI: 10.1002/hep.21145.]

27 **Procaccini NJ**, Al-Osaimi AM, Northup P, Argo C, Caldwell SH. Endoscopic cyanoacrylate versus transjugular intrahepatic portosystemic shunt for gastric variceal bleeding: a single-center U.S. analysis. *Gastrointest Endosc* 2009; **70**: 881-887 [PMID: 19559425 DOI: 10.1016/j.gie.2009.03.1169.]

28 **Kind R**, Guglielmi A, Rodella L, Lombardo F, Catalano F, Ruzzenente A, Borzellino G, Girlanda R, Leopardi F, Pratticò F, Cordiano C. Bucrylate treatment of bleeding gastric varices: 12 years' experience. *Endoscopy* 2000; **32**: 512-519 [PMID: 10917182]

29 **Rockey DC**. Management of gastric varices. *Gastroenterology* 2001; **120**: 1875-1876; discussion 1875-1876 [PMID: 11375970]

30 **Terabayashi H**, Ohnishi K, Tsunoda T, Nakata H, Saito M, Tanaka H, Iida S, Nomura F, Okuda K. Prospective controlled trial of elective endoscopic sclerotherapy in comparison with percutaneous transhepatic obliteration of esophageal varices in patients with nonalcoholic cirrhosis. *Gastroenterology* 1987; **93**: 1205-1209 [PMID: 3315824]

31 **Uflacker R**. Percutaneous transhepatic obliteration of gastroesophageal varices using absolute alcohol. *Radiology* 1983; **146**: 621-625 [PMID: 6600846]

32 **Bengmark S**, Börjesson B, Hoevels J, Joelsson B, Lunderquist A, Owman T. Obliteration of esophageal varices by PTP: a follow-up of 43 patients. *Ann Surg* 1979; **190**: 549-554 [PMID: 384946]

33 **Xiao T**, Chen L, Chen W, Xu B, Long Q, Li R, Li L, Peng Z, Fang D, Wang R. Comparison of transjugular intrahepatic portosystemic shunt (TIPS) alone versus TIPS combined with embolotherapy in advanced cirrhosis: a retrospective study. *J Clin Gastroenterol* 2011; **45**: 643-650 [PMID: 21301360 DOI: 10.1097/MCG.0b013e318203dfb3.]

34 **Sanyal AJ**, Freedman AM, Luketic VA, Purdum PP, Shiffman ML, DeMeo J, Cole PE, Tisnado J. The natural history of portal hypertension after transjugular intrahepatic portosystemic shunts. *Gastroenterology* 1997; **112**: 889-898 [PMID: 9041251]

35 **Barange K**, Péron JM, Imani K, Otal P, Payen JL, Rousseau H, Pascal JP, Joffre F, Vinel JP. Transjugular intrahepatic portosystemic shunt in the treatment of refractory bleeding from ruptured gastric varices. *Hepatology* 1999; **30**: 1139-1143 [PMID: 10534333 DOI: 10.1002/hep.510300523.]

36 **ter Borg PC**, Hollemans M, Van Buuren HR, Vleggaar FP, Groeneweg M, Hop WC, Laméris JS. Transjugular intrahepatic portosystemic shunts: long-term patency and clinical results in a patient cohort observed for 3-9 years. *Radiology* 2004; **231**: 537-545 [PMID: 15044746 DOI: 10.1148/radiol.2312021797.]

P- Reviewer Kim HC **S- Editor** Gou SX
L- Editor Rutherford A **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i5.715

World J Gastroenterol 2013 February 7; 19(5): 715-720
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Endoscopic stent therapy in patients with chronic pancreatitis: A 5-year follow-up study

Andreas Weber, Jochen Schneider, Bruno Neu, Alexander Meining, Peter Born, Stefan von Delius, Monther Bajbouj, Roland M Schmid, Hana Algül, Christian Prinz

Andreas Weber, Jochen Schneider, Bruno Neu, Alexander Meining, Peter Born, Stefan von Delius, Monther Bajbouj, Roland M Schmid, Hana Algül, II Medizinische Klinik und Poliklinik, Klinikum Rechts der Isar, Technische Universität München, 81675 München, Germany
Christian Prinz, II Medizinische Klinik, Gastroenterologie und Hepatologie, Helios Klinikum Wuppertal, Universität Witten/Herdecke, 42283 Wuppertal, Germany
Author contributions: Weber A and Schneider J contributed equally to this work; Weber A, Schneider J, Schmid RM and Prinz C designed the research; Schneider J, Neu B, Meining A and Born P performed the research; Weber A, Bajbouj M, von Delius S analyzed the data; Schneider J, Algül H and Weber A wrote the manuscript.
Correspondence to: Andreas Weber, MD, PhD, II Medizinische Klinik und Poliklinik, Klinikum Rechts der Isar, Technische Universität München, Ismaningerstrasse 22, 81675 München, Germany. andreas.weber@lrz.tum.de
Telephone: +49-89-41405843 Fax: +49-89-41404905
Received: July 29, 2012 Revised: September 11, 2012
Accepted: September 28, 2012
Published online: February 7, 2013

Abstract

AIM: This study analyzed clinical long-term outcomes after endoscopic therapy, including the incidence and treatment of relapse.

METHODS: This study included 19 consecutive patients (12 male, 7 female, median age 54 years) with obstructive chronic pancreatitis who were admitted to the 2nd Medical Department of the Technical University of Munich. All patients presented severe chronic pancreatitis (stage Ⅲ°) according to the Cambridge classification. The majority of the patients suffered intermittent pain attacks. 6 of 19 patients had strictures of the pancreatic duct; 13 of 19 patients had strictures and stones. The first endoscopic retrograde pancreatography (ERP) included an endoscopic sphincterotomy, dilatation of the pancreatic duct, and stent placement. The first control ERP was performed 4 wk after the initial intervention, and the subsequent control ERP was performed after 3 mo to re-evaluate the clinical and morphological conditions. Clinical follow-up was performed annually to document the course of pain and the management of relapse. The course of pain was assessed by a pain scale from 0 to 10. The date and choice of the therapeutic procedure were documented in case of relapse.

RESULTS: Initial endoscopic intervention was successfully completed in 17 of 19 patients. All 17 patients reported partial or complete pain relief after endoscopic intervention. Endoscopic therapy failed in 2 patients. Both patients were excluded from further analysis. One failed patient underwent surgery, and the other patient was treated conservatively with pain medication. Seventeen of 19 patients were followed after the successful completion of endoscopic stent therapy. Three of 17 patients were lost to follow-up. One patient was not available for interviews after the 1st year of follow-up. Two patients died during the 3rd year of follow-up. In both patients chronic pancreatitis was excluded as the cause of death. One patient died of myocardial infarction, and one patient succumbed to pneumonia. All three patients were excluded from follow-up analysis. Follow-up was successfully completed in 14 of 17 patients. 4 patients at time point 3, 2 patients at time point 4, 3 patients at time point 5 and 2 patients at time point 6 and time point 7 used continuous pain medication after endoscopic therapy. No relapse occurred in 57% (8/14) of patients. All 8 patients exhibited significantly reduced or no pain complaints during the 5-year follow-up. Seven of 8 patients were completely pain free 5 years after endoscopic therapy. Only 1 patient reported continuous moderate pain. In contrast, 7 relapses occurred in 6 of the 14 patients. Two relapses were observed during the 1st year, 2 relapses occurred during the 2nd year, one relapse was observed during the 3rd year, one relapse occurred during the 4th year, and one relapse occurred during the 5th follow-up year. Four of these six patients

received conservative treatment with endoscopic therapy or analgesics. Relapse was conservatively treated using repeated stent therapy in 2 patients. Analgesic treatment was successful in the other 2 patients.

CONCLUSION: 57% of patients exhibited long-term benefits after endoscopic therapy. Therefore, endoscopic therapy should be the treatment of choice in patients being inoperable or refusing surgical treatment.

© 2013 Baishideng. All rights reserved.

Key words: Chronic pancreatitis; Pain; Stent therapy; Endoscopic retrograde cholangiopancreatography; Pancreaticolithiasis

Weber A, Schneider J, Neu B, Meining A, Born P, von Delius S, Bajbouj M, Schmid RM, Algül H, Prinz C. Endoscopic stent therapy in patients with chronic pancreatitis: A 5-year follow-up study. *World J Gastroenterol* 2013; 19(5): 715-720 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i5/715.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i5.715

INTRODUCTION

The optimal treatment for chronic pancreatitis is controversial among surgeons and endoscopists. Endoscopic drainage procedures include optional sphincterotomy, stricture dilation, stone removal, and the insertion of a plastic stent. The surgical approaches are classified as drainage and resective procedures. Resective procedures, such as the duodenum-preserving head resections of Beger and Frey, provide good pain control and preserve pancreatic function[1]. Drainage procedures, such as the pancreaticojejunostomy, are comparable to endoscopic treatments because both of these procedures are based on the same pathophysiological idea. The primary goal is to restore pancreatic drainage to lower the pressure within the pancreatic duct and relieve pain[2-4]. Cahen *et al*[5] compared the clinical outcome of patients with endoscopic treatment and patients undergoing pancreaticojejunostomy in a prospective randomized trial. The authors concluded that surgical drainage of the pancreatic duct was more effective than endoscopic treatment. However, some patients are inoperable or refuse surgical treatment. Endoscopic therapy offers as an alternative approach for these patients. We reported previously that endoscopic therapy provided good immediate and short-term benefits to patients with obstructive chronic pancreatitis[6]. The current manuscript provides additional data from a 5-year follow-up to analyze clinical long-term outcomes after the successful completion of endoscopic therapy, including the incidence and treatment of relapse.

MATERIALS AND METHODS

Patients

This section is similar to our previously published 2-year follow-up study[6]. All patients presented severe chronic pancreatitis (stage Ⅲ°) according to the Cambridge classification[7] at the time of sphincterotomy and the majority of the patients (11/19) suffered intermittent pain attacks according to the Amman Score[8]. About 6 of 19 patients had strictures of the pancreatic duct; 13 of 19 patients had strictures and stones.

Methods

The first control endoscopic retrograde pancreatography (ERP) was performed 4 wk after stenting, and the subsequent control ERP was performed after 3 mo to re-evaluate the clinical and morphological conditions. Either a new stent was inserted, or stent therapy was terminated depending on the clinical and morphological conditions.

The long-term effects of stent therapy were analyzed after the successful completion of stent therapy. A short overview of the study protocol is illustrated in Figure 1. Follow-up analyses were conducted from 2004 to 2009. The ethical committee stated that the patient's choice of treatment was protected. Clinical parameters, such as pain and the intake of pain medication, were documented: (1) Prior to endoscopic therapy (time point 1); (2) During endoscopic therapy (time point 2); and (3) Annually during the 5-year follow-up period (time points 3, 4, 5, 6 and 7).

Interviews: The first interview was a two-part session that was conducted during stent therapy. This interview analyzed the technical success of ERP and compared the pain situation prior to and after initial stent therapy (Figure 1). The first session was conducted prior to the first stent implantation (time point 1), and the second session was conducted after the initial stent implantation (time point 2). All 17 patients were interviewed annually during the follow-up period. The interviews primarily evaluated the course of pain. The patients graded their pain on a pain scale from 0 to 10: 0 was no pain, 1-2 slight pain, 3-4 moderate pain, 5-6 strong pain, 7-8 very strong pain, and 9-10 extreme pain. The date and choice of the therapeutic procedure (*e.g.*, conservative pain management, endoscopic or surgical treatment) were documented for instances of relapse.

Statistical analysis

Statistical analysis was not performed. The evaluation of pain was carried out using a pain score in absolute numbers.

RESULTS

Primary outcome, duration and complication rate of stent therapy

This section is similar to our previously published 2-year follow-up study[6].

Long-term follow-up

Nineteen patients with chronic obstructive pancreatitis

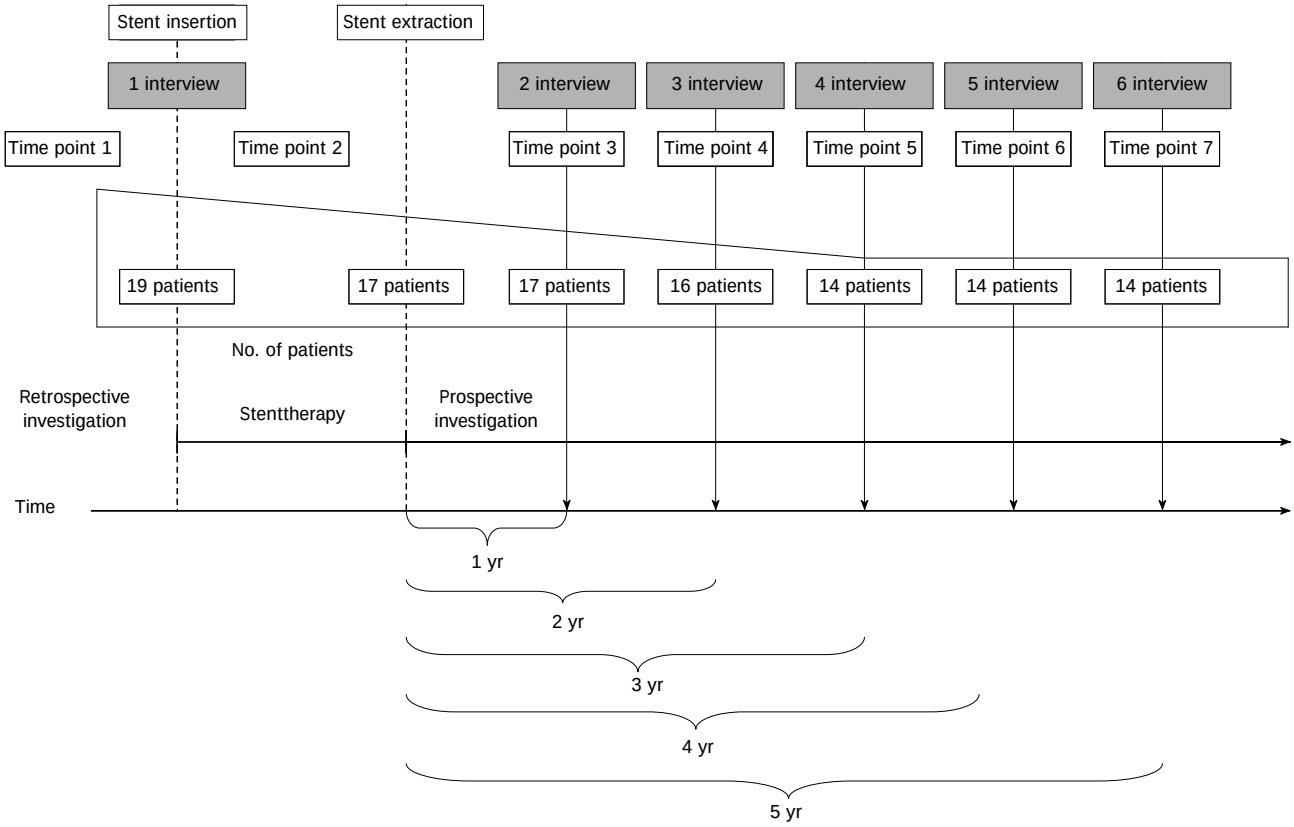


Figure 1 Protocol of the current study, time points of investigation and examination. Nineteen patients with chronic obstructive pancreatitis underwent endoscopic stent therapy. Endoscopic therapy failed in 2 patients. Seventeen of 19 patients showed clinical benefit and were included to follow-up analysis after completion of stent therapy. During the follow-up, patients were interviewed yearly. Three patients were lost to follow-up. One patient was not available any longer and two patients died.

underwent endoscopic stent therapy. Figure 2 is an illustration of a representative ERP procedure. ERP failed in 2 patients in which cannulation or permanent stent therapy was not achieved. One failed patient underwent surgery, and the other patient was treated conservatively with pain medication. We excluded these 2 patients from follow-up analyses. Endoscopic stent therapy was successfully completed in 17 of 19 patients. All 17 patients reported partial or complete pain relief.

The clinical courses of 17 patients were evaluated after the successful completion of stent therapy. Three of the 17 patients were lost during follow-up. One patient was not available for interviews after the 1st year of follow-up (Figure 1). Two patients died during the 3rd year of follow-up, and chronic pancreatitis was excluded as the cause of death. One patient died of myocardial infarction, and one patient succumbed to pneumonia.

Patients consumed the following pain medications during the 5-year follow-up period: paracetamol (500-1000 mg/d), diclofenac (50-100 mg/d), metamizol (500-1000 mg/d) and/or tramadol (100-300 mg/d). 4 patients at time point 3, 2 patients at time point 4, 3 patients at time point 5 and 2 patients at time point 6 and time point 7 used continuous pain medication after endoscopic therapy.

Seven relapses occurred in 6 of the 14 patients (43%). One patient developed 2 relapses during the 1st and 3rd follow-up years. Two relapses were observed during the 1st year, 2 relapses occurred during the 2nd year, one relapse was observed during the 3rd year, one relapse occurred during the 4th year, and one relapse occurred during the 5th follow-up year.

Four of these six patients received conservative treatment with endoscopic therapy or analgesics. Two patients were successfully treated with endoscopic therapy. Two patients achieved pain improvement with analgesic therapy. Only 2 of the 6 patients required surgery.

In contrast, 57% (8/14) of the patients without relapse reported significantly reduced pain or no complaints 5 years after stent extraction. Seven of these patients were completely pain free. Only 1 patient reported continuous moderate pain.

DISCUSSION

Several reviews[9-14] suggest that the majority of physicians consider pancreatic duct stenting a safe and effective therapy for patients with chronic abdominal pain due to chronic pancreatitis. The primary goal of endoscopic therapy is pain relief. Approximately half of the patients in the current study exhibited clinical long-term benefits. Most patients (57%, 8/14) exhibited significantly reduced pain or no pain complaints 5 years after stent extraction. Endoscopic treatment was an effective alternative

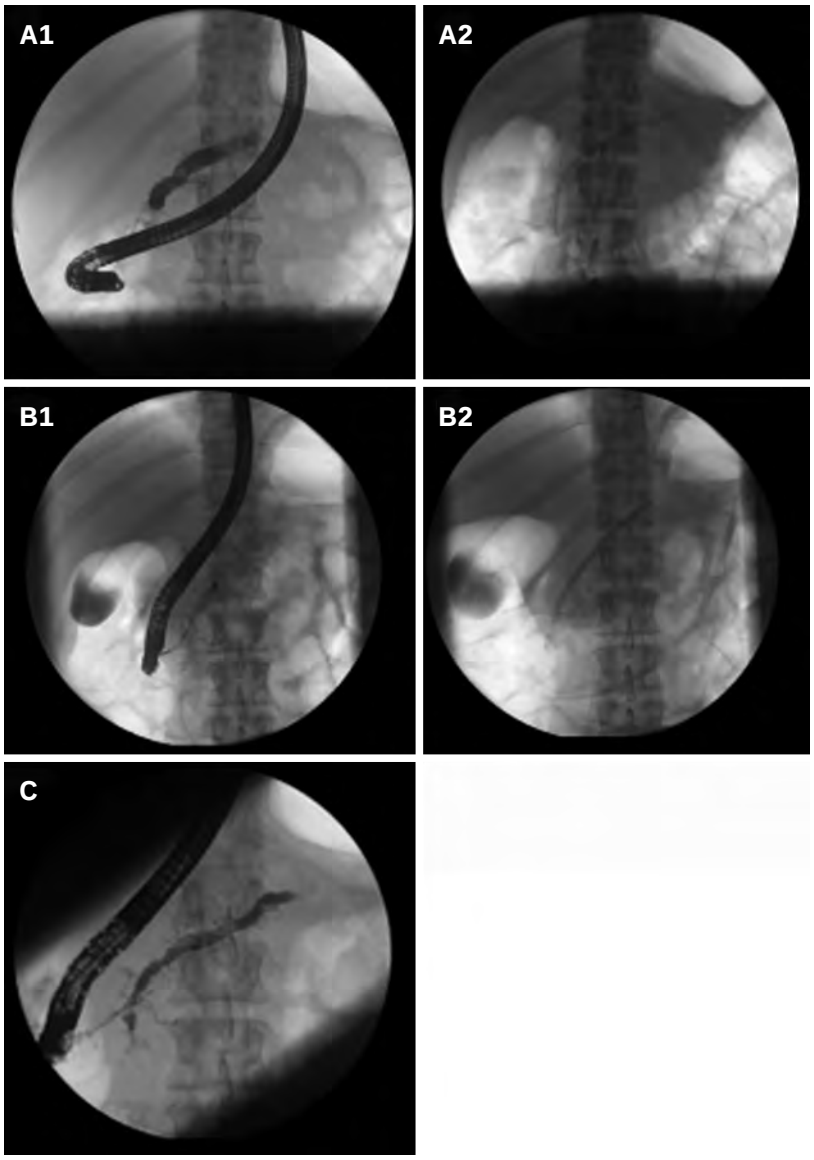


Figure 2 Illustration of a representative endoscopic retrograde pancreatography procedure. A: A dilated pancreatic duct due to a stenosis in the head and several stones in the main duct (A1). The stenosis was passed through with a 5 F catheter followed by a 8.5 F Sohendra Bougie. Subsequently, a 7 F stent was inserted (A2). After initial endoscopic intervention the patient was completely pain free; B: Four weeks later, the patient was readmitted for control endoscopic retrograde pancreatography (ERP). Stones were partially extracted by a dormia basket (B1), and the 7 F stent was replaced by a 10 F stent (B2); C: Three months later, the patient was still free of complaints. ERP revealed a slightly dilated duct with typical stone formations in the corpus and tail of the pancreas. Stones were extracted.

to surgery in instances of relapse. Four of six re-treated patients received conservative treatment with endoscopic or analgesic therapy. Only two of these patients required surgery.

The impact of smoking and alcohol on pain development was not evaluated in this study. However, smoking and alcohol are associated with more a progressive disease course[15-20]. Therefore, the cessation of smoking and alcohol consumption was recommended to all patients in the study. No relationship between the duration of stenting and the occurrence of relapse was observed. However, the small number of patients in this study prohibits valid conclusions. Further studies with a larger number of patients are required.

Surgical procedures seem to be superior to endoscopic therapy. Díte *et al*[21] compared the clinical benefit of endoscopic and surgical therapies in a randomized prospective study. The study included 140 patients with chronic pancreatitis. Endoscopic therapy was performed in 64 patients, and 76 patients underwent surgery. Both therapeutic approaches produced excellent results in initial pain relief (92.1% and 92.2%, respectively). The number of patients with partial or complete pain relief at the 5-year follow-up in the stent group decreased from 92.2% to 65.1%, but 86.2% of patients with surgical procedures reported reduced or no pain. These authors concluded that surgery was superior to endoscopic therapy for long-term pain reduction in patients with painful obstructive chronic pancreatitis. However, 80% of the surgeries were resection procedures. Resection procedures are based on a different pathophysiological concept than endoscopic or surgical drainage. Therefore, these procedures are only

comparable to a limited degree. The prospective randomized study of Cahen *et al*[5] analyzed the clinical benefit of surgical drainage versus endoscopic drainage, which is more suitable for this type of comparison. Nineteen patients underwent endoscopic drainage, and 20 patients received surgical drainage. Two years after intervention, 75% patients with pancreaticojejunostomy were partially or completely pain-free, but complete or partial pain relief was achieved in only 32% of patients with endoscopic therapy. Cahen *et al*[5] concluded that the surgical drainage of pancreatic duct was more effective than endoscopic treatment in patients with obstruction of pancreatic duct due to chronic pancreatitis. In contrast, the data of our current series demonstrated that ERP failed in only 2 of 19 patients. Complete or partial pain relief was achieved in 17 of 19 patients after initial stent insertion. Approximately half of the patients benefited from endoscopic stent therapy 5 years after endoscopic intervention. The less effective outcome of endoscopic stent therapy in the Cahen study may result from the difficulty of managing the endoscopic group. The complication rate of the endoscopic group (58%) was approximately 4 times higher in their study compared to the average results of other studies[22,23].

Many patients present contraindications or refuse to undergo surgery due to the invasiveness of surgical procedures. Consequently, patients who were American Society of Anesthesiologists class Ⅳ, presented with portal vein thrombosis or declined to participate were excluded from the Cahen study[5]. Approximately 12% of patients with chronic pancreatitis exhibit portal hypertension[24]. Izbicki *et al*[25] evaluated the impact of concomitant nonhepatic portal hypertension in chronic pancreatitis on the outcomes after major pancreatic surgery. Patients with portal hypertension required significantly more blood transfusions and longer operative times than their nonhypertensive counterparts. The overall postoperative complication rate was significantly higher in this subgroup. Izbicki concluded that concomitant extrahepatic portal hypertension is a substantial risk in pancreatic surgery for chronic pancreatitis.

The major limitations of this study are the small number of patients, its single-center character and its nonrandomized design. Therefore, randomized long-term follow-up studies with a larger number of patients are required.

In conclusion, endoscopic therapy proved to be a safe and effective alternative to surgery, and endoscopic therapy did not adversely affect the outcome of subsequent surgeries[26]. Our current data demonstrate that endoscopic therapy provided long-term benefits in more than half of the patients (57%) with chronic obstructive pancreatitis. The management of obstructive chronic pancreatitis should be individual. Patients with multiple morbidities profit from low invasive endoscopic therapy. Therefore, endoscopic stent therapy is the treatment of choice in inoperable patients or patients who refuse surgical treatment.

COMMENTS

Background

Obstruction of the pancreatic duct is a common feature of chronic pancreatitis, and it often requires interventional therapy. The optimal treatment for chronic pancreatitis is controversial among surgeons and endoscopists. The primary goal of both surgical and endoscopic drainage procedures is the restoration of pancreatic drainage to lower the pressure within the pancreatic duct and relieve pain.

Research frontiers

This is the first prospective 5-year follow-up assessment of the incidence and treatment of relapse in patients with chronic obstructive pancreatitis after the successful completion of endoscopic stent therapy.

Innovations and breakthroughs

The current data demonstrate that endoscopic therapy provided long-term benefits in more than half of chronic obstructive pancreatitis patients. Most (57 %) of the patients remained free of relapse 5 years after endoscopic treatment. Endoscopic treatment was an effective alternative to surgery in cases of relapse.

Applications

These data suggest that endoscopic therapy is a safe, minimally invasive, and effective procedure in patients who experience pain attacks during chronic pancreatitis. Multimorbid patients benefit from the low invasiveness of endoscopic therapy. Therefore, endoscopic stent therapy should be the treatment of choice in patients who are inoperable or refuse surgical treatment.

Terminology

Chronic pancreatitis is a continuous inflammation of the pancreas that produces morphological changes, such as calcifications, irregularities of the pancreas duct and the formation of pancreatic stones. The pathogenesis of pain in chronic pancreatitis is controversial. Interstitial hypertension, ongoing pancreatic ischemia, neuronal inflammation, and extrapancreatic complications may contribute to the pathogenesis of pain.

Peer review

The current study provides interesting and clinically relevant data. The observed patient group was small, but the differences were obvious. The data support the benefit of long-term endoscopic therapy in a subgroup of patients.

REFERENCES

1 **Hines OJ**, Reber HA. Pancreatic surgery. *Curr Opin Gastroenterol* 2005; **21**: 568-572 [PMID: 16093771 DOI: 00001574-200509000-00007]

2 **Bradley EL**. Pancreatic duct pressure in chronic pancreatitis. *Am J Surg* 1982; **144**: 313-316 [PMID: 7114368 DOI: 0002-9610(82)90008-3]

3 **Madsen P**, Winkler K. The intraductal pancreatic pressure in chronic obstructive pancreatitis. *Scand J Gastroenterol* 1982; **17**: 553-554 [PMID: 7134883]

4 **Okazaki K**, Yamamoto Y, Kagiyama S, Tamura S, Sakamoto Y, Morita M, Yamamoto Y. Pressure of papillary sphincter zone and pancreatic main duct in patients with alcoholic and idiopathic chronic pancreatitis. *Int J Pancreatol* 1988; **3**: 457-468 [PMID: 3221107 DOI: 10.1007/BF02788204]

5 **Cahen DL**, Gouma DJ, Nio Y, Rauws EA, Boermeester MA, Busch OR, Stoker J, Laméris JS, Dijkgraaf MG, Huibregtse K, Bruno MJ. Endoscopic versus surgical drainage of the pancreatic duct in chronic pancreatitis. *N Engl J Med* 2007; **356**: 676-684 [PMID: 17301298 DOI: 356/7/676]

6 **Weber A**, Schneider J, Neu B, Meining A, Born P, Schmid RM, Prinz C. Endoscopic stent therapy for patients with chronic pancreatitis: results from a prospective follow-up study. *Pancreas* 2007; **34**: 287-294 [PMID: 17414050 DOI: 10.1097/mpa.0b013e3180325ba600006676-200704000-00002]

7 **Sarner M**, Cotton PB. Classification of pancreatitis. *Gut* 1984; **25**: 756-759 [PMID: 6735257]

8 **Ammann RW**, Muellhaupt B. The natural history of pain in alcoholic chronic pancreatitis. *Gastroenterology* 1999; **116**: 1132-1140 [PMID: 10220505]

9 **Godil A**, Chen YK. Endoscopic management of benign pan-

creatic disease. *Pancreas* 2000; **20**: 1-13 [PMID: 10630377]
10 **Monkemuller KE**, Kahl S, Malfertheiner P. Endoscopic therapy of chronic pancreatitis. *Dig Dis* 2004; **22**: 280-291 [PMID: 15753611]
11 **Neuhaus H**. Therapeutic pancreatic endoscopy. *Endoscopy* 2000; **32**: 217-225 [PMID: 10718387 DOI: 10.1055/s-2000-94]
12 **Neuhaus H**. Therapeutic pancreatic endoscopy. *Endoscopy* 2004; **36**: 8-16 [PMID: 14722850 DOI: 10.1055/s-2004-814119]
13 **Tringali A**, Boskoski I, Costamagna G. The role of endoscopy in the therapy of chronic pancreatitis. *Best Pract Res Clin Gastroenterol* 2008; **22**: 145-165 [PMID: 18206819 DOI: 10.1016/j.bpg.2007.10.021]
14 **Delhaye M**, Arvanitakis M, Bali M, Matos C, Devière J. Endoscopic therapy for chronic pancreatitis. *Scand J Surg* 2005; **94**: 143-153 [PMID: 16111097]
15 **Maisonneuve P**, Lowenfels AB, Müllhaupt B, Cavallini G, Lankisch PG, Andersen JR, Dimagno EP, Andrén-Sandberg A, Domellöf L, Frulloni L, Ammann RW. Cigarette smoking accelerates progression of alcoholic chronic pancreatitis. *Gut* 2005; **54**: 510-514 [PMID: 15753536]
16 **Maisonneuve P**, Frulloni L, Müllhaupt B, Faitini K, Cavallini G, Lowenfels AB, Ammann RW. Impact of smoking on patients with idiopathic chronic pancreatitis. *Pancreas* 2006; **33**: 163-168 [PMID: 16868482 DOI: 10.1097/01.mpa.0000227916.94073.fc]
17 **Cavallini G**, Talamini G, Vaona B, Bovo P, Filippini M, Rigo L, Angelini G, Vantini I, Riela A, Frulloni L. Effect of alcohol and smoking on pancreatic lithogenesis in the course of chronic pancreatitis. *Pancreas* 1994; **9**: 42-46 [PMID: 8108370]
18 **Yadav D**, Hawes RH, Brand RE, Anderson MA, Money ME, Banks PA, Bishop MD, Baillie J, Sherman S, DiSario J, Burton FR, Gardner TB, Amann ST, Gelrud A, Lawrence C, Elinoff B, Greer JB, O'Connell M, Barmada MM, Slivka A, Whitcomb DC. Alcohol consumption, cigarette smoking, and the risk of recurrent acute and chronic pancreatitis. *Arch Intern Med* 2009; **169**: 1035-1045 [PMID: 19506173 DOI: 10.1001/archinternmed.2009.125]
19 **Schneider KJ**, Scheer M, Suhr M, Clemens DL. Ethanol administration impairs pancreatic repair after injury. *Pancreas* 2012; **41**: 1272-1279 [PMID: 22617711 DOI: 10.1097/MPA.0b013e31824bde37]
20 **Talamini G**, Bassi C, Falconi M, Sartori N, Vaona B, Bovo P, Benini L, Cavallini G, Pederzoli P, Vantini I. Smoking cessation at the clinical onset of chronic pancreatitis and risk of pancreatic calcifications. *Pancreas* 2007; **35**: 320-326 [PMID: 18090237 DOI: 10.1097/mpa.0b013e31812e965e]
21 **Díte P**, Ruzicka M, Zboril V, Novotný I. A prospective, randomized trial comparing endoscopic and surgical therapy for chronic pancreatitis. *Endoscopy* 2003; **35**: 553-558 [PMID: 12822088 DOI: 10.1055/s-2003-40237]
22 **Rösch T**, Daniel S, Scholz M, Huibregtse K, Smits M, Schneider T, Ell C, Haber G, Riemann JF, Jakobs R, Hintze R, Adler A, Neuhaus H, Zavoral M, Zavada F, Schusdziarra V, Soehendra N. Endoscopic treatment of chronic pancreatitis: a multicenter study of 1000 patients with long-term follow-up. *Endoscopy* 2002; **34**: 765-771 [PMID: 12244496 DOI: 10.1055/s-2002-34256]
23 **Dumonceau JM**, Devière J, Le Moine O, Delhaye M, Vandermeeren A, Baize M, Van Gansbeke D, Cremer M. Endoscopic pancreatic drainage in chronic pancreatitis associated with ductal stones: long-term results. *Gastrointest Endosc* 1996; **43**: 547-555 [PMID: 8781931 DOI: S0016-5107(96)70189-X]
24 **Bockhorn M**, Gebauer F, Bogoevski D, Molmenti E, Cataldegirmen G, Vashist YK, Yekebas EF, Izbicki JR, Mann O. Chronic pancreatitis complicated by cavernous transformation of the portal vein: contraindication to surgery? *Surgery* 2011; **149**: 321-328 [PMID: 20684965 DOI: 10.1016/j.surg.2010.06.011]
25 **Izbicki JR**, Yekebas EF, Strate T, Eisenberger CF, Hosch SB, Steffani K, Knoefel WT. Extrahepatic portal hypertension in chronic pancreatitis: an old problem revisited. *Ann Surg* 2002; **236**: 82-89 [PMID: 12131089]
26 **Boerma D**, van Gulik TM, Rauws EA, Obertop H, Gouma DJ. Outcome of pancreaticojejunostomy after previous endoscopic stenting in patients with chronic pancreatitis. *Eur J Surg* 2002; **168**: 223-228 [PMID: 12440760 DOI: 10.1080/11024150260102834]

P- Reviewers Seicean A, Prinz C, Drewes AM, Gerstenmaier JF
S- Editor Wen LL **L- Editor** A **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i5.721

World J Gastroenterol 2013 February 7; 19(5): 721-726
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Australian tertiary care outcomes of entecavir monotherapy in treatment naive patients with chronic hepatitis B

Farzan Fahrtash-Bahin, Viraj C Kariyawasam, Timothy Gray, Karen Byth, Jacob George, Mark W Douglas

Farzan Fahrtash-Bahin, Viraj C Kariyawasam, Jacob George, Mark W Douglas, Storr Liver Unit, Westmead Millennium Institute, University of Sydney at Westmead Hospital, 2145 Sydney, Australia
Karen Byth, Department of Biostatistics, School of Public Health, University of Sydney, 2145 Sydney, Australia
Timothy Gray, Mark W Douglas, Centre for Infectious Diseases and Microbiology and Sydney Emerging Infections and Biosecurity Institute, University of Sydney at Westmead Hospital, 2145 Sydney, Australia
Author contributions: Fahrtash-Bahin F collected data, analyzed data and wrote up manuscript; Kariyawasam VC collected data, analyzed data and wrote up manuscript; Gray T planned study, collected data and reviewed manuscript; Byth K analyzed data; George J planned study and reviewed manuscript; Douglas MW planned study, supervised data collection and analysis and revised manuscript.
Correspondence to: Mark W Douglas, MBBS, PhD, FRACP, Storr Liver Unit, Westmead Millennium Institute, University of Sydney at Westmead Hospital, PO Box 412, Westmead, 2145 Sydney, Australia. mark.douglas@sydney.edu.au
Telephone: +61-2-98457705 Fax: +61-2-96357582
Received: August 25, 2012 Revised: October 25, 2012
Accepted: November 14, 2012
Published online: February 7, 2013

Abstract

AIM: To evaluate the long-term treatment outcomes of entecavir monotherapy in treatment naive patients in an Australian tertiary care setting.

METHODS: A retrospective analysis of treatment naive patients receiving entecavir monotherapy through Westmead Hospital was performed. Patients were excluded if they had received previous treatment with another nucleoside or nucleotide analogue, were pregnant or less than 18 years old.

RESULTS: Out of 336 patients, 163 patients fulfilled the selection criteria. Range of follow up was 3-46 mo (mean 26 mo). 134 patients (82.2%) had pre-treatment biopsies, with 26 patients (16.0 %) demonstrating F3-4 fibrosis. In total, 153 patients (93.9%) achieved at least Partial Virological Suppression (PVS), with 134 patients (82.2%) achieving complete virological suppression. The cumulative CVS and PVS rates at 36 mo were 82.1% and 96.4%, respectively. 3 patients (1.8%) failed to achieve PVS, while 5 patients (3.0%) developed virological rebound. 128 patients (78.5%) maintained CVS throughout follow up. Predictors of CVS included lower baseline DNA level (P = 0.001), hepatitis B virus e antigen negative status (P = 0.001) and increasing age at treatment (log rank 0.001). No significant adverse effects were reported necessitating cessation of entecavir.

CONCLUSION: Entecavir monotherapy is efficacious and safe in an Australian tertiary care setting. Resistance and rebound rates are very low. This is similar to data from controlled and uncontrolled trials around the world.

© 2013 Baishideng. All rights reserved.

Key words: Chronic hepatitis B; Entecavir; Australia; Asia-Pacific; Monotherapy; Hepatitis B virus; Antivirals

Fahrtash-Bahin F, Kariyawasam VC, Gray T, Byth K, George J, Douglas MW. Australian tertiary care outcomes of entecavir monotherapy in treatment naive patients with chronic hepatitis B. *World J Gastroenterol* 2013; 19(5): 721-726 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i5/721.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i5.721

INTRODUCTION

An estimated 350 to 400 million people worldwide have chronic hepatitis B virus (HBV) infection, a leading cause of morbidity and mortality. Australia has an ethnically diverse population, with many migrants from the Asia-Pacific region, where the majority of hepatitis B related deaths occur[1].

The number of people living with chronic hepatitis B in Australia is estimated to be between 153 000 and 175 000, a prevalence of 0.7% to 0.8%[2,3]. In 2010, 228 incident cases of hepatitis B and 6878 notifications of hepatitis B were reported to the Australian government's National Notifiable Diseases Surveillance System[4]. This reflects the major burden of disease in people with childhood acquired chronic hepatitis B.

In chronic HBV infection the goals of antiviral therapy are to suppress HBV DNA and reduce hepatic inflammation [alanine aminotransferase (ALT)], with the aim of preventing progression of liver fibrosis and achieving immune control [hepatitis B e antigen (HBeAg) and/or hepatitis B surface antigen (HBsAg) loss/conversion]. This reduces mortality due to cirrhosis, liver failure and HCC. Antiviral agents available include pegylated interferon, lamivudine, adefovir, entacavir, telbivudine and tenofovir.

Entecavir is an oral deoxyguanosine analog with potent activity against HBV[5]. In multiple clinical trials, entacavir has been found to be highly efficacious in treating nucleoside naive and lamividine refractory patients[6-8]. Emergence of drug resistance during antiviral therapy is well described for HBV, particularly with lamivudine, with resistance appearing in 20% of patients after 1 year and 70% after 5 years[9]. Entecavir has a higher genetic barrier to resistance than lamivudine, with quoted resistance rates of only 1%-2% in treatment naive patients after 5 years[10]. However, cross-resistance with lamivudine is a problem, and in patients who have failed lamivudine, entecavir resistance appears in up to 8% after 5 years[10,11].

The rates of entecavir response and resistance in Australia are unknown. Most published data are from large clinical trials, which do not necessarily reflect the rates seen in "real world" clinical practice. These trials were almost exclusively performed in the Northern Hemisphere, and do not reflect the unique, diverse migrant population seen in Australia. Furthermore, much of this data is based on a 1 mg dose of entecavir, rather than 0.5 mg now recommended for treatment naive patients.

The aim of this study was to evaluate the efficacy, rates of viral resistance and treatment outcomes of entacavir monotherapy in an Australian tertiary referral centre, outside of a clinical trial environment.

MATERIALS AND METHODS

Data collection and clinical setting

Data was entered retrospectively into a database incorporating all patients receiving entecavir through the Westmead Hospital pharmacy (Sydney, Australia) between 1 November 2006 and 31 July 2010.

Selection criteria

All patients receiving entecavir through the Westmead Hospital pharmacy were considered for analysis. Patients were included if they met the following criteria: (1) eligible for entecavir 0.5 mg daily according to AASLD guidelines; (2) HBV infection for greater than 6 mo based on serology; and (3) deranged liver function tests or at least mild fibrosis on liver biopsy (> F2 on Scheuer classification).

Exclusion criteria were: (1) previous treatment with other nucleoside analogues, nucleotide analogues or trial medication (except for interferon); (2) pregnancy; (3) age less than 18 years; (4) hepatitis delta co-infection; and (5) Prophylactic entecavir during immunosuppression (*e.g.*, chemotherapy).

Definitions

Viral load was measured in international units/mL, using the Cobas Taqman assay (12 IU/mL) (Roche Diagnostics, Branchburg, NJ).

Complete and Partial Virologic Suppression were defined as HBV DNA < 12 IU/mL and 12-2000 IU/mL, respectively. Virologic rebound was defined as an increase in viral load > 1 $\log_{10}$ from the previous value, in a patient with initial virologic suppression[12]. Cumulative rates of suppression were calculated by the formula P = 1 - (1 - n1/N1) (1- n2/N2). . .(1 - nx/Nx), where P is the cumulative probability that the event will occur, nx is the number of cases at year x, and Nx is the number of patients still followed up at year x[13].

The Scheuer classification system for grading and staging of chronic hepatitis was used for patients that underwent liver biopsy.

Statistical analysis and ethical consideration

All statistical analyses were performed using SPSS (version 16, Chicago IL). The study was approved by the Westmead Hospital Human Research Ethics Committee.

RESULTS

A total of 336 patients were included in the database, which collected data from 2006 to 2010. 89 patients were excluded due to past or current treatment with another agent. A further 83 patients were excluded as they received treatment for less than 3 mo, were less than 18 years old, received prophylactic entecavir, had hepatitis delta co-infection or were pregnant. Thus, 163 patients were included for analysis. The patient demographics are summarised in Table 1.

Treatment setting

All patients were reviewed at least four-monthly by one of six hepatologists at Westmead Hospital Liver Clinic, with assessment of HBV DNA, viral serology, liver function tests and routine haematological and renal laboratory evaluation. Relevant 6-monthly HCC surveillance was also performed, with serum alpha-fetoprotein and hepatic ultrasound.

The mean and median duration of follow up were 26 and 23 mo, respectively. The range of follow up was 3-46 mo, with 50% of patients followed for greater than 24 mo.

Table 1 Baseline demographics (*n* = 163) *n* (%)

Age (yr)	
Mean age	52.0 (24-86)
Mean age at start	47.4 (20-81)
Gender	
Males	113 (69.3)
Females	50 (30.7)
Pre-treatment Biopsies	134 (82.2)
Duration of therapy (mo)	
Range	3-46
Mean	25.63
Median	23
Patients > 24 mo	79 (48.5)
Patients > 36 mo	49 (30.1)
Co-infection	
Hepatitis C	1 (0.61)
HBV DNA load (IU/mL)	
> 100 000	95 (58.3)
2000-100 000	45 (27.6)
12-2000	23 (14.1)
HBe antigen status	41 (25.2)
e antigen positive	112 (68.7)
e antigen negative	10 (6.1)
Unknown	
ALT (U/L)	
10-40	39 (23.9)
40-400	118 (72.4)
> 400	5 (3.01)
Unknown	1 (0.61)
Fibrosis score	0-10 (6.13)
135 (82.82%)	1-61 (37. 4)
	2-38 (23.3)
	3-17 (10.4)
	4-9 (5.52)
	Unknown-27 (16.6)

HBV: Hepatitis B virus; ALT: Alanine aminotransferase.

Response to treatment

The response to entecavir treatment over time is detailed in Table 2, with a graphical summary of virological suppression in Figure 1. In total, 153 patients (93.9%) achieved partial virological suppression (PVS), with 134 patients (82.2%) achieving complete virological suppression (CVS). The cumulative CVS and PVS rates at 36 mo were 82.1% and 96.4%, respectively. Three patients (1.84%) failed to achieve partial or complete suppression (mean duration of therapy 8 mo). Five patients (3.01%) developed virological rebound (mean duration of therapy 29 mo, 3 females, 4 eAg negative, median fibrosis score 1 (biopsies in 4 patients), mean baseline DNA 9.71×10^6 IU/mL). Four of the 5 patients that developed virological rebound had achieved CVS. Reasons for rebound included non-compliance in 4 patients and suspected entecavir resistance in 1 patient. Specific viral resistance testing was not performed. Four patients (2.45%) were changed to another agent due to failed virologic suppression or virologic rebound. Of these, 3 patients (1.84%) were changed to tenofovir and 1 patient (0.61%) to adefovir, with CVS and PVS achieved in 2 patients each, respectively.

One hundred and twenty eight patients (78.53%) maintained CVS throughout follow up. One hundred and twenty nine patients (79.14%) achieved PVS at 12 mo. The annual HBeAg positive to negative seroconversion rate was 14.3%. One patient (HBeAg negative) had loss of surface antigen, resulting in an annual surface antigen loss rate of 0.3% for HBeAg negative patients and 0.2% for all patients, respectively.

Predictors of virological response

The time to achieve CVS was related to baseline DNA levels ($P = 0.001$) and HBeAg status at baseline ($P = 0.001$) (Figure 2).

The mean and median times to CVS were 11.4 (9.77-13.09) and 6 (5.32-6.69) mo, respectively. For HBeAg negative patients the mean time was 6 mo (5.35-6.65), while for HBeAg positive patients it was 12 mo (3.42-20.58) (log rank < 0.001).

There was a statistically significant difference in median time taken to achieve CVS based on age and HBeAg status (Table 3). There was no significant difference in loss of HBeAg based on age (log rank 0.559).

There was no significant effect of duration of therapy, fibrosis score or gender on time to CVS, HBeAg conversion or ALT normalisation. There was a trend towards faster ALT normalisation based on increased age (log rank 0.053).

HCC

Among the 163 patients in the study, 4 patients (2.45%) developed HCC (mean time to development 19.9 mo), while in 6 (3.68%) cases the diagnosis preceded commencement of therapy. Two patients with HCC had a pre-treatment biopsy which did not show cirrhosis (F2 and F3, respectively).

Treatment discontinuation and safety

Twenty one patients (12.88%) failed to follow up in clinic during the study period and thus did not receive ongoing entecavir through the hospital pharmacy. There were no reports of entecavir being ceased due to adverse effects.

DISCUSSION

This study gives an important insight into outcomes of entecavir monotherapy for treatment naive patients with chronic hepatitis B in an Australian tertiary referral setting, with up to 3.5 years follow-up. To our knowledge it is the first study to describe the efficacy, tolerability and resistance rates of entecavir in treatment naive patients in the Australian tertiary care setting. Daily monotherapy with 0.5 mg entecavir was efficacious, with 94% of patients achieving at least partial suppression, and 82% achieving undetectable HBV DNA levels. Only 2% of patients failed to reach any significant reduction in viral load, while 3% developed virologic rebound. Viral resistance testing was not performed. No significant adverse events were reported, and treatment discontinuation was not due to adverse events.

Table 2 Follow up of DNA values and cumulative rates of complete and partial virological suppression *n* (%)

Month	0	6	12	18	24	30	36	42
Patients with DNA	163 (100)	158 (96.9)	141 (86.5)	121 (74.2)	105 (64.4)	76 (46.6)	56 (34.4)	24 (14.7)
Complete virological suppression	0/169 (0)	104/158 (65.8)	117/141 (83.0)	96/121 (74.4)	94/105 (89.5)	65/76 (85.5)	46/56 (82.1)	19/24 (79.2)
Cumulative CVS	0%	66.7%	88.8%	85.1%	94.8%	93.2%	92.2%	91.6%
Partial virological suppression	22/163 (13.5)	141/158 (89.2)	133/141 (94.3)	117/121 (90.7)	100/105 (95.2)	75/76 (98.7)	54/56 (96.4)	23/24 (95.8)
Cumulative PVS	13.5%	89.2%	94.9%	92.1%	96.3%	99.0%	97.3%	96.6%

CVS: Complete virological suppression; PVS: Partial virological suppression.

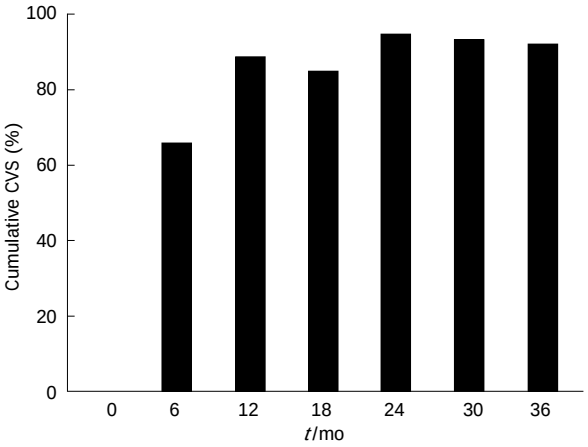


Figure 1 Total cumulative virologic suppression. CVS: Complete virological suppression.

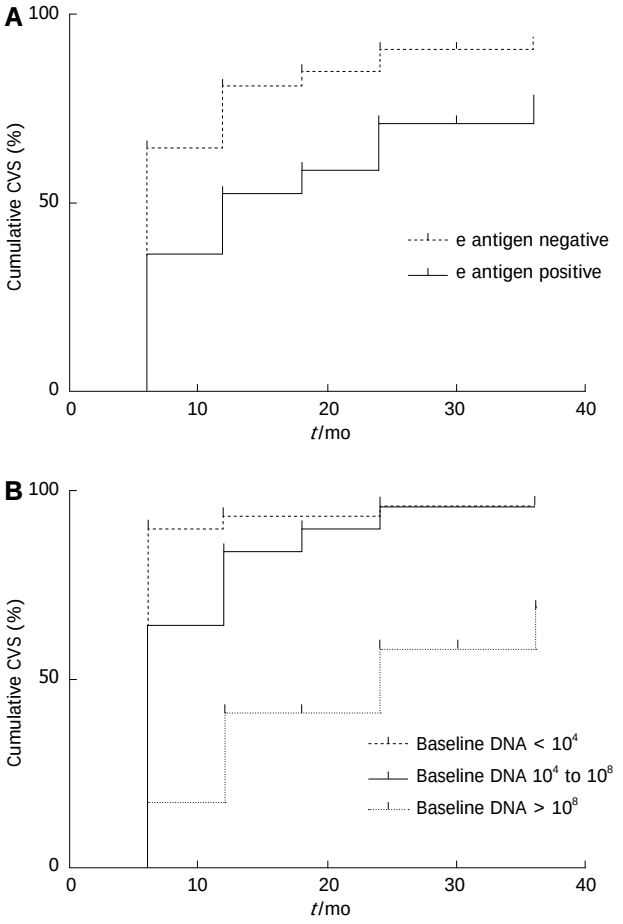


Figure 2 Predictors of complete virological suppression. A: Hepatitis B e antigen status; B: Baseline hepatitis B virus DNA. CVS: Complete virological suppression.

These results are consistent with earlier registration trials, which demonstrated suppression of HBV-DNA to undetectable levels in 67% of HBeAg-positive patients after 12 mo, and in 90% of HBeAg-negative patients[7,8]. Subsequent studies confirmed durable and increasing viral suppression on entecavir, with undetectable HBV DNA achieved by 94% of HBeAg-positive patients over 5 years of treatment, and in 95% of HBeAg-negative patients over 3 years of treatment[14,15]. Clinical trials have also demonstrated minimal emergence of viral resistance (1.2%), after up to 6 years of entecavir treatment[10,16].

Pol *et al*[17] analysed the efficacy and tolerability of entecavir in routine clinical practice settings, by comparing data from 5 international cohorts. In these studies CVS was achieved in 76%-96% of patients, with virologic rebound in 0.6%-4%, and excellent overall tolerability and safety. Therefore the findings from our study are consistent with international data from other "real life" clinical settings, as well as earlier registration trials, which applied more stringent selection criteria.

As has been shown in previous studies, we found that the likelihood of achieving CVS was increased in patients with lower baseline DNA levels and e antigen negative status[18,19]. Amongst patients with baseline HBV DNA $> 10^8$ the CVS was only 67.5% compared to 92% for patients with baseline HBV DNA $< 10^4$. This is most likely explained by the high proportion of eAg positive cases (82%) in the high baseline DNA group compared to the low baseline DNA group (10.5%). The time to achieve CVS was also significantly related to these factors. An interesting and novel finding was that the time taken to achieve CVS was significantly related to age. In patients older than 40 years old the median time to achieve CVS was 6 mo, whereas in patients younger than 40 years old it was 12 mo. This relationship was maintained following HBeAg status stratification, and may reflect better compliance in the older population groups.

A strength of this study is the high proportion of patients in whom pre-treatment liver biopsies were performed (82%). For the duration of this study, liver biopsy

Table 3 Complete virological suppression based on age and HBeAg status

Age-log rank 0.001 (median)		
< 40 yr-	40-55 yr-	> 55 yr-
12 mo (9.25-14.75)	6 mo (5.01-6.99)	6 mo (5.24-6.76)
Hepatitis B e antigen negative status-log rank 0.19 (median)		
< 40 yr-	40-55 yr-	> 55 yr-
6 mo (4.05-7.95)	6 mo (4.99-7.02)	6 mo (5.59-6.41)
Hepatitis B e antigen positive status-log rank 0.19 (median)		
< 40 yr-	40-55 yr-	> 55 yr-
18 mo (7.37-28.63)	18 mo (14.17-26.47)	6 mo (6.5-17.5)
Hepatitis B e antigen seroconversion rate based on age-log rank 0.559 (median)		
< 40 yr-	40-55 yr-	> 55 yr-
36 mo (20.55-51.45)	30 mo (18.46-41.54)	6 mo (9.8-32.2)

was a requirement under the Australian Pharmaceutical Benefit Scheme to access subsidised entacavir treatment. Somewhat surprisingly the degree of fibrosis was not significantly associated with patients achieving CVS, or the time to achieve CVS.

Although there was an association between DNA suppression and ALT normalisation, our study did not take into account other variables that could influence transaminase changes, including anthropomorphic measurements, alcohol intake and intercurrent illness.

The study is limited by its retrospective analysis of medical records and the lack of sufficient follow up data for 13% of patients in the pharmacy registry. Furthermore only a minority of the patients included in the analysis (15%) had completed more than 36 mo of therapy. Finally, viral resistance testing was not performed for patients that failed to achieve PVS or had virologic rebound, as it was not routinely available in our clinic during the study period. The wide range in follow up is mainly due to late commencement in the study period with less time for follow up, drop out of patients and limited treatment duration.

In conclusion, entecavir monotherapy is efficacious and well tolerated for the treatment of patients with chronic hepatitis B infection in Australia. In our cohort baseline HBV DNA levels and HBeAg status influenced time to complete virologic suppression, consistent with previous studies. These factors are not independent, however, as HBeAg negative status most likely correlates with lower baseline HBV DNA levels. There was a very low incidence of failure to suppress DNA, and no apparent emergence of viral resistance on therapy. Our study supports the recommendations that 0.5 mg daily entecavir monotherapy is an appropriate first line agent for patients with treatment naive chronic hepatitis B in Australia.

COMMENTS

Background

An estimated 350 to 400 million people worldwide have chronic hepatitis B virus (HBV) infection, a leading cause of morbidity and mortality. Australia has an ethnically diverse population, with many migrants from the Asia-Pacific region, where the majority of hepatitis B related deaths occur.

Research frontiers

Entecavir is an oral antiviral agent with potent activity against HBV. The rates of entecavir response and resistance in Australia are unknown. Most published data are from large clinical trials, which do not necessarily reflect the rates seen in "real world" clinical practice.

Innovations and breakthroughs

Out of 163 patients, 153 patients (93.9%) achieved at least partial suppression of HBV DNA, with 134 patients (82.2%) achieving complete suppression. Predictors of complete suppression included lower baseline DNA level, HBV e antigen negative status and increasing age at treatment. No significant adverse effects were reported necessitating cessation of entecavir.

Applications

The study results suggest that entecavir monotherapy is efficacious and safe in an Australian tertiary care setting. Resistance and rebound rates are very low. This is similar to data from controlled and uncontrolled trials around the world.

Terminology

Hepatitis B is a viral infection that can lead to persistent inflammation and scarring of the liver. This has significant adverse outcomes including cirrhosis of the liver and liver cancer. Entecavir is a antiviral agent that prevents proliferation of the hepatitis B virus.

Peer review

Well described cohort of entecavir treated patients in clinical practice from Australia; This is an interesting retrospective study from Australia confirms the efficacy and safety of entecavir in clinical practice.

REFERENCES

1 **Lavanchy D**. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *J Viral Hepat* 2004; **11**: 97-107 [PMID: 14996343 DOI: 10.1046/j.1365-2893.2003.00487.x]

2 **Gidding HF**, Warlow M, MacIntyre CR, Backhouse J, Gilbert GL, Quinn HE, McIntyre PB. The impact of a new universal infant and school-based adolescent hepatitis B vaccination program in Australia. *Vaccine* 2007; **25**: 8637-8641 [PMID: 18054127 DOI: 10.1016/j.vaccine.2007.07.046]

3 **O'Sullivan BG**, Gidding HF, Law M, Kaldor JM, Gilbert GL, Dore GJ. Estimates of chronic hepatitis B virus infection in Australia, 2000. *Aust N Z J Public Health* 2004; **28**: 212-216 [PMID: 15707165 DOI: 10.1111/j.1467-842X.2004.tb00697.x]

4 **NNDSS Annual Report Writing Group**. Australia's notifiable disease status, 2010: Annual report of the National Notifiable Diseases Surveillance System. *Commun Dis Intell* 2012; **36**: 1-69 [PMID: 23153082]

5 **Innaimo SF**, Seifer M, Bisacchi GS, Standring DN, Zahler R, Colonno RJ. Identification of BMS-200475 as a potent and selective inhibitor of hepatitis B virus. *Antimicrob Agents Chemother* 1997; **41**: 1444-1448 [PMID: 9210663]

6 **Chang TT**, Gish RG, Hadziyannis SJ, Cianciara J, Rizzetto M, Schiff ER, Pastore G, Bacon BR, Poynard T, Joshi S, Kleszczewski KS, Thiry A, Rose RE, Colonno RJ, Hindes RG. A dose-ranging study of the efficacy and tolerability of entecavir in Lamivudine-refractory chronic hepatitis B patients. *Gastroenterology* 2005; **129**: 1198-1209 [PMID: 16230074 DOI: 10.1053/j.gastro.2005.06.055]

7 **Chang TT**, Gish RG, de Man R, Gadano A, Sollano J, Chao YC, Lok AS, Han KH, Goodman Z, Zhu J, Cross A, DeHertogh D, Wilber R, Colonno R, Apelian D. A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2006; **354**: 1001-1010 [PMID: 16525137 DOI: 10.1056/NEJMoa051285]

8 **Lai CL**, Shouval D, Lok AS, Chang TT, Cheinquer H, Goodman Z, DeHertogh D, Wilber R, Zink RC, Cross A, Colonno R, Fernandes L. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. *N Engl J Med* 2006; **354**: 1011-1020 [PMID: 16525138 DOI: 10.1056/NEJMoa051287]

9 **Lok AS**, Lai CL, Leung N, Yao GB, Cui ZY, Schiff ER, Dien-

stag JL, Heathcote EJ, Little NR, Griffiths DA, Gardner SD, Castiglia M. Long-term safety of lamivudine treatment in patients with chronic hepatitis B. *Gastroenterology* 2003; **125**: 1714-1722 [PMID: 14724824 DOI: 10.1053/j.gastro.2003.09.033]

10 **Tenney DJ**, Rose RE, Baldick CJ, Pokornowski KA, Eggers BJ, Fang J, Wichroski MJ, Xu D, Yang J, Wilber RB, Colonno RJ. Long-term monitoring shows hepatitis B virus resistance to entecavir in nucleoside-naive patients is rare through 5 years of therapy. *Hepatology* 2009; **49**: 1503-1514 [PMID: 19280622 DOI: 10.1002/hep.22841]

11 **Sherman M**, Yurdaydin C, Simsek H, Silva M, Liaw YF, Rustgi VK, Sette H, Tsai N, Tenney DJ, Vaughan J, Kreter B, Hindes R. Entecavir therapy for lamivudine-refractory chronic hepatitis B: improved virologic, biochemical, and serology outcomes through 96 weeks. *Hepatology* 2008; **48**: 99-108 [PMID: 18537189 DOI: 10.1002/hep.22323]

12 **Lok AS**, Zoulim F, Locarnini S, Bartholomeusz A, Ghany MG, Pawlotsky JM, Liaw YF, Mizokami M, Kuiken C. Antiviral drug-resistant HBV: standardization of nomenclature and assays and recommendations for management. *Hepatology* 2007; **46**: 254-265 [PMID: 17596850 DOI: 10.1002/hep.21698]

13 **Pawlotsky JM**, Dusheiko G, Hatzakis A, Lau D, Lau G, Liang TJ, Locarnini S, Martin P, Richman DD, Zoulim F. Virologic monitoring of hepatitis B virus therapy in clinical trials and practice: recommendations for a standardized approach. *Gastroenterology* 2008; **134**: 405-415 [PMID: 18242209 DOI: 10.1053/j.gastro.2007.11.036]

14 **Shouval D**, Lai CL, Chang TT, Cheinquer H, Martin P, Carosi G, Han S, Kaymakoglu S, Tamez R, Yang J, Tenney D, Brett-Smith H. Relapse of hepatitis B in HBeAg-negative chronic hepatitis B patients who discontinued successful entecavir treatment: the case for continuous antiviral therapy. *J Hepatol* 2009; **50**: 289-295 [PMID: 19070393 DOI: 10.1016/j.jhep.2008.10.017]

15 **Chang TT**, Lai CL, Kew Yoon S, Lee SS, Coelho HS, Carrilho FJ, Poordad F, Halota W, Horsmans Y, Tsai N, Zhang H, Tenney DJ, Tamez R, Iloeje U. Entecavir treatment for up to 5 years in patients with hepatitis B e antigen-positive chronic hepatitis B. *Hepatology* 2010; **51**: 422-430 [PMID: 20049753 DOI: 10.1002/hep.23327]

16 **Tenney DJ**, Pokornowski KA, Rose RE, Baldick CJ, Eggers BJ, Fang J, Wichroski MJ, Diva UA, Xu D, Wilber RB, Brett-Smith H, Iloeje UH. W1805 Entecavir Maintains a High Genetic Barrier to HBV Resistance Through 6 Years in Na-iVE Patients. *Gastroenterol* 2009; **136** (Suppl 1): A-865 [DOI: 10.1016/S0016-5085(09)63991-3]

17 **Pol S**, Lampertico P. First-line treatment of chronic hepatitis B with entecavir or tenofovir in 'real-life' settings: from clinical trials to clinical practice. *J Viral Hepat* 2012; **19**: 377-386 [PMID: 22571899 DOI: 10.1111/j.1365-2893.2012.01602.x]

18 **Wong GL**, Wong VW, Chan HY, Tse PC, Wong J, Chim AM, Yiu KK, Chu SH, Chan HL. Undetectable HBV DNA at month 12 of entecavir treatment predicts maintained viral suppression and HBeAg-seroconversion in chronic hepatitis B patients at 3 years. *Aliment Pharmacol Ther* 2012; **35**: 1326-1335 [PMID: 22506552 DOI: 10.1111/j.1365-2036.2012.05098.x]

19 **Yao GB**, Ren H, Xu DZ, Zhou XQ, Jia JD, Wang YM, Chen CW. Virological, serological and biochemical outcomes through 3 years of entecavir treatment in nucleoside-naive Chinese chronic hepatitis B patients. *J Viral Hepat* 2010; **17** Suppl 1: 51-58 [PMID: 20586934]

P- Reviewer Lampertico P **S- Editor** Jiang L **L- Editor** A **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i5.727

World J Gastroenterol 2013 February 7; 19(5): 727-735
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Can mosapride citrate reduce the volume of lavage solution for colonoscopy preparation?

Masahiro Tajika, Yasumasa Niwa, Vikram Bhatia, Shinya Kondo, Tsutomu Tanaka, Nobumasa Mizuno, Kazuo Hara, Susumu Hijioka, Hiroshi Imaoka, Koji Komori, Kenji Yamao

Masahiro Tajika, Yasumasa Niwa, Shinya Kondo, Tsutomu Tanaka, Department of Endoscopy, Aichi Cancer Center Hospital, Nagoya 464-8681, Japan
Vikram Bhatia, Department of Medical Hepatology, Institute of Liver and Biliary Sciences, New Delhi 110070, India
Nobumasa Mizuno, Kazuo Hara, Susumu Hijioka, Hiroshi Imaoka, Kenji Yamao, Department of Gastroenterology, Aichi Cancer Center Hospital, Nagoya 464-8681, Japan
Koji Komori, Department of Gastroenterological surgery, Aichi Cancer Center Hospital, Nagoya 464-8681, Japan
Author contributions: Tajika M contributed to study conception and design, analysis and interpretation of the data, drafting of the article; Niwa Y and Yamao K contributed to critical revision of the article for important intellectual content and final approval of the article; Bhatia V contributed to critical revision of the article and drafting of the article; and Tajika M, Kondo S, Tanaka T, Mizuno N, Hara K, Hijioka S, Imaoka H performed the research and collected the data; Komori K contributed data analysis.
Correspondence to: Masahiro Tajika, MD, PhD, Department of Endoscopy, Aichi Cancer Center Hospital, 1-1 Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan. mtajika@aichi-cc.jp
Telephone: +81-52-7626111 Fax: +81-52-7635233
Received: September 6, 2012 Revised: October 10, 2012
Accepted: November 14, 2012
Published online: February 7, 2013

Abstract

AIM: To evaluate the possibility of reducing the volume of polyethylene glycol (PEG)-electrolyte solution using adjunctive mosapride citrate for colonoscopy preparation.

METHODS: This was a single-center, prospective, randomized, investigator-blinded, non-inferiority study involving 252 patients of both sexes, aged from 20 to 80 years, scheduled for screening or diagnostic colonoscopy in our department. A total of 126 patients was randomized to receive 1.5 L PEG-electrolyte solution plus 15 mg of mosapride (1.5 L group), and 126 received 2 L PEG-electrolyte solution plus 15 mg of mosapride (2 L group). Patients completed a questionnaire on the acceptability and tolerability of the bowel preparation process. The efficacy of bowel preparation was assessed using a 5-point scale based on the Aronchick scale. The primary end point was adequate bowel preparation rates (score of excellent/good/fair) *vs* (poor/inadequate). Acceptability and tolerability, as well as disease detection, were secondary end points.

RESULTS: A total of 244 patients was included in the analysis. There were no significant differences between the 2 L and 1.5 L groups in age, sex, body mass index, number of previous colonoscopies, and the preparation method used previously. The adequate bowel preparation rates were 88.5% in the 2 L group and 82.8% in the 1.5 L group [95% lower confidence limit (LCL) for the difference = -14.5%, non-inferiority P = 0.019] in the right colon. In the left colon, the adequate bowel preparation rates were 89.3% in the 2 L group and 81.1% in the 1.5 L group (95% LCL = -17.0%, non-inferiority P = 0.066). Compliance, defined as complete (100%) intake of the PEG solution, was significantly higher in the 1.5 L group than in the 2 L group (96.8% *vs* 85.7%, P = 0.002). The proportion of abdominal distension (none/mild/moderate/severe) was significantly lower in the 1.5 L group than in the 2 L group (36/65/22/3 *vs* 58/48/18/2, P = 0.040). Within the subgroup who had undergone colonoscopy previously, a significantly higher number of patients in the 1.5 L group than in the 2 L group felt that the current preparation was easier than the previous one (54.1% *vs* 28.0%, P = 0.001). The disease detection rate was not significantly different between the two groups.

CONCLUSION: Although the 1.5 L group had better acceptability and tolerability, 15 mg of mosapride may be insufficient to compensate for a 0.5-L reduction of PEG solution.

© 2013 Baishideng. All rights reserved.

Key words: Mosapride citrate; Bowel preparation; Polyethylene glycol-electrolyte solution; Prokinetics; Colonoscopy

Tajika M, Niwa Y, Bhatia V, Kondo S, Tanaka T, Mizuno N, Hara K, Hijioka S, Imaoka H, Komori K, Yamao K. Can mosapride citrate reduce the volume of lavage solution for colonoscopy preparation? *World J Gastroenterol* 2013; 19(5): 727-735 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i5/727.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i5.727

INTRODUCTION

Polyethylene glycol (PEG)-electrolyte solution is widely used worldwide for bowel cleansing. By consensus of the American Society for Gastrointestinal Endoscopy, the American Society of Colon and Rectal Surgeons, and the Society of American Gastrointestinal and Endoscopic Surgeons, PEG-electrolyte solution is the gold standard for colonoscopic bowel preparation (Grade I A), and aqueous sodium phosphate (NaP) is an alternative regimen to PEG-electrolyte solutions (Grade I A)[1]. Several meta-analyses on the available bowel preparations have favored NaP, concluding that it was effective and better tolerated by patients than PEG-electrolyte solution[2-4]. However, the disadvantages of NaP are its associated side effects. Significant changes in serum electrolyte levels[5], even in patients without renal failure, have prompted recommendations for serum electrolyte evaluation prior to the administration of NaP[6,7].

On the other hand, osmotically balanced electrolyte lavage solutions offer safe and effective cleansing, but volume-related discomfort and adverse experiences have decreased the percentage of patients completing the pre-examination preparation[1,8,9]. This is mainly due to the large volumes of fluid required for bowel preparation, the unpleasant taste, and an increase in the incidence of side effects[10]. Although 3-4 L of this solution is used in Western countries, approximately 2 L of this solution, along with a laxative, is usually considered adequate for bowel preparation in Japan. Despite the lower volume in Japan, the need to drink such large volumes of liquid with an unpalatable taste has a negative impact on patient compliance[11]. Therefore, more effective bowel preparation regimens for colonoscopy are required to improve the acceptability and tolerability of the procedure.

Mosapride citrate (mosapride) is a selective 5-hydroxytryptamine 4 (5-HT4) receptor agonist. Mosapride enhances gastric emptying and motility by facilitating acetylcholine release from the enteric cholinergic neurons, without blocking the dopaminergic D2 receptors[12]. It is known to be effective in gastroesophageal reflux disease[13], functional gastrointestinal disorders, such as functional dyspepsia[14], chronic gastritis with delayed gastric emptying, and diabetic gastroparesis[15]. Since 5-HT4 receptors are also located in the human colon and rectum[16,17], mosapride is also expected to have a prokinetic effect on the colo-rectum. A few clinical studies have reported that mosapride in combination with PEG-electrolyte solution may enhance bowel cleansing and improve patient acceptability and tolerability[18,19]. Furthermore, we previously conducted a randomized, double-blind, placebo-controlled study with mosapride in addition to PEG-electrolyte solution and demonstrated that co-administration of mosapride with PEG-electrolyte solution improved the quality of bowel preparation for colonoscopy, especially in patients without severe constipation[20]. Among the subgroup that had undergone previous colonoscopy, a significantly higher number of mosapride-group patients than placebo-group patients felt that the current preparation was easier. However, mosapride could not improve symptoms such as nausea, abdominal distension, abdominal pain, and willingness to repeat the same regimen compared with placebo. In short, mosapride did not sufficiently improve patient acceptability and tolerability. Therefore, it appears that it is necessary to reduce the volume of PEG-electrolyte solution to improve patient acceptability and tolerability.

The aim of this study was to evaluate the reduction of PEG-electrolyte solution volume when combined with mosapride citrate for colonoscopy preparation.

MATERIALS AND METHODS

This was a prospective, randomized, investigator-blinded study, comparing 1.5 L PEG plus mosapride (1.5 L group), with 2 L PEG-electrolyte solution plus mosapride (2 L group) dosing for patients who underwent colonoscopy. All patients provided written, informed consent prior to entering the study. The study was conducted at Aichi Cancer Center Hospital (ACCH), Nagoya, from January 2010 to June 2010, and was reviewed and approved by the ethics committee of ACCH. This trial was registered in an international clinical trial registry (UMIN000001556).

Study population

All consecutive outpatients of both sexes, aged 20 to 80 years, who were scheduled for screening or diagnostic colonoscopy at ACCH were evaluated for inclusion in the study. Patients with the following clinical features were excluded: significant cardiac, renal, hepatic, or metabolic co-morbidities, ascites, severe constipation (< 2 bowel movements a week), known allergy to PEG-electrolyte solution, history of gastric stapling or bypass procedure, or a history of prior colonic or rectal surgery. Patients were excluded if there was a suspected diagnosis of intestinal obstruction because of advanced colorectal cancer.

Randomization and blinding

Patients were randomly allocated to receive one of two different bowel preparation regimens using a computer-generated random-number list. Patients were randomized in block sizes of two, with serially numbered, sealed, opaque envelopes. Concealed allocation was accom-

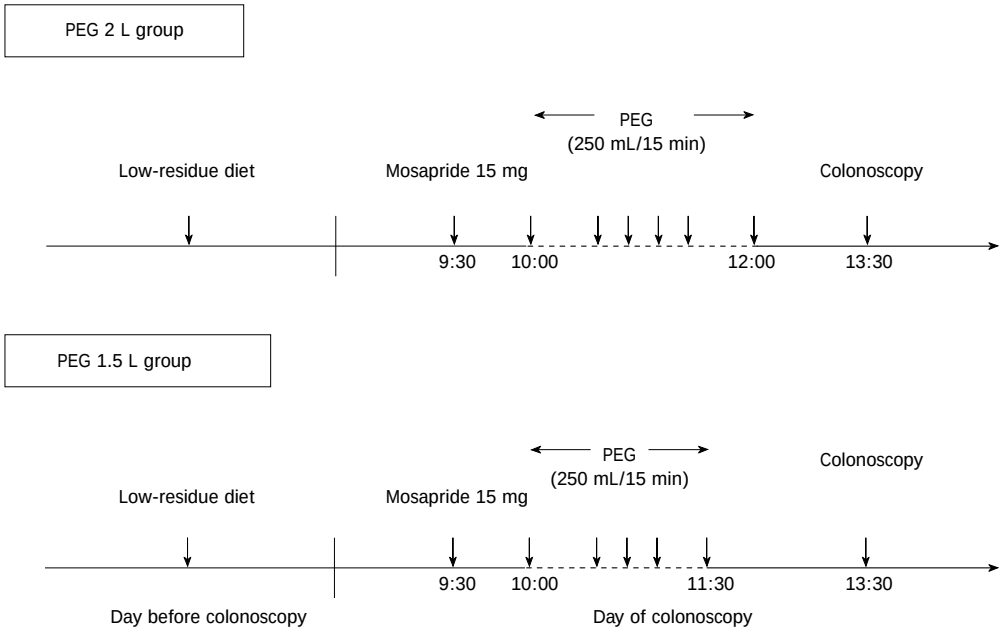


Figure 1 Steps in preparation for colonoscopy. PEG: Polyethylene glycol.

plished through non-research personnel who were not involved in this study. Patients were instructed not to discuss their bowel preparation with anyone other than the unblinded research assistant. With the exceptions of the patient and the unblinded research assistant, all other individuals participating in this study, including the endoscopists and endoscopy nurses, were blinded to the allocated treatment group. Comparisons between the 1.5 L group and the 2 L group were made in an investigator-blind fashion.

Bowel preparation methods

The day before colonoscopy, all patients were instructed to eat a pre-packaged, low residue diet (Enimaclin CS; Horii Pharmaceutical Ind., Ltd., Osaka, Japan) that consisted of a lunch, snack, and dinner, and asked to drink more than 2 liters of clear liquid. On the day of the colonoscopy, all participants reported to the endoscopy room at 9:00 am and received in-hospital bowel preparation. In-hospital preparation was important to ensure uniformity and remove any confounding caused by poor patient adherence. More than 10 toilet facilities were made available in the endoscopy unit for patient comfort. Six mosapride tablets (15 mg) (Gasmotin; Dainippon Sumitomo Pharma Co., Ltd. Osaka, Japan) were administered orally with water at half past nine. After 30 min, both groups were instructed to drink 0.25 L of PEG-electrolyte solution (Niflec; Ajinomoto Pharmaceuticals Co., Ltd. Tokyo, Japan) every 15 min (Figure 1). Colonoscopies were performed from half past thirteen, and the start times were recorded for each patient.

Evaluation of bowel preparation

The efficacy of bowel preparation was assessed using the Aronchick scale[21]. Participating endoscopists were trained to use the Aronchick scale to achieve a good level of agreement. Investigators performed calibration exercises involving more than 20 colonoscopies prior to study commencement, based on their interpretation of scale anchors, to ensure that their findings agreed. The final assessment of bowel preparation was divided into two categories, adequate and failure. Bowel preparation rated as fair, good, or excellent, based on the Aronchick scale, was considered adequate; poor or inadequate ratings were considered failure. After colonoscopy, two observers, one who was the operator and the other who was a fellow in the procedure room, decided the score by mutual agreement. They scored the quality of the preparation on the right side (proximal to splenic flexure) colon and on the left side (distal to splenic flexure) colon and rectum separately. If the decision was discordant, a third expert reviewer graded and scored the recorded images later, and this evaluation was used in the final analysis. Twelve experienced colonoscopists carried out all colonoscopy procedures, each of whom had performed more than 1000 colonoscopies.

During or immediately following the colonoscopy, the investigator completed a physician questionnaire regarding assessment of bowel preparation, amount of irrigation fluid used, time needed to reach the cecum, and ease of insertion to the cecum and difficulty in observing the lumen of the colo-rectum because of peristalsis.

Patient tolerance and other measurements

The nursing staff recorded the time required to drink the indicated volume of lavage solution. They also recorded the time and number of bowel movements from the start of ingestion to the appearance of clear excretion. Until one hour after finishing the PEG-electrolyte solution plus mosapride, the nursing staff checked patients' excretions.

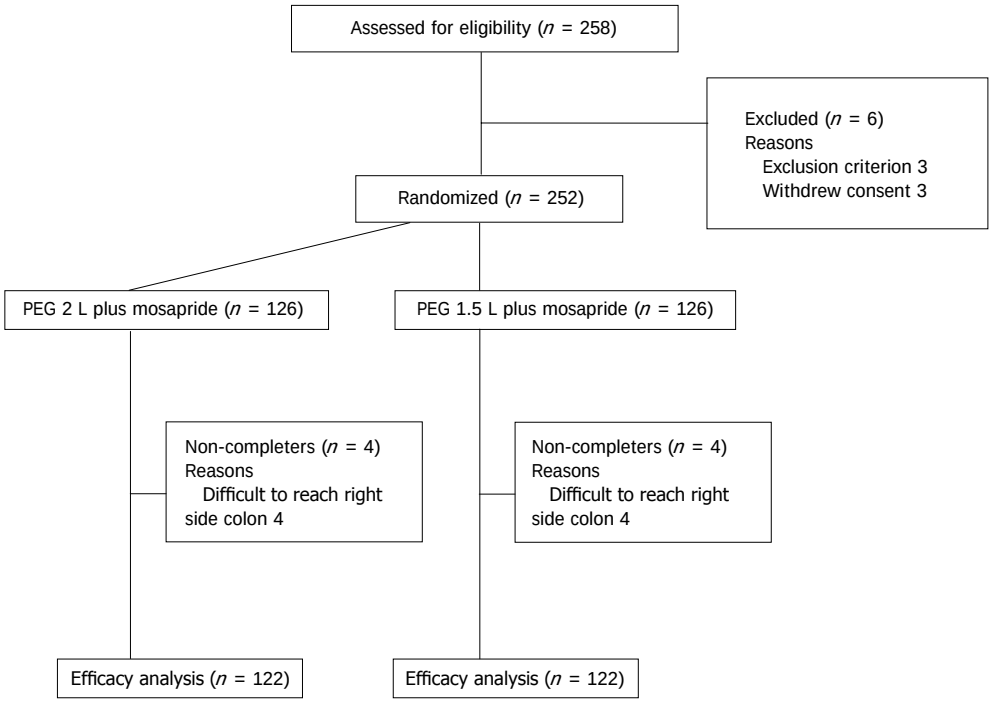


Figure 2 Patient disposition flow chart. PEG: Polyethylene glycol.

If there was a solid stool with muddy excretions or no excretion at that time, the patient was given an additional preparation, such as additional PEG-electrolyte solution or enemas. The patients who received an additional preparation were defined by the Aronchick scale as inadequate. The patient questionnaire consisted of 20 questions[20]. The adverse events were scored using a 4-point scale, where 1 = none, 2 = mild, 3 = moderate, and 4 = severe. The patients completed the questionnaire form before undergoing colonoscopy and submitted it to the nursing staff.

End points

The primary end point was the difference in the rate of adequate colon cleansing between the 1.5 L PEG-electrolyte solution plus 15 mg of mosapride (1.5 L group) and the 2 L PEG-electrolyte solution plus 15 mg of mosapride (2 L group). Secondary end points included differences in patients' acceptability and tolerance of solutions, time to first defecation, frequency of defecation, complete time for colonic preparation, time needed to reach the cecum, amount of irrigation fluid used, subjective difficulty in colonoscopy insertion to the cecum and in observing the lumen of the colo-rectum because of peristalsis, and disease detection rates.

Statistical analysis

Based on a previous study[20], the adequate bowel preparation rate for the PEG-electrolyte solution plus mosapride was expected to be less than 80%. It was expected that about 80% of the 1.5 L group would be judged adequate, and the non-inferiority margin was set at -15%. This study was designed to have 80% power to establish non-inferiority (using a one-sided significance level of 0.025 and a target sample size of 250).

The primary efficacy analysis was based on an intent-to-treat analysis and included patients who were randomized and received any treatment. In patients in this group, the preparation was classified as adequate or inadequate based on the colonoscopists' score of cleansing. Patients who did not undergo colonoscopy because of preparation-related adverse events, or preparation failure, or in whom the right colon could not be reached because of bowel obstruction or technical reasons were excluded. The rates of adequate preparation were compared between the groups by χ^2 test or Fisher's exact test for categorical variables.

For the secondary end points, the Mann-Whitney *U* test was used to compare continuous variables. Categorical variables were tested using the corrected χ^2 test or 2-sided Fisher's exact tests as appropriate. The criterion for significance was $P < 0.05$.

All statistical analyses were performed using Statistical Analysis Software (SAS Ver. 9.2 for the PC, SAS Institute Inc., Cary, NC, United States).

RESULTS

Patients' characteristics

A total of 252 patients was randomized into two groups (Figure 2). Although 252 patients were analyzed, colonoscope insertion to the right colon failed in 4 patients in each group (advanced stenosing cancer in two, and patient refusal in six because of pain). These eight patients were excluded from the efficacy analysis. The baseline characteristics of the patients are shown in Table

Table 1 Baseline characteristics

Variable	2.0 L	1.5 L	*P* value
Patients (*n*)	126	126	
Age (yr, mean ± SD)	65.3 ± 9.9	66.3 ± 9.6	NS
< 60	30	25	
60 - < 70	51	44	NS
⩾ 70	45	53	
Male	77	67	NS
Female	49	59	
Indication (*n*)			
Screening	40	41	
Surveillance	67	63	NS
Diagnostic	19	22	
Body mass index (kg/m^2)	22.5 + 3.2	22.3 + 2.7	NS
Previous colonoscopy			
None (first time)	44	41	NS
⩾ 2	82	85	
Previous preparation for colonoscopy (*n*)			
2L PEG	82	85	NS

PEG: Polyethylene glycol; NS: Not significant.

Table 2 Overall colon-cleansing efficacy *n* (%)

Variable	Right side colon		Left side colon and rectum		*P* value[1]	
	2.0 L	1.5 L	2.0 L	1.5 L	Right	Left
Patients (*n*)	122	122	122	122		
Overall score (*n*)						
Excellent	36	22	48	37		
Good	52	38	49	45		
Fair	20	41	12	17	0.006	NS
Poor	3	3	2	5		
Inadequate	11	18	11	18		
No. adequate	108 (88.5)	101 (82.8)	109 (89.3)	99 (81.1)	NS	NS

[1]*P* value by χ^2 test. PEG: Polyethylene glycol; NS: Not significant.

1. There was no significant difference in age, sex, body mass index, number of previous colonoscopies, and the preparation method used previously between the 2.0 L and 1.5 L groups.

Bowel cleansing efficacy

The efficacy of bowel preparation is shown in Table 2. The adequate bowel preparation rates were 88.5% in the 2 L group and 82.8% in the 1.5 L group (95% lower confidence limit, lower confidence limit (LCL), for the difference = -14.5%, non-inferiority $P = 0.019$) in the right side colon. In the left colon, the adequate bowel preparation rates were 89.3% in the 2 L group and 81.1% in the 1.5 L group (95% LCL = -17.0%, non-inferiority $P = 0.066$). In the right side colon, there were significant differences in the proportion of the overall colon-cleansing score between the two groups ($P = 0.006$). Eleven patients (8.7%) required additional preparation in the 2 L group. On the other hand, 18 patients (14.3%) required additional preparation in the 1.5 L group. However, there was no significant difference in required additional preparation between the two groups.

As shown in Table 3, there were no differences in frequency of defecation, time needed to reach the cecum, elapsed time from last fluid intake to colonoscopy, amount of irrigation fluid used, and subjective difficulties in insertion to the cecum and in observing the lumen of the colo-rectum between the two groups.

Patient acceptability, tolerability, and safety

There was no significant difference in compliance as defined by > 80% intake of the prescribed PEG-electrolyte solution volume. However, complete (100%) intake of the PEG solution was significantly higher in the 1.5 L group than in the 2 L group ($P = 0.002$) (Table 3). The proportion of abdominal distension was significantly less in the 1.5 L group than in the 2 L group ($P = 0.040$), but symptoms such as nausea, vomiting, abdominal pain, and circulatory reactions were similar in both groups. The proportion of patients willing to repeat the same preparation regimen was significantly higher in the 1.5 L group ($P = 0.034$). Furthermore, among the subgroup of patients who had undergone colonoscopy more than twice previously, a significantly higher number of patients in the 1.5 L group than in the 2 L group felt that the current preparation was easier than in the past ($P = 0.001$).

Disease detection rate

In this study, 11 colorectal cancers were detected in 11 patients (4.4%), 4 (3.2%) in the 2 L group and 7 (5.6%) in the 1.5 L group (Table 4). A total of 177 polyps was detected in 74 patients (58.7%) in the 2 L group, and 187 polyps were detected in 73 patients (57.9%) in the 1.5 L group. The proportions of polyps by size and location were similar in the two groups.

DISCUSSION

In this study, 1.5 L PEG-electrolyte solution plus mosapride was found to be non-inferior to 2 L PEG-electrolyte solution plus mosapride with respect to adequate bowel preparation rates only in the right colon, not in the entire colo-rectum. On the other hand, patient tolerability, especially abdominal distension, and acceptability were superior in the 1.5 L group compared to the 2 L group.

This is the first study, to the best of our knowledge, that has evaluated the effect of mosapride when used in conjunction with reduced dose, 1.5 L PEG-electrolyte solution for colonoscopy preparation. We previously conducted a randomized, double-blind, placebo-controlled study with mosapride in addition to PEG-electrolyte solution and demonstrated that co-administration of mosapride with PEG-electrolyte solution improved the quality of bowel preparation for colonoscopy, especially in patients without severe constipation[20]. On the other hand, the beneficial effect of mosapride on gastric emptying[22] was expected to ameliorate nausea, vomiting, and fullness of the abdomen during bowel preparation. Mishima *et al*[19] showed that administration of mosapride prior to PEG-electrolyte solution significantly decreased the incidence of uncomfortable abdominal symptoms. However, there were no significant differences in the

Table 3 Results of preparation, endoscopic findings and patient questionnaire *n* (%)

Variable	2.0 L	1.5 L	*P* value
Patients	126	126	
Time to first defecation (min, mean ± SD)	55.7 ± 27.4	56.4 ± 27.8	NS
Frequency of defecation (times, median, quartile)	7 (4-15)	7 (4-15)	NS
Time to preparation (min, mean ± SD)	157.3 ± 51.9	159.6 ± 57.1	NS
Elapsed time from last fluid intake to colonoscopy (min, mean ± SD)	169.4 ± 56.5	179.7 ± 61.1	NS
Cecal intubation rate	122 (96.8)	122 (96.8)	NS
Insertion time (min, median, quartile)[1]	11.4 (3-76)	10.1 (3-47)	NS
Feel of peristalsis	20 (16.4)	25 (20.5)	NS
Amount of irrigation fluid			
None	38	41	NS
< 50 mL	74	74	
50-100 mL	9	9	
> 100 mL	5	2	
Compliance > 80%	121 (96.0)	125 (99.2)	NS
100% intake	108 (85.7)	122 (96.8)	0.002
Any symptom			
Nausea (none/mild/moderate/severe)	109/13/3/1	117/5/3/1	NS
Vomiting (none/mild/moderate/severe)	0/0/1/0	0/0/0/0	NS
Distension (none/mild/moderate/severe)	36/65/22/3	58/48/18/2	0.04
Abdominal pain (none/mild/moderate/severe)	98/26/2/0	107/18/1/0	NS
Circulatory reactions (none/mild/moderate/severe)	0	0	NS
Willingness to repeat			
The same preparation regimen (much/fair/never)	78/19/29	97/12/17	0.034
How easy/difficult to take preparation compared to previous (easy/no difference/difficult)	23/54/5	46/36/3	0.001

[1]Insertion time was calculated without including the patients whose cecal portion was not examined. NS: Not significant.

Table 4 Characteristics of the endoscopic diagnosis *n* (%)

Variable	2.0 L		1.5 L		*P* value (2.0 L *vs* 1.5 L)	
	Right side colon	Left side colon	Right side colon	Left side colon	Right	Left
Patients	126		126			
Cancer patients	3	1	5	2[1]	NS	NS
Polyp patients	74 (58.7)		73 (57.9)			
Proportion of polyps						
< 5 mm	60	62	65	70		
5-10 mm	15	29	20	19	NS	NS
> 10 mm	6	5	8	5		
Total polyps per study arm	81	96	93	94	NS	NS
Polyps per patient, mean ± SD	0.71 ± 1.19	0.79 ± 1.20	0.78 ± 1.19	0.78 ± 1.11	NS	NS
Diverticulosis	30		37			

[1]Advanced stenosing cancer. NS: Not significant.

frequencies of these symptoms between the mosapride group and the placebo group in the previous study[20]. Therefore, we think that there is a need to reduce the volume of PEG-electrolyte solution to improve patients' acceptability and tolerability. In the present study, it was assumed that 2 L PEG-electrolyte solution plus mosapride was the standard regimen for bowel preparation based on the results of the previous study. Thus, the study was designed to compare a 1.5 L PEG group with a 2 L PEG group.

In the present study, the patients' acceptability and tolerability were superior in the 1.5 L group. The 0.5 L reduction of PEG-electrolyte solution significantly improved patients' acceptability and tolerability; 100% intake of PEG-electrolyte solution was significantly higher in the 1.5 L group than in the 2 L group. For Japanese patients with relatively smaller physiques than Western patients, 2 L PEG-electrolyte solution may be too much to drink. With respect to adverse events, abdominal distension was more common than nausea, vomiting, and abdominal pain. The proportion of abdominal distension was significantly improved in the 1.5 L group compared with the 2 L group. This may be the reason why willingness to repeat the same preparation regimen was significantly higher in the 1.5 L group, and a significantly higher number of patients in the 1.5 L group than in the 2 L group felt that the current preparation was easier.

Although 0.5-L volume reduction improved patient acceptability and tolerability, it would not make sense to decrease the volume of solution if the adequate bowel

preparation rates were worse, and it prolonged the time to preparation. In the present study, 1.5 L PEG was non-inferior to 2 L PEG with respect to adequate bowel preparation rates in the right colon, but the proportion for the overall colon-cleansing score was significantly higher in the 2 L PEG group than in the 1.5 L PEG group. Furthermore, although there was no significant difference between the two groups, 18 patients required additional preparation in the 1.5 L group compared with 11 patients in the 2 L group. One of the reasons why the times to preparation were similar in the two groups is that it took longer for the patients who required additional preparation in the 1.5 L group compared with the 2 L group. From these results, we cannot help but recognize that the dose of 15 mg may be insufficient to compensate for the 0.5-L reduction in PEG solution with respect to cleansing efficacy. In the present study, 15 mg of mosapride was given for colonoscopy preparation. The dose of 15 mg per day is the recommended usual dosage of mosapride citrate for adult patients with chronic gastritis. Since the effects of mosapride are reported to be dose-dependent[23], additional studies that address the optimal dosage are required to clarify the best bowel preparation method for colonoscopy.

Over the years, many researchers have investigated several different combinations and dosages of prokinetic agent or laxatives in search of acceptable, tolerable, and efficacious low-volume bowel preparation that may lead to a better experience for the patient and a more thorough colonoscopic examination[24-26]. Cisapride has been used as a prokinetic agent along with lavage solution for bowel preparation and has been demonstrated to shorten the required time period for precolonoscopic bowel preparation and to decrease the lavage solution volume[27,28], although these results have been difficult to reproduce[29]. However, cisapride was withdrawn from the market because of severe cardiac effects[30]. Other prokinetic agents, including metoclopramide and tegaserod, have been co-administered with oral lavage solution in an attempt to improve the quality of bowel preparation and patient tolerance to lavage solution through increasing the amplitude of gastric contraction and peristalsis of small intestine, and shortening transit time[31,32]. However, the effect of these agents had not yet been clearly established, and the results of studies that have evaluated these agents have thus far been contradictory[33]. The effects of prokinetic agents with the reported timings and doses may not be enough to compensate for the large volume of PEG solution.

Stimulant laxatives such as bisacodyl and magnesium citrate have been used as adjuncts to low-volume PEG-electrolyte solution, achieving results similar to those with full-volume PEG-electrolyte solution[8,34]. Recently, Cohen *et al*[35] compared a reduced-dose 2 L PEG formulation plus ascorbic acid with 2 L PEG formulation plus bisacodyl. The authors found that the use of PEG plus ascorbic acid resulted in better colon cleansing and higher adenoma detection rates than PEG plus bisacodyl. Moreover, Repici *et al*[36] compared a new iso-osmotic sulphate-free formulation (2 L formulation of PEG-citrate-simethicone) in combination with bisacodyl with 2 L formulation PEG plus ascorbic acid. The authors reported that low-volume PEG-citrate-simethicone with bisacodyl provided better bowel cleansing and similar tolerability and acceptance compared with PEG plus ascorbic acid. Unfortunately, these low-volume formulations are currently not available in Japan. In the previous study[20], we selected mosapride from among several prokinetic agents because it is a highly selective agonist for 5-HT4 receptors and does not affect other receptors, including dopamine D2 receptors. However, the results of this study did not demonstrate the efficacy of mosapride in reducing lavage solution volume. A combination with some laxatives may improve the cleansing efficacy of our low-volume 1.5 L PEG formulation plus mosapride.

Few studies designed to assess the quality of bowel preparation for colonoscopy have also examined the disease detection rates, including adenoma detection rates[37-39]. Previous studies demonstrated that a better bowel preparation led to a higher rate of colon lesion detection, enhancing the ability to discern smaller lesions and thus improving the thoroughness of colonoscopy[37,38]. In the present study, there were no differences between the two groups in the polyp detection rate, the proportion of the size of polyps, total polyps per study arm, and polyps per patient. These findings may lead to the conclusion that the efficacy of bowel preparation with 1.5 L PEG is non-inferior to 2 L PEG with respect to bowel cleansing. However, polyp detection rates are indeed affected by several variables, such as patients' background, colonoscopy indication, and endoscopist technique, as well as endoscopy technology, that would introduce uncertainly into the results of this study. Additional studies are necessary to demonstrate the relationship between bowel preparation and the adenoma detection rate.

There are several limitations to consider in interpreting the present results. First, the study was conducted in a single hospital with a small number of patients. Although we hypothesized that the non-inferiority margin was -15%, that margin might be inappropriate. The sample size may have been too small to elucidate the non-inferiority of 1.5 L PEG, which may explain why non-inferiority in only a limited part of colon could be demonstrated. Second, for the evaluation of bowel preparation, the Aronchick scale was used. The merit of the Aronchick scale is that in cases in which additional bowel preparation was needed, such cases could be defined as "inadequate" using the Aronchick scale. However, the Aronchick scale was designed to assess bowel preparation of the entire colon. In the present study, it was scored separately on the right side colon and left side colon; a different scoring system, such as the Ottawa scale[40] and the Boston scale[41], that evaluates different colon segments individually and generates a summary score may have been more appropriate. Finally, biochemical parameters were not evaluated in the two groups. However, co-administration of mosapride at a dose of 40 mg and

PEG-electrolyte solution is already approved in Japan for barium enema examination preparation[42], based on its excellent safety profile.

In conclusion, co-administration of mosapride with 1.5 L PEG-electrolyte solution was non-inferior to mosapride with 2.0 L PEG-electrolyte solution for adequate bowel preparation rates only in the right colon, although better acceptability and tolerability compared to the larger PEG-electrolyte solution volumes were found. A mosapride dose of 15 mg may provide insufficient cleansing efficacy to compensate for a 0.5-L reduction in PEG-electrolyte solution.

COMMENTS

Background

Although polyethylene glycol (PEG) electrolyte solution has been used for colonoscopy preparation since 1980, the need to drink large volumes is a limiting factor that affects patient tolerance. Low-volume bowel preparation regimens for colonoscopy are reported to improve patients' acceptance and compliance.

Research frontiers

Mosapride citrate (mosapride) is a selective 5-hydroxytryptamine 4 (5-HT4) receptor agonist. Mosapride enhances gastric emptying and motility by facilitating acetylcholine release from enteric cholinergic neurons, without blocking the dopaminergic D2 receptors. Since 5-HT4 receptors are also located in the human colon and rectum, mosapride is also expected to have a prokinetic effect on the colo-rectum.

Innovations and breakthroughs

The present randomized trial compared 1.5 L PEG plus mosapride with 2 L PEG plus mosapride dosing for patients who underwent colonoscopy in terms of cleansing effectiveness, patient compliance, tolerability, and disease detection rates.

Applications

The low-volume preparation (1.5 L PEG) represents a valid alternative to high-volume preparations (2 L PEG) with regard to patient compliance and tolerability. However, optimal visualization of colonic wall seems to be one of the primary advantages of high-volume preparations. A mosapride dose of 15 mg may provide insufficient cleansing efficacy to compensate for a 0.5-L reduction in PEG solution.

Terminology

PEG-electrolyte solution: PEG-electrolyte solution is used worldwide for bowel cleansing. Approximately 2 L of this oral solution with some laxatives are usually required for adequate bowel preparation in Japan.

Peer review

This randomized trial compared 1.5 L PEG plus mosapride with 2 L PEG plus mosapride in terms of cleansing effectiveness, patient acceptability, physical tolerability, and endoscopic findings. This is an interesting and well written study. The conclusion sounds good and useful for the general practice.

REFERENCES

1 **Wexner SD**, Beck DE, Baron TH, Fanelli RD, Hyman N, Shen B, Wasco KE. A consensus document on bowel preparation before colonoscopy: prepared by a task force from the American Society of Colon and Rectal Surgeons (ASCRS), the American Society for Gastrointestinal Endoscopy (ASGE), and the Society of American Gastrointestinal and Endoscopic Surgeons (SAGES). *Gastrointest Endosc* 2006; **63**: 894-909 [PMID: 16733101 DOI: 10.1016/j.gie.2006.03.918]

2 **Young CJ**, Simpson RR, King DW, Lubowski DZ. Oral sodium phosphate solution is a superior colonoscopy preparation to polyethylene glycol with bisacodyl. *Dis Colon Rectum* 2000; **43**: 1568-1571 [PMID: 11089594]

3 **Lee J**, McCallion K, Acheson AG, Irwin ST. A prospective randomised study comparing polyethylene glycol and sodium phosphate bowel cleansing solutions for colonoscopy. *Ulster Med J* 1999; **68**: 68-72 [PMID: 10661631]

4 **Hsu CW**, Imperiale TF. Meta-analysis and cost comparison of polyethylene glycol lavage versus sodium phosphate for colonoscopy preparation. *Gastrointest Endosc* 1998; **48**: 276-282 [PMID: 9744604]

5 **Verghese VJ**, Ayub K, Qureshi W, Taupo T, Graham DY. Low-salt bowel cleansing preparation (LoSo Prep) as preparation for colonoscopy: a pilot study. *Aliment Pharmacol Ther* 2002; **16**: 1327-1331 [PMID: 12144583]

6 **DiPalma JA**, Buckley SE, Warner BA, Culpepper RM. Biochemical effects of oral sodium phosphate. *Dig Dis Sci* 1996; **41**: 749-753 [PMID: 8674396]

7 **Sharma VK**, Schaberg JW, Chockalingam SK, Vasudeva R, Howden CW. The effect of stimulant laxatives and polyethylene glycol-electrolyte lavage solution for colonoscopy preparation on serum electrolytes and hemodynamics. *J Clin Gastroenterol* 2001; **32**: 238-239 [PMID: 11246353]

8 **Belsey J**, Epstein O, Heresbach D. Systematic review: adverse event reports for oral sodium phosphate and polyethylene glycol. *Aliment Pharmacol Ther* 2009; **29**: 15-28 [PMID: 18729847 DOI: 10.1111/j.1365-2036.2008.03837.x]

9 **Pox C**, Schmiegel W, Classen M. Current status of screening colonoscopy in Europe and in the United States. *Endoscopy* 2007; **39**: 168-173 [PMID: 17327977 DOI: 10.1055/s-2007-966182]

10 **Gili M**, Roca M, Ferrer V, Obrador A, Cabeza E. Psychosocial factors associated with the adherence to a colorectal cancer screening program. *Cancer Detect Prev* 2006; **30**: 354-360 [PMID: 16963195 DOI: 10.1016/j.cdp.2006.06.005]

11 **Harewood GC**, Wiersema MJ, Melton LJ. A prospective, controlled assessment of factors influencing acceptance of screening colonoscopy. *Am J Gastroenterol* 2002; **97**: 3186-3194 [PMID: 12492209 DOI: 10.1111/j.1572-0241.2002.07129.x]

12 **Yoshida N**, Omoya H, Oka M, Furukawa K, Ito T, Karasawa T. AS-4370, a novel gastrokinetic agent free of dopamine D2 receptor antagonist properties. *Arch Int Pharmacodyn Ther* 1989; **300**: 51-67 [PMID: 2533479]

13 **Ruth M**, Finizia C, Cange L, Lundell L. The effect of mosapride on oesophageal motor function and acid reflux in patients with gastro-oesophageal reflux disease. *Eur J Gastroenterol Hepatol* 2003; **15**: 1115-1121 [PMID: 14501621 DOI: 10.1097/01.meg.0000085480.12407.de]

14 **Mizuta Y**, Shikuwa S, Isomoto H, Mishima R, Akazawa Y, Masuda J, Omagari K, Takeshima F, Kohno S. Recent insights into digestive motility in functional dyspepsia. *J Gastroenterol* 2006; **41**: 1025-1040 [PMID: 17160514 DOI: 10.1007/s00535-006-1966-z]

15 **Asakawa H**, Hayashi I, Fukui T, Tokunaga K. Effect of mosapride on glycemic control and gastric emptying in type 2 diabetes mellitus patients with gastropathy. *Diabetes Res Clin Pract* 2003; **61**: 175-182 [PMID: 12965107]

16 **McLean PG**, Coupar IM. Stimulation of cyclic AMP formation in the circular smooth muscle of human colon by activation of 5-HT4-like receptors. *Br J Pharmacol* 1996; **117**: 238-239 [PMID: 8789374]

17 **Sakurai-Yamashita Y**, Yamashita K, Kanematsu T, Taniyama K. Localization of the 5-HT(4) receptor in the human and the guinea pig colon. *Eur J Pharmacol* 1999; **383**: 281-285 [PMID: 10594320]

18 **Nakashima M**, Okumura S, Iizuka H, Ohmae Y, Sagawa T, Kudo T, Masuo T, Kobayashi R, Marubashi K, Ishikawa T, Oshimoto H, Yoshida M, Motegi K, Sakamoto T, Iesaki K, Mori M. Mosapride Citrate for Colonoscopy Preparation with Lavage. *Kitakanto Med J* 2002; **52**: 111-115

19 **Mishima Y**, Amano Y, Okita K, Takahashi Y, Moriyama N, Ishimura N, Furuta K, Ishihara S, Adachi K, Kinoshita Y. Efficacy of prokinetic agents in improving bowel preparation for colonoscopy. *Digestion* 2008; **77**: 166-172 [PMID: 18577886 DOI: 10.1159/000141040]

20 **Tajika M**, Niwa Y, Bhatia V, Kawai H, Kondo S, Sawaki A,

Mizuno N, Hara K, Hijioka S, Matsumoto K, Kobayashi Y, Saeki A, Akabane A, Komori K, Yamao K. Efficacy of mosapride citrate with polyethylene glycol solution for colonoscopy preparation. *World J Gastroenterol* 2012; **18**: 2517-2525 [PMID: 22654449 DOI: 10.3748/wjg.v18.i20.2517]

21 **Aronchick CA**, Lipshutz WH, Wright SH, Dufrayne F, Bergman G. A novel tableted purgative for colonoscopic preparation: efficacy and safety comparisons with Colyte and Fleet Phospho-Soda. *Gastrointest Endosc* 2000; **52**: 346-352 [PMID: 10968848 DOI: 10.1067/mge.2000.108480]

22 **Kanaizumi T**, Nakano H, Matsui Y, Ishikawa H, Shimizu R, Park S, Kuriya N. Prokinetic effect of AS-4370 on gastric emptying in healthy adults. *Eur J Clin Pharmacol* 1991; **41**: 335-337 [PMID: 1804650]

23 **Mine Y**, Morikage K, Oku S, Yoshikawa T, Shimizu I, Yoshida N. Effect of mosapride citrate hydrate on the colon cleansing action of polyethylene glycol electrolyte lavage solution (PEG-ELS) in guinea pigs. *J Pharmacol Sci* 2009; **110**: 415-423 [PMID: 19602846]

24 **Sharma VK**, Chockalingham SK, Ugheoke EA, Kapur A, Ling PH, Vasudeva R, Howden CW. Prospective, randomized, controlled comparison of the use of polyethylene glycol electrolyte lavage solution in four-liter versus two-liter volumes and pretreatment with either magnesium citrate or bisacodyl for colonoscopy preparation. *Gastrointest Endosc* 1998; **47**: 167-171 [PMID: 9512283]

25 **Adams WJ**, Meagher AP, Lubowski DZ, King DW. Bisacodyl reduces the volume of polyethylene glycol solution required for bowel preparation. *Dis Colon Rectum* 1994; **37**: 229-233; discussion 233-234 [PMID: 8137669]

26 **DiPalma JA**, Wolff BG, Meagher A, Cleveland Mv. Comparison of reduced volume versus four liters sulfate-free electrolyte lavage solutions for colonoscopy colon cleansing. *Am J Gastroenterol* 2003; **98**: 2187-2191 [PMID: 14572566 DOI: 10.1111/j.1572-0241.2003.07690.x]

27 **Lazarczyk DA**, Stein AD, Courval JM, Desai D. Controlled study of cisapride-assisted lavage preparatory to colonoscopy. *Gastrointest Endosc* 1998; **48**: 44-48 [PMID: 9684663]

28 **Ueda S**, Iishi H, Tatsuta M, Oda K, Osaka S. Addition of cisapride shortens colonoscopy preparation with lavage in elderly patients. *Aliment Pharmacol Ther* 1994; **8**: 209-214 [PMID: 8038353]

29 **Martínek J**, Hess J, Delarive J, Jornod P, Blum A, Pantoflickova D, Fischer M, Dorta G. Cisapride does not improve precolonoscopy bowel preparation with either sodium phosphate or polyethylene glycol electrolyte lavage. *Gastrointest Endosc* 2001; **54**: 180-185 [PMID: 11474387]

30 **Tonini M**, De Ponti F, Di Nucci A, Crema F. Review article: cardiac adverse effects of gastrointestinal prokinetics. *Aliment Pharmacol Ther* 1999; **13**: 1585-1591 [PMID: 10594392]

31 **Rhodes JB**, Engstrom J, Stone KF. Metoclopramide reduces the distress associated with colon cleansing by an oral electrolyte overload. *Gastrointest Endosc* 1978; **24**: 162-163 [PMID: 348558]

32 **Sanaka MR**, Super DM, Mullen KD, Ferguson DR, McCullough AJ. Use of tegaserod along with polyethylene glycol electrolyte solution for colonoscopy bowel preparation: a prospective, randomized, double-blind, placebo-controlled study. *Aliment Pharmacol Ther* 2006; **23**: 669-674 [PMID: 16480406 DOI: 10.1111/j.1365-2036.2006.02790.x]

33 **Brady CE**, DiPalma JA, Pierson WP. Golytely lavage--is metoclopramide necessary? *Am J Gastroenterol* 1985; **80**: 180-184 [PMID: 3976636]

34 **Ell C**, Fischbach W, Bronisch HJ, Dertinger S, Layer P, Rünzi M, Schneider T, Kachel G, Grüger J, Köllinger M, Nagell W, Goerg KJ, Wanitschke R, Gruss HJ. Randomized trial of low-volume PEG solution versus standard PEG + electrolytes for bowel cleansing before colonoscopy. *Am J Gastroenterol* 2008; **103**: 883-893 [PMID: 18190651 DOI: 10.1111/j.1572-0241.2007.01708.x]

35 **Cohen LB**, Sanyal SM, Von Althann C, Bodian C, Whitson M, Bamji N, Miller KM, Mavronicolas W, Burd S, Freedman J, Aisenberg J. Clinical trial: 2-L polyethylene glycol-based lavage solutions for colonoscopy preparation - a randomized, single-blind study of two formulations. *Aliment Pharmacol Ther* 2010; **32**: 637-644 [PMID: 20626383 DOI: 10.1111/j.1365-2036.2010.04390.x]

36 **Repici A**, Cestari R, Annese V, Biscaglia G, Vitetta E, Minelli L, Trallori G, Orselli S, Andriulli A, Hassan C. Randomised clinical trial: low-volume bowel preparation for colonoscopy - a comparison between two different PEG-based formulations. *Aliment Pharmacol Ther* 2012; **36**: 717-724 [PMID: 22924336 DOI: 10.1111/apt.12026]

37 **Harewood GC**, Sharma VK, de Garmo P. Impact of colonoscopy preparation quality on detection of suspected colonic neoplasia. *Gastrointest Endosc* 2003; **58**: 76-79 [PMID: 12838225 DOI: 10.1067/mge.2003.294]

38 **Froehlich F**, Wietlisbach V, Gonvers JJ, Burnand B, Vader JP. Impact of colonic cleansing on quality and diagnostic yield of colonoscopy: the European Panel of Appropriateness of Gastrointestinal Endoscopy European multicenter study. *Gastrointest Endosc* 2005; **61**: 378-384 [PMID: 15758907]

39 **Chiu HM**, Lin JT, Wang HP, Lee YC, Wu MS. The impact of colon preparation timing on colonoscopic detection of colorectal neoplasms--a prospective endoscopist-blinded randomized trial. *Am J Gastroenterol* 2006; **101**: 2719-2725 [PMID: 17026559 DOI: 10.1111/j.1572-0241.2006.00868.x]

40 **Rostom A**, Jolicoeur E. Validation of a new scale for the assessment of bowel preparation quality. *Gastrointest Endosc* 2004; **59**: 482-486 [PMID: 15044882]

41 **Lai EJ**, Calderwood AH, Doros G, Fix OK, Jacobson BC. The Boston bowel preparation scale: a valid and reliable instrument for colonoscopy-oriented research. *Gastrointest Endosc* 2009; **69**: 620-625 [PMID: 19136102 DOI: 10.1016/j.gie.2008.05.057]

42 **Futei S**, Sugino Y, Kuribayashi S, Imai Y, Ueno F, Hibi T, Mitsushima T. [New preparation method for barium enema: efficacy and administration of oral intestinal lavage solution with gastrointestinal prokinetic agent]. *Nihon Igaku Hoshasen Gakkai Zasshi* 2004; **64**: 22-30 [PMID: 14994507]

P- Reviewers Bechtold ML, Martinek J, Castro FJ
S- Editor Gou SX **L- Editor** A **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i5.736

World J Gastroenterol 2013 February 7; 19(5): 736-741
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Effectiveness of gastric cancer screening programs in South Korea: Organized *vs* opportunistic models

Beom Jin Kim, Chae Heo, Byoung Kwon Kim, Jae Yeol Kim, Jae Gyu Kim

Beom Jin Kim, Chae Heo, Jae Yeol Kim, Jae Gyu Kim, Department of Internal Medicine, Chung-Ang University College of Medicine, Heukseok-dong, Dongjak-gu, Seoul 156-755, South Korea
Byoung Kwon Kim, Department of Pathology, Green Cross Reference Laboratory, 314 Pojung-dong, Giheung-gu, Yongin-si, Kyunggi-do 446-913, South Korea
Author contributions: Kim BJ designed the research and wrote the paper; Heo C and Kim JY collected and analyzed the data; Kim BK analyzed the data; Kim JG designed the research.
Supported by The Chung-Ang University Research Grants in 2011
Correspondence to: Jae Gyu Kim, MD, PhD, Department of Internal Medicine, Chung-Ang University College of Medicine, Heukseok-dong, Dongjak-gu, Seoul 156-755, South Korea. jgkimd@cau.ac.kr
Telephone: +82-2-62993147 Fax: +82-2-7499150
Received: October 10, 2012 Revised: December 14, 2012
Accepted: December 22, 2012
Published online: February 7, 2013

Abstract

AIM: To investigate the outcome and effectiveness of two screening programs, National Cancer Screening Program (NCSP) and opportunistic screening (OS), for the detection of gastric cancer.

METHODS: A total of 45 654 subjects underwent upper endoscopy as part of the NCSP or OS at the Chung-Ang University Healthcare System in Korea between January 2007 and December 2010. The study population was comprised of subjects over the age of 40 years. More specifically, subjects who took part in the NCSP were Medicaid recipients and beneficiaries of the National Health Insurance Corporation. Still photographs from the endoscopies diagnosed as gastric cancer were reviewed by two experienced endoscopists.

RESULTS: The mean age of the screened subjects was 55 years for men and 54 years for women. A total of 126 cases (0.28%) of gastric cancer were detected from both screening programs; 100 cases (0.3%) from NCSP and in 26 cases (0.2%) from OS. The proportion of early gastric cancer (EGC) detected in NCSP was higher than that in OS (74.0% *vs* 53.8%, $P = 0.046$). Among the 34 416 screenees in NCSP, 6585 (19.1%) underwent upper endoscopy every other year as scheduled. Among the 11 238 screenees in OS, 3050 (27.1%) underwent upper endoscopy at least once every two years during the study period. The detection rate of gastric cancer was found to be significantly higher during irregular follow-up than during regular follow-up in both screening programs (0.3% *vs* 0.2%, $P = 0.036$). A higher incidence of EGC than advanced gastric cancer was observed during regular follow-up compared with irregular follow-up.

CONCLUSION: Compliance to the screening program is more important than the type of screening system used.

© 2013 Baishideng. All rights reserved.

Key words: Gastric cancer; National Cancer Screening Program; Opportunistic screening; Early gastric cancer

Kim BJ, Heo C, Kim BK, Kim JY, Kim JG. Effectiveness of gastric cancer screening programs in South Korea: Organized *vs* opportunistic models. *World J Gastroenterol* 2013; 19(5): 736-741 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i5/736.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i5.736

INTRODUCTION

Gastric cancer is the second most common cause of cancer death worldwide, and East Asian countries, such

as China and Japan, have high incidence rates of gastric cancer[1,2]. Although the incidence has declined in recent decades, gastric cancer remains the most frequently diagnosed form of cancer in South Korea[3-6]. The symptoms of gastric cancer appear quite late; in fact, a large number of advanced gastric cancer cases, as well as most early gastric cancer (EGC) cases, show no symptoms. Thus, gastric cancer screening is extremely important for the early detection of gastric cancer.

Screening can be defined as the systematic application of a test or inquiry to identify individuals at sufficient risk of a specific disorder who will benefit from further investigation or direct preventive action, among those who have not sought medical attention[7]. Some countries, such as Japan and South Korea, provide gastric cancer screening to populations with an average risk to reduce the disease burden.

Cancer screening may be offered in an organized or opportunistic model[8]. An organized model is primarily distinguished from opportunistic screening (OS) in that invitations to screening are issued from population registers[9]. Organized screening programs have nationally implemented guidelines defining who should be invited, how frequently they should be screened, and how any abnormalities detected on screening should be followed up and treated[8,10]. In South Korea, a nationwide gastric cancer screening program began in 1999 as part of the National Cancer Screening Program (NCSP). NCSP provides screening services free of charge for Medicaid enrollees and people with National Health Insurance with a premium below 50%. The NCSP recommends biennial upper endoscopy or upper gastrointestinal series for men and women aged $\geqslant$ 40 years.

In addition to the organized screening program, OS is also widely available in South Korea. OS programs involve various options in terms of the items screened, intervals between screening, and costs, depending on the preference by the individual, but these services are entirely paid by the users[11]. OS has been highly implemented owing to the low cost and easy accessibility of endoscopy. However, OS involves fewer formal decisions on whether to screen, whom to screen, and at what intervals the screening should be done. Moreover, the evidence and efficiency of this examination tend to be underestimated.

Although screening programs are conducted widely in South Korea, the utilization of such screening programs, including both organized and opportunistic programs, is still unclear[11]. Even though there have been some studies carried out on the characteristics of gastric cancer detected by mass screening, there is neither a study evaluating the efficiency of gastric cancer screening programs or comparison between organized and OS. Therefore, in this study, we investigated the current features of gastric cancer screening programs in South Korea, in terms of the outcome and effectiveness of NCSP and OS conducted at a single center.

MATERIALS AND METHODS

Subjects

The present study was conducted by reviewing medical records. A total of 45 991 subjects underwent upper endoscopy as part of the NCSP or OS at the Chung-Ang University Healthcare System in South Korea between January 2007 and December 2010. The study population was comprised of subjects over the age of 40 years. More specifically, subjects who took part in the NCSP were Medicaid recipients and beneficiaries of the National Health Insurance Corporation. Still photographs from the endoscopies diagnosed as gastric cancer were reviewed by two experienced endoscopists. The gross shape of the lesion was determined as either EGC or advanced gastric cancer (AGC). If there was a discrepancy between the two endoscopists' findings, a final decision was made after further discussion.

Statistical analysis

Statistical analyses in this study were conducted using the SPSS version 12.0 software package (SPSS, Chicago, IL, United States). Categorical data analysis was conducted using the χ^2 test or Fisher's exact test. Continuous data were analyzed using Student's *t*-test. Continuous variables measured in this study are expressed as mean ± SD. All *P* values were 2-tailed. *P* values of less than 0.05 were considered statistically significant. The study was approved by the Institutional Review Board of the Chung-Ang University Medical Center in South Korea.

RESULTS

Demographics of the subjects

A total of 45 991 subjects were screened for gastric cancer during the study period. Of these, 34 481 subjects were included in NCSP and 11 238 subjects in OS. In NCSP, 275 subjects were excluded due to previous gastric surgery (n = 233) and endoscopic resection (n = 42). In OS, 62 subjects were excluded due to previous gastric surgery (n = 45) and endoscopic resection (n = 17). A total of 45 654 subjects (34 416 in NCSP and 11 238 in OS) were subsequently enrolled in this study. The mean age of the screened subjects was 55.1 years for men and 54.4 years for women. The male to female ratio of the screened subjects was 0.7:1. The mean age of the subjects was 56.2 ± 9.2 years in NCSP and 50.0 ± 8.0 years in OS, respectively. In addition, the male to female ratio was 0.5 in NCSP and 1.4 in OS, respectively. The demographics of the subjects are shown in Table 1.

Participation rate of the subjects

In NCSP, a total of 6585 subjects (19.1%) underwent upper endoscopy every other year as scheduled. Their mean age was 58.7 ± 8.3 years, and the male to female ratio was 0.5:1. In contrast, in OS, a total of 3050 subjects (27.1%) underwent upper endoscopy at least once

Table 1 Demographics of participants *n* (%)

	National Cancer Screening Program	Opportunistic screening	*P* value
No. of subjects	34 416 (75.4)	11 238 (24.6)	
Age (yr), mean ± SD	56.2 ± 9.2	50.0 ± 8.0	< 0.001
40-49	8723 (25.4)	6159 (54.8)	
50-59	12 491 (36.3)	3623 (32.2)	
60-69	10 645 (30.9)	1165 (10.4)	
70+	2557 (7.4)	291 (2.6)	
Sex			< 0.001
Male	11 987 (34.8)	6668 (59.3)	
Female	22 429 (65.2)	4570 (40.7)	
No. of regular follow-up	6585 (19.1)	3050 (27.1)	< 0.001
Gastric cancer	100 (0.3)	26 (0.2)	0.299
Endoscopic feature			0.046
EGC	74 (74.0)	14 (53.8)	
AGC	26 (26.0)	12(46.2)	

EGC: Early gastric cancer; AGC: Advanced gastric cancer.

Table 2 Outcomes according to compliance to screening systems *n* (%)

	Regular follow-up	Irregular follow-up	*P* value
No. of subjects	9635 (21.1)	36 019 (78.9)	
Age (yr), mean ± SD	54.4 ± 9.3	55.7 ± 9.1	< 0.001
40-49	2783 (28.9)	12 099 (33.6)	
50-59	3413 (35.4)	12 701 (35.2)	
60-69	2789 (28.9)	9021 (25.1)	
70+	650 (6.8)	2198 (6.1)	
Sex			< 0.001
Male	4170 (43.3)	14 485 (40.2)	
Female	5465 (56.7)	21 534 (59.8)	
Gastric cancer	17 (0.2)	109 (0.3)	0.036
Endoscopic feature			0.942
EGC	12 (70.6)	76 (69.7)	
AGC	5 (29.4)	33 (30.3)	

EGC: Early gastric cancer; AGC: Advanced gastric cancer.

every two years during the study period. Their mean age was 49.4 ± 7.1 years, and the male to female ratio was 1.3:1. Among these subjects, 140 subjects (0.6%) had undertaken upper endoscopy every year during the study period.

Gastric cancer detection rate

As shown in Table 1, gastric cancer was diagnosed in 126 (0.28%) of 45 654 subjects (87 men and 39 women). Their mean age was 60.3 ± 9.5 years. Of these, 88 subjects (0.19%) were endoscopically diagnosed with EGC and 38 subjects (0.08%) with AGC. The proportion of EGCs among the total cases of gastric cancer was 69.8%. Figure 1 shows the annual EGC/AGC ratio in each system.

A total of 100 cases of gastric cancer found in NCSP included 74 cases of EGC (74%) and 26 cases of AGC (26%). The EGC/AGC ratio was 2.9. On the other hand, 14 cases of EGC (53.8%) and 12 cases of AGC (46.2%) were found in OS. The EGC/AGC ratio was 1.2. Hence, the detection rate of EGC in NCSP was significantly higher than that in OS ($P = 0.046$).

Outcomes according to compliance

In this study, we defined regular follow-up as satisfying the biennial checks in NCSP and a check-up at least once every two years during the period in OS. Table 2 shows the outcomes according to compliance to the systems. As a result, their detection rates in regular follow-up were 0.12% for EGC and 0.05% for AGC, respectively. With regard to compliance to the system, EGC was found more than AGC during regular follow-up compared with irregular follow-up in both NCSP and OS.

Among the 100 cases of gastric cancer in NCSP, 10 cases (83.3%) of EGC and 2 cases (16.7%) of AGC were found during regular follow-up. On the other hand, 64 cases (72.7%) of EGC and 24 cases (27.3%) of AGC were found during irregular follow-up. As for OS, 2 cases (40%) of EGC and 3 cases (60%) of AGC were found during regular follow-up, whereas 12 cases (57.1%) of EGC and 9 cases (42.9%) of AGC were found during irregular follow-up. Annual follow-up revealed no cases of gastric cancer among the 140 subjects.

DISCUSSION

In this study, we measured the effectiveness of gastric cancer screening between NCSP and OS. The results showed no significant difference in the gastric cancer detection rate between NCSP and OS. However, the proportion of EGC was higher in NCSP than in OS. In addition, regular check-ups were important regardless of the screening system.

Screening has become an important component in health promotion programs along with the progress of diagnostic technology aimed at early detection of diseases[7]. Screening has the potential to produce benefits in preventing morbidity and mortality as long as the screening program fulfills certain conditions.

The epidemiology and natural history of the disease, including development from the latent to declared disease, should be adequately understood; and there should be a detectable risk factor, disease marker, latent period, or early symptomatic stage. All cost-effective primary prevention interventions should have been implemented as far as practicable. Therefore, many countries have developed a variety of screening programs as part of their preventive health services according to the recommendations of specialized committees[7]. For example, national cancer control programs should be organized to ensure that a large proportion of the target group is screened and that those individuals in whom abnormalities are observed receive appropriate diagnosis and therapy. Agreement should be reached on the guidelines for proper application of national cancer control programs. The purpose of gastric cancer screening is to reduce the cancer-related mortality, and the effect of gastric cancer screening should be evaluated on the basis of this purpose. From the perspective of the South Korean public

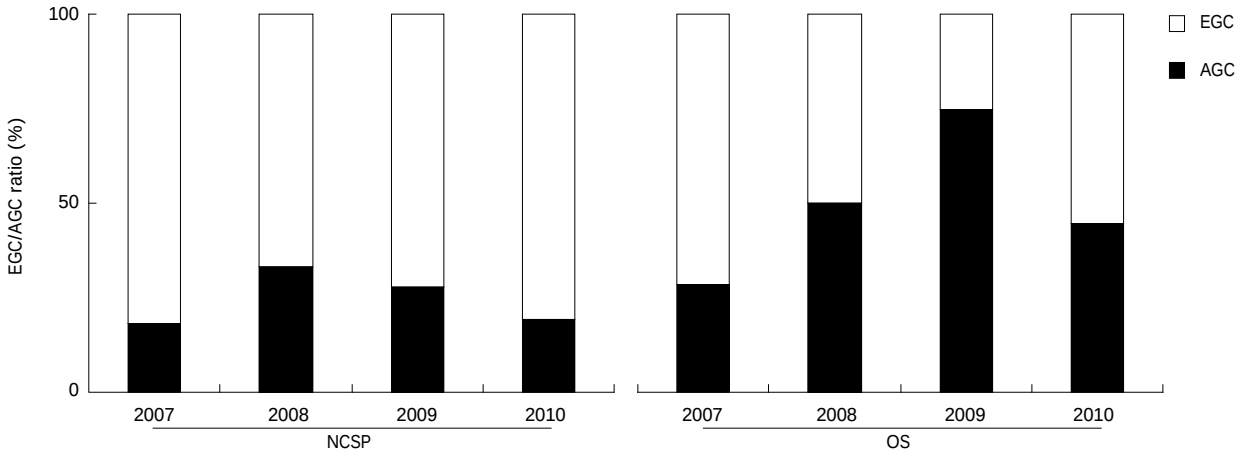


Figure 1 Annual early gastric cancer/advanced gastric cancer ratio in National Cancer Screening Program and opportunistic screening. The proportion of early gastric cancers (EGCs) among a total of 126 gastric cancers was 69.8%. The EGC/advanced gastric cancer (AGC) ratio in National Cancer Screening Program (NCSP) was 2.9, while the EGC/AGC ratio in opportunistic screening (OS) was 1.2. The detection rate of EGC in NCSP was significantly higher than that in OS (P = 0.046).

health care system, reducing the gastric cancer-related mortality rate appears to have the utmost importance[12].

Recently, endoscopy has replaced photofluorography as the initial mass screening method in South Korea. This method has become increasingly useful for gastric cancer screening owing to its high detection rate[13]. Considering the circumstances in South Korea that the cost of endoscopy is as low as that of photofluorography and the incidence of gastric cancer is still high, endoscopy is regarded as the most cost-effective screening method in South Korea. However, gastric cancer screening using endoscopy as a mass screening method has not yet been reported.

NCSP in South Korea has been available for several years, and has played a role in the early detection of gastric cancer. Apart from NCSP, OS has also contributed to screening gastric cancer in South Korea. Although the contents and process of the screening program are similar to those of the NCSP, there are some distinctive features in OS[7]. For this reason, some criticize that this unestablished guideline may drive the population into unnecessary socioeconomic consumption. Our hospital has run both NSCP and OS in the same healthcare system, which is rare in South Korea. A total of 8 well-trained gastroenterologists with at least 5 year of endoscopy experience performed upper endoscopy using a flexible endoscope (Q260 or Q240, Olympus Optical Co., Tokyo, Japan) in our healthcare system (NCSP and OS). They rotated between NCSP and OS as scheduled. Hence, there was little qualitative difference between NCSP and OS. Thus, this study could be regarded as a community-based study conducted for the local population.

So far, numerous studies on gastric cancer screening have focused on either OS or NCSP. Therefore, to our knowledge, this is the first study to compare the efficiency of two gastric cancer screening programs, NCSP and OS, in South Korea.

In this study, a total of 126 cases (0.28%) of gastric cancer were detected from both screening programs; 100 cases (0.3%) from NCSP and in 26 cases (0.2%) from OS during the study period. Screening for gastric cancer revealed that the proportion of EGC detected in NCSP was higher than that in OS (74.0% *vs* 53.8%, P = 0.046).

Kim *et al*[12] reported a significant relationship between the history of gastric examination and severity of gastric cancer. This suggests that gastric cancer screening is effective in detecting early stage gastric cancer. Unfortunately, an appropriate interval for screening gastric cancer has not been determined[14,15]. Despite the lack of evidence, screening for gastric cancer is conducted every other year in South Korea. Therefore, we compared the gastric cancer detection rates between NCSP and OS according to the frequency of check-ups.

We classified the subjects in each system according to the number of tests performed during the study period. This implies compliance to their target screening program. Regular follow-up was defined as satisfying the biennial check-ups in NCSP and a check-up at least once every two years during the period in OS. As a result, only 21.1% of subjects underwent endoscopy.

In this study, compliance to gastric cancer screening programs was determined to be as low as 19.1% in NCSP and 27.1% in OS, respectively. Hence, OS seems to be more effective regarding the examination interval. However, the detection rate of gastric cancer was significantly higher during irregular follow-up than during regular follow-up, regardless of the type of system (0.3% *vs* 0.2%, P = 0.036). While gastric cancer detected during the screening appears to have a good prognosis, several biases should be carefully assessed. Generally, individuals taking part in screening programs are healthier than those who do not. Selection bias has been discussed as a major limitation of case-control studies in which infor-

mation on confounding factors were not obtained[16,17]. Ideally, the effectiveness of a screening program is evaluated by an interventional study in which subjects are randomly allocated to screened and unscreened groups[18]. Due to the wide implementation of gastric cancer screening, however, no such interventional study has been carried out.

The present study had some limitations. First, this study had little information on risk factors. Second, gastric cancer was classified as EGC and AGC based on endoscopic findings, instead of surgical findings. Finally, this study did not cover the clinical course after diagnosis, including stage work-up, treatment, and prognosis, including survival. However, the present study reflects the features of presently available gastric cancer screening programs and may have an influence on future plans for cancer control.

In conclusion, this study demonstrated that NCSP was an effective screening system comparable to OS in the early detection of gastric cancer. The results suggest that compliance to the screening program is more important than the type of screening system itself. However, further studies on the efficiency and analysis of cost-effectiveness will be needed for successful progression of both systems. Furthermore, more comprehensive analyses with extensive nationwide data are warranted.

COMMENTS

Background

Gastric cancer remains one of the leading causes of cancer-related death worldwide. Currently, screening is performed in countries where gastric cancer is highly prevalent such as South Korea and Japan. In South Korea, cancer screening is widely available in two systems; organized cancer screening program, *e.g.*, National Cancer Screening Program (NCSP) and opportunistic screening (OS). However, there are no comparative studies on the effectiveness and outcomes of these two systems.

Research frontiers

Apart from government-led mass screening programs, OS programs by individual needs using an endoscopic examination have become common. However, OS has not been estimated in terms of clinical usefulness as well as cost-effectiveness. This study evaluated the effectiveness of screening systems with regard to early detection of gastric cancer and compliance to the system.

Innovations and breakthroughs

So far, most epidemiologic studies on gastric cancer screening have dealt with screening tools, screening interval and the cost-effectiveness of mass screening. However, little attention has been paid to the differences between two screening systems, *e.g.*, organized and opportunistic models. The authors made a comparison between these two systems and suggest that regular check-up is essential in the early detection of gastric cancer regardless of the screening system used.

Applications

The authors demonstrated that regular check-up is more important than the screening system in the early detection of gastric cancer. This work is interesting and will help health professionals establish a practical program for gastric cancer screening. Furthermore, it will contribute to the exploration of future strategies for gastric cancer prevention and control.

Terminology

NCSP is a cancer screening program which has been conducted by the South Korean government since 1999. OS is a screening program which depends on requests from individuals and has no guidelines.

Peer review

The authors have compared the effectiveness of NCSP and OS for gastric cancer screening. This paper has some valuable information and is worthy of publication.

REFERENCES

1 **Lee HY**, Park EC, Jun JK, Choi KS, Hahm MI. Comparing upper gastrointestinal X-ray and endoscopy for gastric cancer diagnosis in Korea. *World J Gastroenterol* 2010; **16**: 245-250 [PMID: 20066745]

2 **Shin A**, Kim J, Park S. Gastric cancer epidemiology in Korea. *J Gastric Cancer* 2011; **11**: 135-140 [PMID: 22076217 DOI: 10.5230/jgc.2011.11.3.135]

3 **Leung WK**, Wu MS, Kakugawa Y, Kim JJ, Yeoh KG, Goh KL, Wu KC, Wu DC, Sollano J, Kachintorn U, Gotoda T, Lin JT, You WC, Ng EK, Sung JJ. Screening for gastric cancer in Asia: current evidence and practice. *Lancet Oncol* 2008; **9**: 279-287 [PMID: 18308253 DOI: 10.1016/S1470-2045(08)70072-X]

4 **Choi KS**, Kwak MS, Lee HY, Jun JK, Hahm MI, Park EC. Screening for gastric cancer in Korea: population-based preferences for endoscopy versus upper gastrointestinal series. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 1390-1398 [PMID: 19383892 DOI: 10.1158/1055-9965.EPI-08-0940]

5 **Lee KS**, Oh DK, Han MA, Lee HY, Jun JK, Choi KS, Park EC. Gastric cancer screening in Korea: report on the national cancer screening program in 2008. *Cancer Res Treat* 2011; **43**: 83-88 [PMID: 21811423 DOI: 10.4143/crt.2011.43.2.83]

6 **Kang JM**, Shin DW, Kwon YM, Park SM, Park MS, Park JH, Son KY, Cho BL. Stomach cancer screening and preventive behaviors in relatives of gastric cancer patients. *World J Gastroenterol* 2011; **17**: 3518-3525 [PMID: 21941419 DOI: 10.3748/wjg.v17.i30.3518]

7 **Kim HS**, Shin DW, Lee WC, Kim YT, Cho B. National screening program for transitional ages in Korea: a new screening for strengthening primary prevention and follow-up care. *J Korean Med Sci* 2012; **27** Suppl: S70-S75 [PMID: 22661875 DOI: 10.3346/jkms.2012.27.S.S70]

8 **Lee HY**, Park EC, Jun JK, Hahm MI, Jung KW, Kim Y, Han MA, Choi KS. Trends in socioeconomic disparities in organized and opportunistic gastric cancer screening in Korea (2005-2009). *Cancer Epidemiol Biomarkers Prev* 2010; **19**: 1919-1926 [PMID: 20647409 DOI: 10.1158/1055-9965.EPI-09-1308]

9 **Miles A**, Cockburn J, Smith RA, Wardle J. A perspective from countries using organized screening programs. *Cancer* 2004; **101**: 1201-1213 [PMID: 15316915 DOI: 10.1002/cncr.20505]

10 **Park B**, Choi KS, Lee YY, Jun JK, Seo HG. Trends in Cancer Screening Rates among Korean Men and Women: Results from the Korean National Cancer Screening Survey (KNCSS), 2004-2011. *Cancer Res Treat* 2012; **44**: 113-120 [PMID: 22802749 DOI: 10.4143/crt.2012.44.2.113]

11 **Park B**, Lee HY, Choi KS, Lee YY, Jun JK, Park EC. Cancer screening in Korea, 2010: results from the Korean National Cancer Screening Survey. *Asian Pac J Cancer Prev* 2011; **12**: 2123-2128 [PMID: 22292663]

12 **Kim YS**, Park HA, Kim BS, Yook JH, Lee MS. Efficacy of screening for gastric cancer in a Korean adult population: a case-control study. *J Korean Med Sci* 2000; **15**: 510-515 [PMID: 11068986]

13 **Kubota H**, Kotoh T, Masunaga R, Dhar DK, Shibakita M, Tachibana M, Kohno H, Nagasue N. Impact of screening survey of gastric cancer on clinicopathological features and survival: retrospective study at a single institution. *Surgery* 2000;

128: 41-47 [PMID: 10876184 DOI: 10.1067/msy.2000.106812]
14 **Weiss NS**, Etzioni R. Estimating the influence of rescreening interval on the benefits associated with cancer screening: approaches and limitations. *Epidemiology* 2002; **13**: 713-717 [PMID: 12410014 DOI: 10.1097/01.EDE.0000029603.52822.D3]
15 **Hahm MI**, Choi KS, Lee HY, Jun JK, Oh D, Park EC. Who participates in the gastric cancer screening and on-time re-screening in the National Cancer Screening Program? A population-based study in Korea. *Cancer Sci* 2011; **102**: 2241-2247 [PMID: 21895871 DOI: 10.1111/j.1349-7006.2011.02090.x]
16 **Oshima A**, Hirata N, Ubukata T, Umeda K, Fujimoto I. Evaluation of a mass screening program for stomach cancer with a case-control study design. *Int J Cancer* 1986; **38**: 829-833 [PMID: 3793262]
17 **Fukao A**, Tsubono Y, Tsuji I, HIsamichi S, Sugahara N, Takano A. The evaluation of screening for gastric cancer in Miyagi Prefecture, Japan: a population-based case-control study. *Int J Cancer* 1995; **60**: 45-48 [PMID: 7814150]
18 **Mizoue T**, Yoshimura T, Tokui N, Hoshiyama Y, Yatsuya H, Sakata K, Kondo T, Kikuchi S, Toyoshima H, Hayakawa N, Tamakoshi A, Ohno Y, Fujino Y, Kaneko S. Prospective study of screening for stomach cancer in Japan. *Int J Cancer* 2003; **106**: 103-107 [PMID: 12794764 DOI: 10.1002/ijc.11183]

P- Reviewer Park SH **S- Editor** Song XX
L- Editor Webster JR **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i5.742

World J Gastroenterol 2013 February 7; 19(5): 742-749
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Probiotics increase T regulatory cells and reduce severity of experimental colitis in mice

Hai-Mei Zhao, Xiao-Ying Huang, Zhi-Qin Zuo, Qi-Hong Pan, Mei-Ying Ao, Feng Zhou, Hong-Ning Liu, Zhi-Yong Liu, Duan-Yong Liu

Hai-Mei Zhao, Zhi-Qin Zuo, Qi-Hong Pan, Mei-Ying Ao, Feng Zhou, Duan-Yong Liu, Jiangxi University of Traditional Chinese Medicine Science and Technology College, Nanchang 330025, Jiangxi Province, China
Xiao-Ying Huang, Hong-Ning Liu, Zhi-Yong Liu, Jiangxi University of Traditional Chinese Medicine, Nanchang 330006, Jiangxi Province, China
Author contributions: Zhao HM and Huang XY contributed equally to this work; Zhao HM, Huang XY, Liu HN and Liu DY designed the research; Zhao HM, Huang XY, Zuo ZQ, Pan QH, Ao MY, Zhou F and Liu ZY performed the research; Liu HN and Liu DY contributed to the new reagents and analytic tools; Zhao HM, Huang XY and Liu DY analyzed the data; Zhao HM and Liu DY wrote the paper.
Supported by The Project of Science and Technology Project from Department of Education of Jiangxi Province, No. GJJ1056
Correspondence to: Duan-Yong Liu, Associate Professor, Jiangxi University of Traditional Chinese Medicine Science and Technology College, 666 Fusheng Road, Nanchang 330025, Jiangxi Provence, China. liuduanyong@163.com
Telephone: +86-791-86526385 Fax: +86-791-86588664
Received: August 21, 2012 Revised: December 13, 2012
Accepted: December 25, 2012
Published online: February 7, 2013

Abstract

AIM: To investigate the effect of probiotics on regulating T regulatory cells and reducing the severity of experimental colitis in mice.

METHODS: Forty C57/BL mice were randomly divided into four groups. Colitis was induced in the mice using 2,4,6-trinitrobenzene sulfonic acid (TNBS). After 10-d treatment with Bifico capsules (combined bifidobacterium, lactobacillus and enterococcus), body weight, colonic weight, colonic weight index, length of colon, and histological scores were evaluated. $CD4^+CD25^+Foxp3^+T$ cell in mesenteric lymph nodes were measured by flow cytometry, and cytokines in colonic tissue homogenates were analyzed by a cytometric bead array.

RESULTS: The colonic weight index and the colonic weight of colitis mice treated with Bifico were lower than that of TNBS-induced mice without treatment. However, colonic length and percent of body weight amplification were higher than in TNBS-induced mice without treatment. Compared with TNBS-induced mice without treatment, the level of $CD4^+CD25^+Foxp3^+T$ cells in mesenteric lymph nodes, the expression of interleukin (IL)-2, IL-4 and IL-10 in colonic tissues from colitis mice treated with Bifico were upregulated, and tumor necrosis factor-α and interferon-γ were downregulated.

CONCLUSION: Probiotics effectively treat experimental colitis by increasing $CD4^+CD25^+Foxp3^+T$ cell and regulating the balance of Th1 and Th2 cytokines in the colonic mucosa.

© 2013 Baishideng. All rights reserved.

Key words: Probiotics; Ulcerative colitis; $CD4^+CD25^+T$ cell; Cytokine

Zhao HM, Huang XY, Zuo ZQ, Pan QH, Ao MY, Zhou F, Liu HN, Liu ZY, Liu DY. Probiotics increase T regulatory cells and reduce severity of experimental colitis in mice. *World J Gastroenterol* 2013; 19(5): 742-749 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i5/742.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i5.742

INTRODUCTION

Ulcerative colitis (UC) belongs to the inflammatory bowel disease (IBD) group, which are chronic, relapsing-remitting gastrointestinal diseases of unknown etiology[1,2]. The primary treatments of UC (san azosulfamide, immunodepressants and surgery) are usually not well tolerated,

have side effects and show a high relapse rate[3,4]. Many reports have indicated that the non-specific inflammation of UC is associated with bacterial overgrowth and dysbiosis[5,6]. As live microbial feed supplements, probiotics can beneficially affect the host by improving the balance of the intestinal microbial flora to effectively and securely treat UC in human and murids[1,7,8]. A pivotal event in the development of UC is that high sensitivity to intestinal symbiotic microbial flora induces serious autoimmunity enteritis, which inhibits the function or lowers the level of $CD4^+CD25^+$T cells. Re-import of $CD4^+CD25^+$T cells may prevent the occurrence or aggravation of IBD[9]. This could be accomplished by secreting suppressor cytokines to recovering immunological tolerance and maintain the stability of internal environment in the intestines[10,11].

To explore the effects of probiotics on regular intestinal mucosal internal environment, we observed the level of $CD4^+CD25^+$T cells in mesenteric lymph nodes and the expression of related cytokines in colonic mucosa from mice with colitis induced by 2,4,6-trinitrobenzene sulfonic acid (TNBS).

MATERIALS AND METHODS

Reagents

TNBS (batch number: 2508-19-2) was purchased from Sigma (St. Louis, MO, United States). Bifico Capsules (batch number: 20101002), which combine bifidobacterium, lactobacillus and enterococcus, was purchased from Bifico Pharma (Shanghai, China). Mesalazine (batch number: 110201) was purchased from Sunflower Pharma (Jiamusi, China). The mouse regulatory T cell staining kit (batch number: 88-8111-40) was purchased from eBioscience CO (San Diego, CA, United States). The BD cytometric bead array (CBA) and mouse Th1/Th2/Th17 Cytokine Kit (batch number: 23-12380-00) was from BD Biosciences (San Jose, CA, United States).

Experimental design

Forty C57/BL mice (half male and half female, weighing 22-26 g, the animal certificate number was SCXK 2009-0004) were provided from Sino-British Sippr/BK Lab Animal Co. Ltd. (Changsha, China), and randomly divided into four groups of ten animals each: the normal group (mice were challenged and administrated with physiological saline), the model group (mice were induced by TNBS without treatment), the Bifico group (mice were induced by TNBS and treated with 345 mg/kg Bifico capsules), the Mesalazine group (mice were induced by TNBS and treated with 300 mg/kg mesalazine). All animals were housed at 20 ± 1 ℃ with a humidity of 50% ± 5% in a 12 h light/dark cycle and fed *ad libitum* with standard mouse feed and water throughout the experiments. The experimental protocols were performed according to the Guidelines of the Jiangxi University of Traditional Chinese Medicine Animal Research Committee.

Experimental colitis in mice was induced with TNBS Solution (100 mg·kg^{-1} body TNBS dissolved in 0.15 mL 30% ethanol) by enema[12]. The mice were maintained in a head-down position for 1 min. After 24 h, mice in the treated groups were administrated Bifico capsules (345 mg/kg) or mesalazine (300 mg/kg) by lavage for 10 d, while mice in the normal and model group received the equivalent volume of physiological saline.

To evaluate the therapeutic effect of probiotics treatment on experimented colitis, body weight, colonic weigh index [colonic weigh index (%) = colonic weigh/body weigh × 100%], colonic length and histological score were analyzed. The histological scoring was performed by Professor Chun Xiao of the pathological department from Jiangxi University of Traditional Chinese Medicine, and was a total score of inflammatory cell infiltration and tissue damage, according to the study of Schmidt and his colleagues[13]. Infiltration scores were as follows: 0, no infiltration; 1, increased number of inflammatory cells in the lamina propia; 2, inflammatory cells extending into the submucosa; 3, transmural inflammatory infiltrates; and for tissue damage: 0, no mucosal damage; 1, discrete epithelial lesions; 2, erosions or focal ulcerations; 3, severe mucosal damage with extensive ulceration extending into the bowel wall[13].

On day 11, mice were euthanized, and mesenteric lymph nodes were separated and triturated with cold physiological saline in a mortar into a cell suspension. The cell suspension was filtered through a 300-micron screen mesh to measure $CD4^+CD25^+Foxp3^+$T cells. The colon from the ileocecal junction to the anus was excised, its length was measured, and then it was cut open longitudinally at the mesenteric attachment, washed with 5 mL of 0.01 mol/L phosphate buffered solution (PBS) (pH 7.4) to remove the fecal remnants and weighted to compute the colonic weigh index. Partial colons were fixed in PBS-buffered 4% paraformaldehyde, and then processed for paraffin embedding and cut into 5-μm thick sections. The sections were stained with hematoxylin and eosin. Other colons were made into tissue homogenates to analyze the expressions of the cytokines.

Assay of $CD4^+CD25^+Foxp3^+$T cells in mesenteric lymph nodes

Cells from mesenteric lymph nodes were resuspended in eFluor® NC Flow Cytometry Staining Buffer at a final cell concentration of 2×10^7/mL. fluorescein isothiocyanate anti-mouse $CD4^+$Ab (RM4-5, 0.125 μg/sample; eBioscience) and APC anti-mouse $CD25^+$Ab (PC61.5, 0.25ug/sample; eBioscience) were added to the cell suspension. Cells were centrifuged at 300 × *g* at 4 ℃ for 5 min, fixed using Fix/Perm Buffer (eBioscience) for at least 12 h at 4 ℃, and then incubated with PE-anti-mouse $Foxp3^+$Ab (FJK-16s, 0.5 μg/sample; eBioscience) for 30 min in the dark at 4 ℃. Cells labeled with PE rat IgG2a were used as the isotype negative control. Fluorescence-activated cell sorting analysis was performed on a FACSCalibur (BD Biosciences)[14].

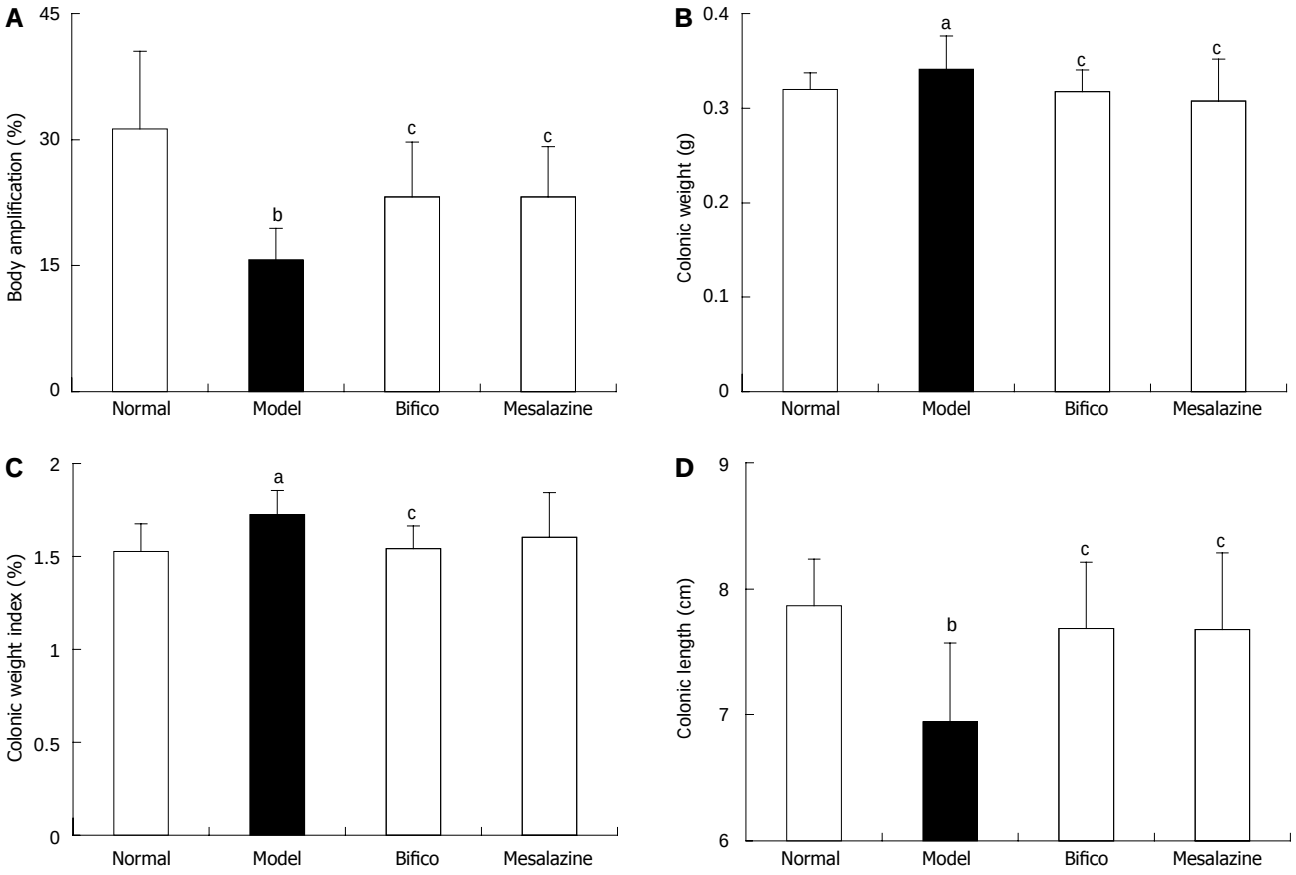


Figure 1 Change of body weight amplification, colonic weight index, colonic weight and colonic length. A: Percent of body weight amplification; B: Colonic weight; C: Colonic weight index; D: Colonic length. Normal: In the normal group, the animals were administrated with physiological saline; Model: In the model group, the animals were challenged with 2,4,6-trinitrobenzene sulfonic acid (TNBS) without treatment; Bifico: In the Bifico group, the animals were challenged by TNBS and treated with Bifico at 345 mg/kg; Mesalazine: In the Mesalazine group, the animals were challenged by TNBS and treated with 300 mg/kg Mesalazine. Data are means ± SE (n = 10). [a]$P < 0.05$, [b]$P < 0.01$ *vs* the normal group; [c]$P < 0.05$ *vs* the model group.

Determination of cytokines in colonic tissue homogenates

The concentrations of cytokines in colonic tissue homogenates were measured using a BD CBA Mouse Th1/Th2/Th17 Cytokine Kit according to the manufacturer's protocol. Fifty microliters of homogenates or standards were incubated with 50 μL of capture beads for 1h at room temperature, and then mixed with 50 μL of phycoerythrobilin (PE)-labeled tumor necrosis factor (TNF)-α, interleukin (IL)-4, interferon (IFN)-γ, IL-10, IL-6, IL-17 and IL-2 detection antibodies and incubated for 2 h at room temperature to form a sandwich complex. Following incubation, 1 mL of washing buffer (BD Biosciences Pharmingen, United States) was added to each tube, and the mean fluorescence intensity was detected using flow cytometry (FACSCalibur, B. D. Co, United States). Data were analyzed using BD Cytometric BeadArray analysis software[15].

Statistical analysis

Statistical analyses were performed using SPSS for Windows, version 15.0. Data were presented as means ± SD. Statistical analysis was performed using ANOVA and the F-test. $P < 0.05$ or $P < 0.01$ were considered significant.

RESULTS

Probiotics relieved macroscopic severity of TNBS-induced colitis in mice

Body weight amplifications of mice in the normal, Bifico and mesalazine groups were higher than that of the model group (31.28 ± 9.23, 23.20 ± 6.51, 23.17 ± 6.05, *vs* 15.73 ± 3.73, $P < 0.05$) (Figure 1A); however, the colonic weight index (CWI) and colonic weight (CW) in the Bifico group decreased compared with the model group (CWI: 1.53 ± 0.15, 1.54 ± 0.12 *vs* 1.72 ± 0.13, $P < 0.05$; CW: 0.32 ± 0.02, 0.32 ± 0.02 *vs* 0.36 ± 0.03, $P < 0.05$) (Figure 1B and C). The colonic lengths of mice in the normal, Bifico and mesalazine groups were significantly higher compared with those in the model group (7.86 ± 0.37, 7.68 ± 0.53, 7.68 ± 0.61, *vs* 6.94 ± 0.63, $P < 0.05$) (Figure 1D).

Probiotics attenuated histological severity of TNBS-induced colitis in mice

As shown in Figure 2A, integral colonic epithelium and no inflammatory cell infiltration were observed in the slice from the normal mice. However, ulceration, colonic epithelium loss, incrassate intestinal wall and abundant

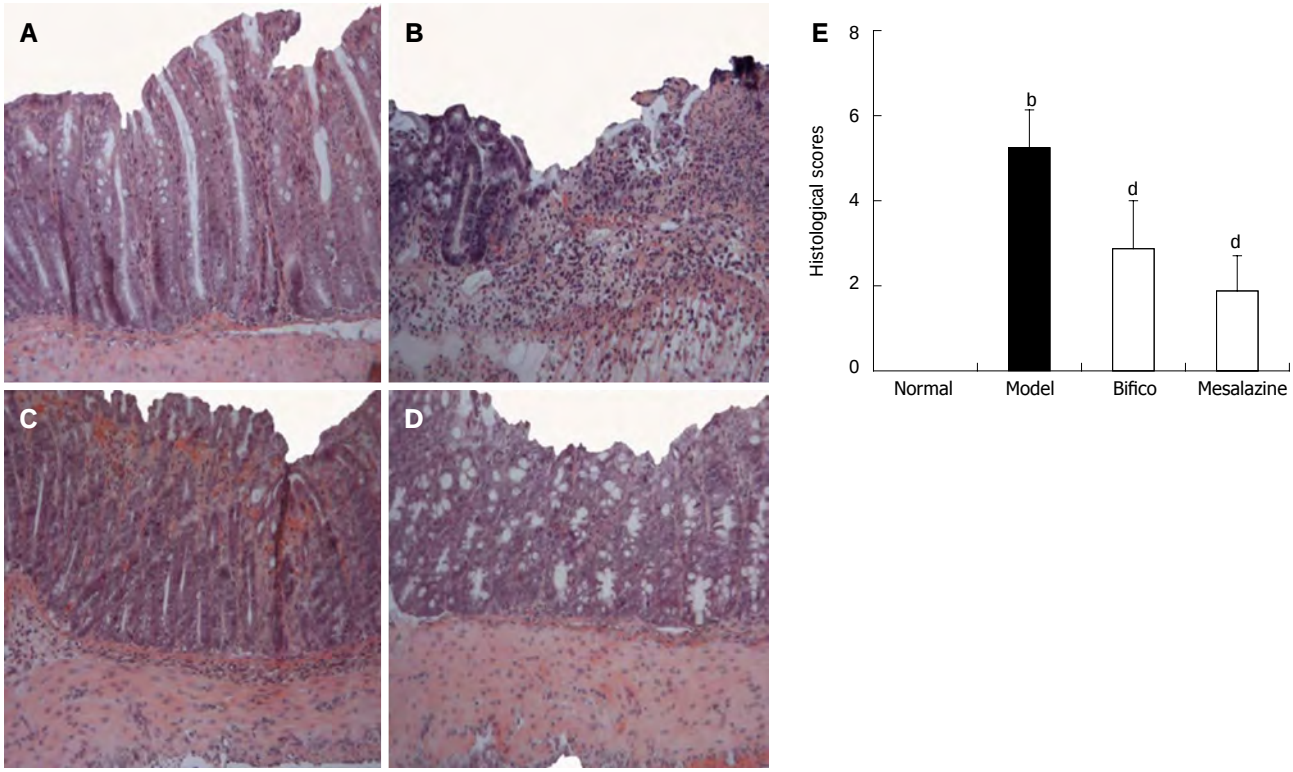


Figure 2 Representative histological images and scores of mice. A: The normal group; B: The model group; C: The bifico group; D: The mesalazine group; E: Histological images and scores (hematoxylin and eosin, light microscope, × 100). Normal: In the normal group, the animals were administrated with physiological saline; Model: In the model group, the animals were challenged with 2,4,6-trinitrobenzene sulfonic acid (TNBS) without treatment; Bifico: In the Bifico group, the animals were challenged by TNBS and treated with Bifico at 345 mg/kg; Mesalazine: In the Mesalazine group, the animals were challenged by TNBS and treated with 300 mg/kg mesalazine. Data are means ± SE (n = 10). $^{b}P < 0.01$ *vs* the normal group; $^{d}P < 0.01$ *vs* the model group.

inflammatory cell infiltration were observed in the colon of mice in the model group (Figure 2B). Meanwhile, the above histological symptoms were remarkably relieved with fewer infiltrated inflammatory cell and restored epithelium in slices from colitis mice treated with Bifico and mesalazine (Figure 2C and D). Compared with the model group, the histological score in the Bifico and mesalazine groups decreased statistically significantly (2.88 ± 1.33, 1.88 ± 0.83 *vs* 5.25 ± 0.89, $P < 0.01$) (Figure 2E). The results indicated that Bifico effectively restrained colonic injury of experimented colitis mice.

Probiotics improved the level of $CD4^+CD25^+Foxp3^+$T cells in mesenteric lymph nodes

To identify the developmental capacity of $CD4^+CD25^+$T regulatory cells, the level of $CD4^+CD25^+Foxp3^+$T cells in mesenteric lymph nodes was analyzed by flow cytometry. As shown in Figure 3, the level of $CD4^+CD25^+Foxp3^+$T cells in the model group was decreased compared with the normal group (29.70 ± 1.87 *vs* 34.73 ± 4.04, $P < 0.05$). But in the Bifico and mesalazine groups, the levels of $CD4^+CD25^+Foxp3^+$T cells were higher than in the model group (34.46 ± 4.11, 32.18 ± 1.44 *vs* 29.70 ± 1.87, $P < 0.05$).

Probiotics regulated the expression of cytokines in colonic tissues

Compared with the normal group, the expressions of TNF-α and IFN-γ in colonic tissues from mice in the model group were significantly increased (TNF-α: 32.67 ± 7.12 *vs* 18.33 ± 4.55, $P < 0.01$; IFN-γ: 28.90 ± 6.13 *vs* 10.24 ± 3.74, $P < 0.01$) (Figure 4A and B), and they were significantly decreased in the Bifico and mesalazine groups compared with the model (TNF-α: 16.87 ± 2.70, 16.96 ± 1.72 *vs* 32.67 ± 7.12, $P < 0.05$; IFN-γ: 10.46 ± 5.44, 5.20 ± 2.10 *vs* 28.90 ± 6.13, $P < 0.01$) (Figure 4A and B). Meanwhile, the expressions of IL-2, IL-4 and IL-10 in the model group were lower than in the normal group (IL-2: 2.00 ± 0.17 *vs* 2.40 ± 0.34, $P < 0.05$; IL-4: 1.58 ± 0.16 *vs* 1.84 ± 0.24, $P < 0.05$; IL-10: 7.50 ± 2.96 *vs* 13.35 ± 2.89, $P < 0.05$) (Figure 4C-E). Compared with the model group, the three cytokines were upregulated in the Bifico and mesalazine groups (IL-2: 2.52 ± 0.40, 2.32 ± 0.11 *vs* 2.00 ± 0.17, $P < 0.05$; IL-4: 1.92 ± 0.34, 1.82 ± 0.20 *vs* 1.58 ± 0.16, $P < 0.05$; IL-10: 14.19 ± 4.14, 12.25 ± 3.41 *vs* 7.50 ± 2.96, $P < 0.05$) (Figure 4C-E). The expressions of IL-6 and IL-17 in colonic tissues from colitis mice without treatment were higher than in the normal group (IL-6: 4.69 ± 1.47 *vs* 2.35 ± 0.19, $P < 0.01$; IL-17: 5.94 ± 1.54 *vs* 3.37 ± 0.96, $P < 0.01$); however, in the two treated groups, they were no different compared with the model.

DISCUSSION

In the present study, the curative effect of probiotics treatment on experimental colitis was demonstrated by

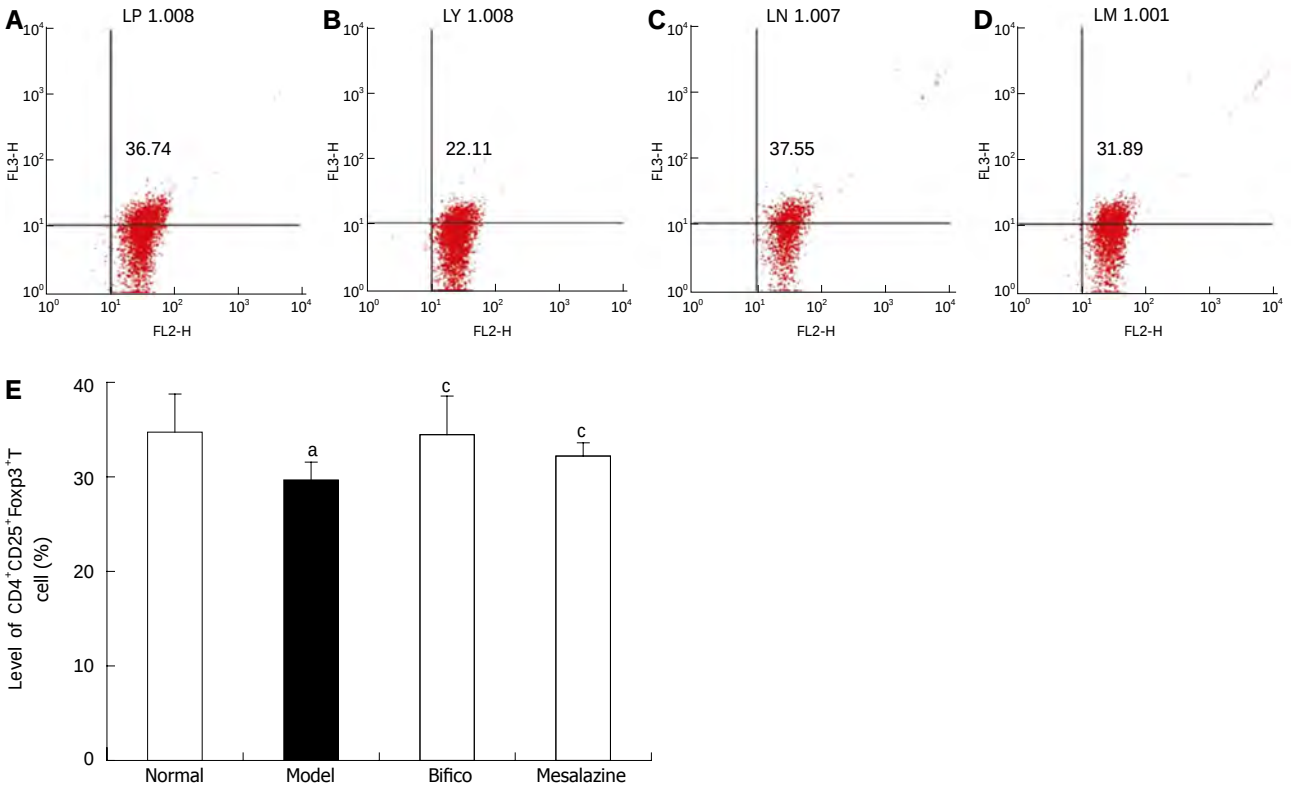


Figure 3 Representative flow plots and level of $CD4^+CD25^+Foxp3^+$T cell. A: The normal group; B: The model group; C: The bifico group; D: The mesalazine group; E: The level of $CD4^+CD25^+Foxp3^+$T. Normal: In the normal group, the animals were administrated with physiological saline; Model: In the model group, the animals were challenged with 2,4,6-trinitrobenzene sulfonic acid (TNBS) without treatment; Bifico: In the Bifico group, the animals were challenged by TNBS and treated with Bifico at 345 mg/kg; Mesalazine: In the Mesalazine group, the animals were challenged by TNBS and treated with 300 mg/kg Mesalazine. Data are means ± SE (n = 10). $^aP < 0.05$ *vs* the normal group; $^cP < 0.05$ *vs* the model group.

the increased body weight, the increased length of the colon, and decreasing histological scores followed by restored pathological characters. Probiotics treatment for 10 d inhibited the tendency to decrease $CD4^+CD25^+$T cells in mesenteric lymph nodes, downregulated the expression of TNF-α and IFN-γ, and upregulated the products of IL-2, IL-4 and IL-10 in colonic mucosa from TNBS-induced colitis mice.

Many studies have indicated that dysregulation of intestinal microflora plays an important role in initiating and perpetuating colonic inflammation in the development of human UC[16-18]. Findings from animal dextran sulfate sodium (DSS)- or TNBS-colitis models suggest that the intestinal microflora plays a key role in the abnormal immune response leading to mucosal injury in experimental colitis, because germ-free animals do not mount an intestinal inflammatory response[1,19-20]. Recently, probiotic therapy has been recognized as a safe and effective treatment for patients with UC. Lin-Lin Chen and their colleagues demonstrated that every strain of three probiotics in Bifico Capsules (combined bifidobacterium, lactobacillus and enterococcus) had a beneficial effect on DSS-induced experimental colitis in mice[21].

$CD4^+CD25^+$T regulatory (Treg) cells are critical for maintaining immune homeostasis and establishing tolerance to foreign, non-pathogenic antigens, including commensal bacteria and food, and are identified by their constitutive expression of Foxp3. $CD4^+CD25^+$T cell secretes suppressive cytokines, such as IL-2, IL-10 and TGF-β, to regulate the balance of Th1/Th2 cells and maintain mucosal immunity in the intestine[22-24]. Evidence has shown that non-functional, absent Tregs or genetic mutations in Foxp3 induces hypersensitivity to bacterial antigens[10,11], and destroys the balance of intestinal mucosal immunity to inflammatory injury followed by lymphocytic infiltration of the intestinal mucosa[25,26]. $CD4^+CD25^+Foxp3^+$ T cells increased in the colon of paracmasis patients with UC[22]. These data indicated that Tregs would be a promising target for treating UC, because increasing the activity of appropriate Tregs in the gut should help to restore inflamed colonic tissues[27].

In the present study, we found that the level of $CD4^+CD25^+Foxp3^+$ T cells was downregulated in mesocolic lymph nodes from experimented colitis mice without treatment, while IL-2, IL-10 and IL-4 were hypo-expressed in colonic tissues in the model group. The results showed that the suppressed function of $CD4^+CD25^+Foxp3^+$T cell induced disturbance of Th1/Th2 cytokines in the colonic mucosa, which leads to increased susceptibility to symbiotic bacteria, overexpression of proinflammatory factors (IL-1, TNF-α, IFN-γ), finally resulting in inflammatory injury and ulceration. After 10-d of treatment by probiotics, impaired colonic mucosa was restored with epithelial

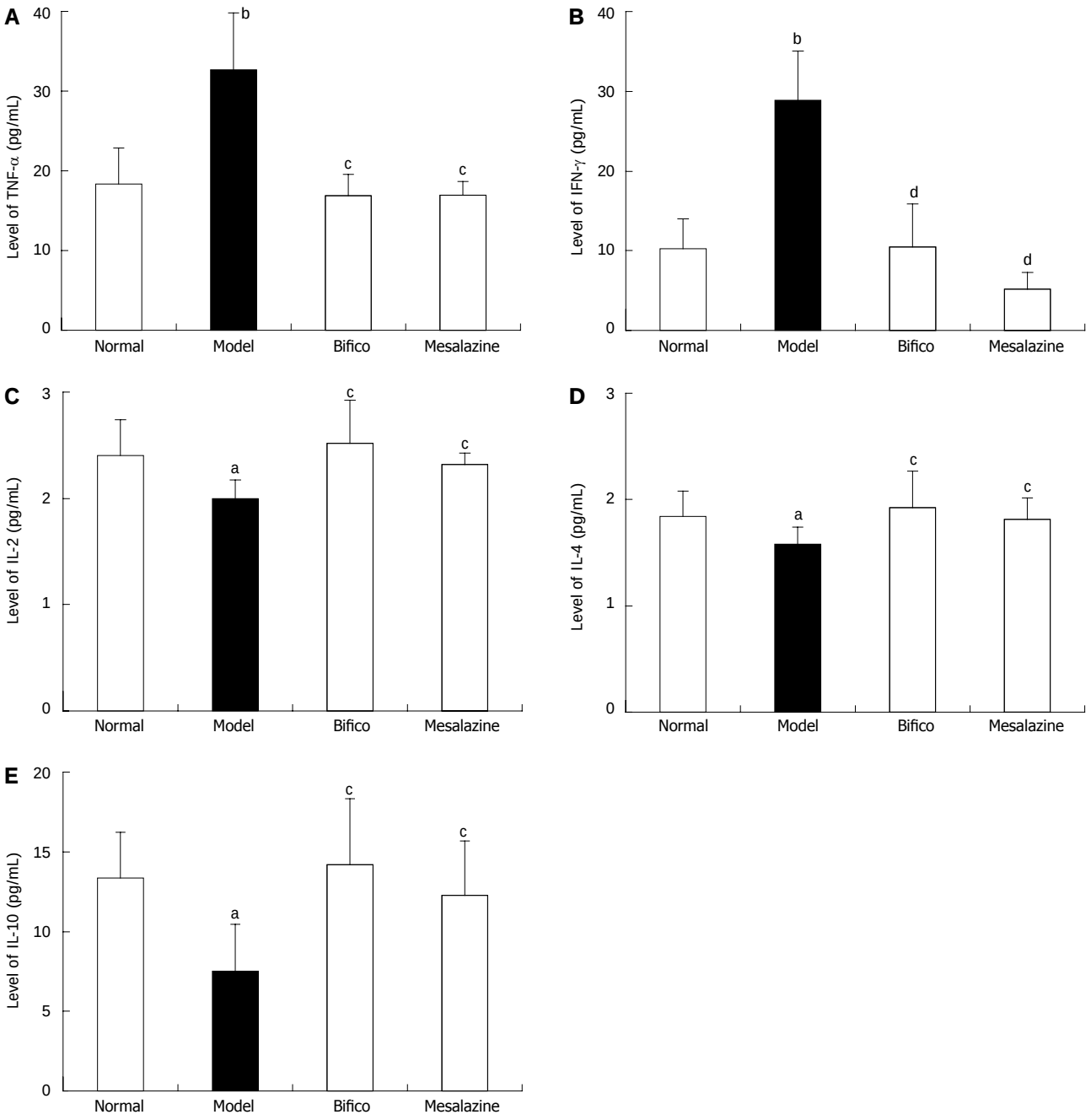


Figure 4 Expression of cytokines in colonic mucosa. A: Level of tumor necrosis factor (TNF)-α; B: Level of interferon (IFN)-γ; C: Level of interleukin (IL)-2; D: Level of IL-4; E: Level of IL-10. Normal: In the normal group, the animals were administrated with physiological saline; Model: In the model group, the animals were challenged with 2,4,6-trinitrobenzene sulfonic acid (TNBS) without treatment; Bifico: In the Bifico group, the animals were challenged by TNBS and treated with Bifico at 345 mg/kg; Mesalazine: In the Mesalazine group, the animals were challenged by TNBS and treated with 300 mg/kg Mesalazine. Data are the means ± SE (n = 10). $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* the normal group; $^{c}P < 0.05$, $^{d}P < 0.01$ *vs* the model group.

hyperplasia, intact colonic epithelia and fewer infiltrating inflammatory cells infiltration. Probiotics effectively treated mice with colitis. $CD4^{+}CD25^{+}Foxp3^{+}T$ cells were upregulated, with high expression of IL-10, IL-4 and IL-2, and TNF-α and IFN-γ were decreased in colonic mucosa from colitis mice treated by probiotics. In the study, probiotics improved the quantity of $CD4^{+}CD25^{+}Foxp3^{+}T$ cells, and restored the suppressed function of $CD4^{+}CD25^{+}Foxp3^{+}T$ cells. The Treg cells secreted more IL-10 and IL-4, competitively bound with IL-2, and enhanced the immune tolerance of intestinal mucosa to resist bacterial antigens and decreased the activity of proinflammatory factors (as TNF-α and IFN-γ), thereby renewing the immunological barrier of the colonic mucosa. Probiotics alleviated colonic inflammatory injury of colitis mice by alleviating the suppression of or increasing the quantity of $CD4^{+}CD25^{+}Foxp3^{+}T$ cell.

In summary, Probiotics effectively treated experimental colitis by improving $CD4^{+}CD25^{+}Foxp3^{+}T$ cell and regulating the balance of Th1/Th2 cytokines in the co-

lonic mucosa.

COMMENTS

Background

Ulcerative colitis (UC) is a chronic, inflammatory disease of the colonic mucosa, characterized by a relapsing-remitting course; however, the cause is largely unknown. Dysregulation of intestinal microflora and hypofrontality of T regulatory cell play important roles in pathogenicity of UC.

Research frontiers

The barrier of intestinal mucosal immunity may become damaged, which may be primarily caused by decreased inhibition of T regulatory cell by bacterial antigens. This suggested that regulating the function of T regulatory cells and/or the balance of intestinal microflora could potentially be used to treat UC.

Innovations and breakthroughs

Previous studies have highlighted that re-imported T regulatory cells may prevent occurrence or aggravation of UC. In the present study, probiotics (Bifico) inhibited the decreased tendency of $CD4^+CD25^+Foxp3^+$T cell induced by 2,4,6-trinitrobenzene sulfonic acid to effectively treat experimental colitis, followed by regulation of the balance of Th1/Th2 cytokines in the colonic mucosa.

Applications

By understanding the effects of probiotics in the treatment experimental colitis, which was mediated by inhibiting the decreased level of T regulatory cell and regulating the balance of Th1/Th2 cytokines, the study provided some evidence of the effect of probiotics on maintaining stable intestinal mucosal immunity in the treatment of UC.

Peer review

The authors investigated the effects of probiotics on treating experimental colitis and explored its potential mechanism by observing the level of T regulatory cell and expression of Th1/Th2 cytokines in colonic mucosa. It has revealed that probiotics effectively treated experimental colitis, which was accomplished by improving $CD4^+CD25^+Foxp3^+$T cell and regulating balance Th1 and Th2 cytokines in colonic mucosa. The results are valuable for exploring the mechanism of treating UC with probiotics.

REFERENCES

1 **Tsuda Y**, Yoshimatsu Y, Aoki H, Nakamura K, Irie M, Fukuda K, Hosoe N, Takada N, Shirai K, Suzuki Y. Clinical effectiveness of probiotics therapy (BIO-THREE) in patients with ulcerative colitis refractory to conventional therapy. *Scand J Gastroenterol* 2007; **42**: 1306-1311 [PMID: 17852859 DOI: 10.1080/00365520701396091]

2 **Ochsenkühn T**, D'Haens G. Current misunderstandings in the management of ulcerative colitis. *Gut* 2011; **60**: 1294-1299 [PMID: 21504997 DOI: 10.1136/gut.2010.218180.]

3 **Lewis JD**, Gelfand JM, Troxel AB, Forde KA, Newcomb C, Kim H, Margolis DJ, Strom BL. Immunosuppressant medications and mortality in inflammatory bowel disease. *Am J Gastroenterol* 2008; **103**: 1428-1435; quiz 1436 [PMID: 18494836 DOI: 10.1111/j.1572-0241.2008.01836.x]

4 **Maul J**, Zeitz M. Ulcerative colitis: immune function, tissue fibrosis and current therapeutic considerations. *Langenbecks Arch Surg* 2012; **397**: 1-10 [PMID: 21479621 DOI: 10.1007/s00423-011-0789-4.]

5 **Gionchetti P**, Rizzello F, Lammers KM, Morselli C, Sollazzi L, Davies S, Tambasco R, Calabrese C, Campieri M. Antibiotics and probiotics in treatment of inflammatory bowel disease. *World J Gastroenterol* 2006; **12**: 3306-3313 [PMID: 16733845]

6 **Bloom SM**, Bijanki VN, Nava GM, Sun L, Malvin NP, Donermeyer DL, Dunne WM, Allen PM, Stappenbeck TS. Commensal Bacteroides species induce colitis in host-genotype-specific fashion in a mouse model of inflammatory bowel disease. *Cell Host Microbe* 2011; **9**: 390-403 [PMID: 21575910 DOI: 10.1016/j.chom.2011.04.009.]

7 **Rust C**, Brand S. PSC: Protect and serve with colitis: does it help the liver to have severe ulcerative colitis? *Gut* 2011; **60**: 1165-1166 [PMID: 21561875 DOI: 10.1136/gut.2011.240309.]

8 **Lagranderie M**, Kluge C, Kiefer-Biasizzo H, Abolhassani M, Nahori MA, Fitting C, Huerre M, Bandeira A, Bercovier H, Marchal G. Mycobacterium bovis Bacillus Calmette-Guérin killed by extended freeze-drying reduces colitis in mice. *Gastroenterology* 2011; **141**: 642-652, 652.e1-4 [PMID: 21683076 DOI: 10.1053/j.gastro.2011.05.002.]

9 **Sujino T**, Kanai T, Ono Y, Mikami Y, Hayashi A, Doi T, Matsuoka K, Hisamatsu T, Takaishi H, Ogata H, Yoshimura A, Littman DR, Hibi T. Regulatory T cells suppress development of colitis, blocking differentiation of T-helper 17 into alternative T-helper 1 cells. *Gastroenterology* 2011; **141**: 1014-1023 [PMID: 21699791 DOI: 10.1053/j.gastro.2011.05.052.]

10 **Singh B**, Read S, Asseman C, Malmström V, Mottet C, Stephens LA, Stepankova R, Tlaskalova H, Powrie F. Control of intestinal inflammation by regulatory T cells. *Immunol Rev* 2001; **182**: 190-200 [PMID: 11722634 DOI: 10.1034/j.1600-065X.2001.1820115.x]

11 **Mottet C**, Uhlig HH, Powrie F. Cutting edge: cure of colitis by CD4+CD25+ regulatory T cells. *J Immunol* 2003; **170**: 3939-3943 [PMID: 12682220]

12 **Segain JP**, Raingeard de la Blétière D, Sauzeau V, Bourreille A, Hilaret G, Cario-Toumaniantz C, Pacaud P, Galmiche JP, Loirand G. Rho kinase blockade prevents inflammation via nuclear factor kappa B inhibition: evidence in Crohn's disease and experimental colitis. *Gastroenterology* 2003; **124**: 1180-1187 [PMID: 12730857 DOI: 10.1016/S0016-5085(03)00283-X.]

13 **Schmidt N**, Gonzalez E, Visekruna A, Kühl AA, Loddenkemper C, Mollenkopf H, Kaufmann SH, Steinhoff U, Joeris T. Targeting the proteasome: partial inhibition of the proteasome by bortezomib or deletion of the immunosubunit LMP7 attenuates experimental colitis. *Gut* 2010; **59**: 896-906 [PMID: 20581238 DOI: 10.1136/gut.]

14 **Aswad F**, Kawamura H, Dennert G. High sensitivity of CD4+CD25+ regulatory T cells to extracellular metabolites nicotinamide adenine dinucleotide and ATP: a role for P2X7 receptors. *J Immunol* 2005; **175**: 3075-3083 [PMID: 16116196]

15 **Sun K**, Wang CS, Guo J, Horie Y, Fang SP, Wang F, Liu YY, Liu LY, Yang JY, Fan JY, Han JY. Protective effects of ginsenoside Rb1, ginsenoside Rg1, and notoginsenoside R1 on lipopolysaccharide-induced microcirculatory disturbance in rat mesentery. *Life Sci* 2007; **81**: 509-518 [PMID: 17655881 DOI: 10.1016/j.lfs.2007.06.008]

16 **Berrebi D**, Languepin J, Ferkdadji L, Foussat A, De Lagausie P, Paris R, Emilie D, Mougenot JF, Cezard JP, Navarro J, Peuchmaur M. Cytokines, chemokine receptors, and homing molecule distribution in the rectum and stomach of pediatric patients with ulcerative colitis. *J Pediatr Gastroenterol Nutr* 2003; **37**: 300-308 [PMID: 12960653]

17 **Hegazy SK**, El-Bedewy MM. Effect of probiotics on pro-inflammatory cytokines and NF-kappaB activation in ulcerative colitis. *World J Gastroenterol* 2010; **16**: 4145-4151 [PMID: 20806430 DOI: 10.3748/wjg.]

18 **Koh SJ**, Kim JS. Prebiotics: germinated barley foodstuff for the prevention of colitis-associated colon cancer? *J Gastroenterol Hepatol* 2011; **26**: 1219-1220 [PMID: 21771059 DOI: 10.1111/j.1440-1746.2011.06769.x.]

19 **Furrie E**, Macfarlane S, Kennedy A, Cummings JH, Walsh SV, O'neil DA, Macfarlane GT. Synbiotic therapy (Bifidobacterium longum/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. *Gut* 2005; **54**: 242-249 [PMID: 15647189 DOI: 10.1136/gut.2004.044834]

20 **Campieri M**, Gionchetti P. Bacteria as the cause of ulcerative colitis. *Gut* 2001; **48**: 132-135 [PMID: 11115835 DOI: 10.1136/gut.48.1.132]

21 **Chen LL**, Wang XH, Cui Y, Lian GH, Zhang J, Ouyang CH, Lu FG. Therapeutic effects of four strains of probiotics on experimental colitis in mice. *World J Gastroenterol* 2009; **15**:

321-327 [PMID: 19140231 DOI: 10.3748/wjg.15.321]
22 **Uhlig HH**, Coombes J, Mottet C, Izcue A, Thompson C, Fanger A, Tannapfel A, Fontenot JD, Ramsdell F, Powrie F. Characterization of Foxp3+CD4+CD25+ and IL-10-secreting CD4+CD25+ T cells during cure of colitis. *J Immunol* 2006; **177**: 5852-5860 [PMID: 17056509]
23 **Liu B**, Tonkonogy SL, Sartor RB. Antigen-presenting cell production of IL-10 inhibits T-helper 1 and 17 cell responses and suppresses colitis in mice. *Gastroenterology* 2011; **141**: 653-662, 662.e1-4 [PMID: 21679711 DOI: 10.1053/j.gastro.2011.04.053.]
24 **Kameyama K**, Nemoto Y, Kanai T, Shinohara T, Okamoto R, Tsuchiya K, Nakamura T, Sakamoto N, Totsuka T, Hibi T, Watanabe M. IL-2 is positively involved in the development of colitogenic CD4+ IL-7R alpha high memory T cells in chronic colitis. *Eur J Immunol* 2010; **40**: 2423-2436 [PMID: 20623548 DOI: 10.1002/eji.200939764.]
25 **Bacchetta R**, Passerini L, Gambineri E, Dai M, Allan SE, Perroni L, Dagna-Bricarelli F, Sartirana C, Matthes-Martin S, Lawitschka A, Azzari C, Ziegler SF, Levings MK, Roncarolo MG. Defective regulatory and effector T cell functions in patients with FOXP3 mutations. *J Clin Invest* 2006; **116**: 1713-1722 [PMID: 16741580 DOI: 10.1172/JCI25112]
26 **McMurchy AN**, Di Nunzio S, Roncarolo MG, Bacchetta R, Levings MK. Molecular regulation of cellular immunity by FOXP3. *Adv Exp Med Biol* 2009; **665**: 30-46 [PMID: 20429414]
27 **Himmel ME**, Yao Y, Orban PC, Steiner TS, Levings MK. Regulatory T-cell therapy for inflammatory bowel disease: more questions than answers. *Immunology* 2012; **136**: 115-122 [PMID: 22348589 DOI: 10.1111/j.1365-2567.2012.03572.x.]

P- Reviewer Rosen MJ **S- Editor** Jiang L **L- Editor** Stewart GJ **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i5.750

World J Gastroenterol 2013 February 7; 19(5): 750-754
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Laparoscopic total mesorectal excision with natural orifice specimen extraction

Quan Wang, Chao Wang, Dong-Hui Sun, Punyaram Kharbuja, Xue-Yuan Cao

Quan Wang, Chao Wang, Dong-Hui Sun, Punyaram Kharbuja, Xue-Yuan Cao, Department of Gastric and Colorectal Surgery, Jilin University First Hospital, Changchun 130021, Jilin Province, China

Author contributions: Wang Q and Cao XY designed the study and carried out most of the study; Cao XY, Wang Q and Wang C wrote the first draft of manuscript; Wang C, Sun DH, Kharbuja P analyzed patient clinical data and provided valuable suggestions in the preparation of the manuscript.

Supported by Jilin University, China, No. 3R211P163428

Correspondence to: Xue-Yuan Cao, MD, PhD, Department of Gastric and Colorectal Surgery, Jilin University First Hospital, No. 71, Xin Min Street, Changchun 130021, Jilin Province, China. ccmzc32jdyycao@yahoo.com.cn
Telephone: +86-431-88783953 Fax: +86-431-88782889

Received: November 20, 2012 Revised: December 20, 2012
Accepted: January 5, 2013
Published online: February 7, 2013

Abstract

AIM: To introduce transvaginal or transanal specimen extraction in laparoscopic total mesorectal excision surgery to avoid an abdominal incision.

METHODS: Between January 2009 and December 2011, 21 patients with rectal cancer underwent laparoscopic radical resection and the specimen was retrieved by two different ways: transvaginal or transanal rectal removal. Transvaginal specimen extraction approach was strictly limited to elderly post-menopausal women who need hysterectomy. Patients aged between 30 and 80 years, with a body mass index of less than 30 kg/m^2, underwent elective surgery. The surgical technique and the outcomes related to the specimen extraction, such as duration of surgery, length of hospital stay, and the complications were retrospectively reviewed.

RESULTS: Laparoscopic resection using a natural orifice removal approach was successful in all of the 21 patients. Median operating time was 185 min (range, 122-260 min) and the estimated blood loss was 48 mL. The mean length of hospital stay was 7.5 d (range, 2-11 d). One patient developed postoperative ileus and had an extended hospital stay. The patient complained of minimal pain. There were no postoperative complications or surgery-associated death. The mean size of the lesion was 2.8 cm (range, 1.8-6.0 cm), and the mean number of lymph nodes harvested was 18.7 (range, 8-27). At a mean follow-up of 20.6 mo (range, 10-37 mo), there were no functional disorders associated with the transvaginal and transanal specimen extraction.

CONCLUSION: Transvaginal or transanal extraction in L-TME is a safe and effective procedure. Natural orifice specimen extraction can avoid the abdominal wall incision and its potential complications.

© 2013 Baishideng. All rights reserved.

Key words: Laparoscopic total mesorectal excision; Natural orifice specimen extraction; Rectum cancer; Transvaginal; Transanal

Wang Q, Wang C, Sun DH, Kharbuja P, Cao XY. Laparoscopic total mesorectal excision with natural orifice specimen extraction. *World J Gastroenterol* 2013; 19(5): 750-754 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i5/750.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i5.750

INTRODUCTION

A number of single and multicenter randomized trials and meta-analysis have demonstrated that the laparoscopic total mesorectal excision (L-TME) was superior to the open approach. Laparoscopic surgery resulted in faster postoperative recovery and fewer long-term complications than open surgery without apparently compromising the long-term oncologic outcomes[1-3]. However, this typically requires an abdominal incision for specimen

removal, which is commonly associated with postoperative pain. Surgical wound complications, mainly infection and postoperative hernia, continue to be major problems after both open and laparoscopic colorectal surgery, and the mini-laparotomy is often the most important source of postoperative pain after L-TME. Avoidance of the abdominal wall extraction site may be desirable to improve the short-term outcome of L-TME and limit the high cost of wound complication management[4-6].

The concept of natural orifice transluminal endoscopic surgery (NOTES) has gained interest in the surgical and gastrointestinal community as a technique to potentially reduce the surgical wound complications and trauma associated with conventional surgery[7,8]. However, NOTES has been limited because of lack of suitable instruments and platforms to facilitate a safe performance of such complex procedures. The combination of standard laparoscopy and specimen extraction through a natural orifice has the potential to reduce wound-related complications[9,10]. Natural orifice specimen extraction (NOSE) using either the anus or vagina as a route eliminates the need for abdominal wall incision for specimen extraction and anastomosis construction[11]. The NOSE concept is appealing as it maximizes the benefits of laparoscopic surgery while reducing potential wound complications and provides experience for cautious progression toward less incision surgery[12].

In this report, we adopted the technique of L-TME and transvaginal or transanal extraction in a series of 21 rectal cancer patients who required anterior rectal resection. Technique, results, morbidity, and mortality were described.

MATERIALS AND METHODS

Study participants

All consecutive patients operated on for rectal cancer using laparoscopic TME anterior rectal resection approach and transanal or transvaginal extraction of the specimen from January 2009 to December 2011 at First Hospital of Jilin University were enrolled in the study. Patients were aged between 30 and 80 years, had a body mass index of less than 30 kg/m^2, and underwent elective surgery. Patients operated on in an emergency setting and those who underwent Hartmann or abdominal perineal resection procedures were excluded. Written informed consent was obtained from all subjects and the study protocol was approved by the Ethics Committee of the First Hospital, Jilin University. The procedures were performed by a team consisting of experienced laparoscopic general surgeon and gynecologist. All patients underwent an oral magnesium citrate bowel preparation the day before surgery.

In this study, inclusion criteria for transvaginal specimen extraction were as follows: It is suitable for elderly post-menopausal women who need hysterectomy; female patients who were diagnosed with uterine myoma along with irregular vaginal bleeding, hematochezia, and receive

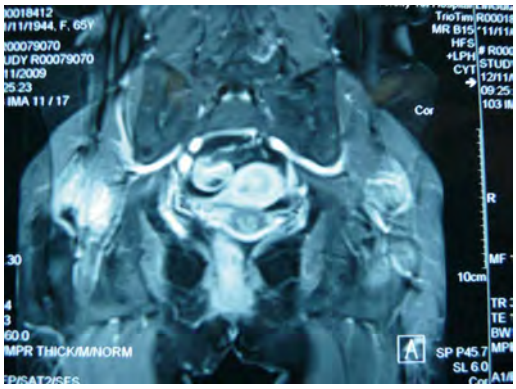


Figure 1 Computed tomography scan showed a rectal tumor combined with uterine myoma in a female patient.

L-TME combined with uterine myomectomy (Figure 1). All patients received preoperative gynecologic examination to rule out vaginal stenosis and congenital abnormalities. Women with a history of endometriosis, narrow vagina, virgins, extensive pelvic adhesions, and lesions > 6 cm, and refusal of the procedure were not considered candidates for the transvaginal removal route. All of the decision was made after consulting with gynecologist.

Transvaginal method

The patients were placed in the supine split leg or modified lithotomy position to allow for vaginal access. Abdominal and vaginal preparations were performed. After vascular control, mesocolic dissection, mobilization of tumor was performed, and the rectum distal to tumor was transected using stapler laparoscopically leaving the proximal rectum in the abdominal cavity. Then, with the assistance of gynecologist, laparoscopic hysterectomy was performed. Next, the specimens were placed in the specimen bag, which is then closed. The proximal rectum with tumor and uterus were delivered transvaginally and the rectum tumor was resected proximally outside body (Figure 2A). The anvil of the circular stapler was then inserted into the proximal colon and closed using purse string suture. The proximal colon was then returned into abdomen with the anvil transvaginally. Bowel anastomosis was completed by inserting the circular stapler via anal canal. Posterior fornix of the vagina was sutured. Final laparoscopic visualization was carried out to ensure hemostasis in the mesentery and inspect the anastomosis site.

Transanal method

The patients were placed in the lithotomy position. Briefly, a three-trocar laparoscopic approach was generally employed for mesenteric dissection and sigmoid colon and rectum mobilization. (1) Laparoscopically TME dissection was done and the upper and lower margins of the tumor determined and rectum was transected by endocutter stapler. Then, the anus was dilated, rectal stump was washed out with a 500 mL povidone-iodine normal saline solution; (2) After transanal lavage was performed,

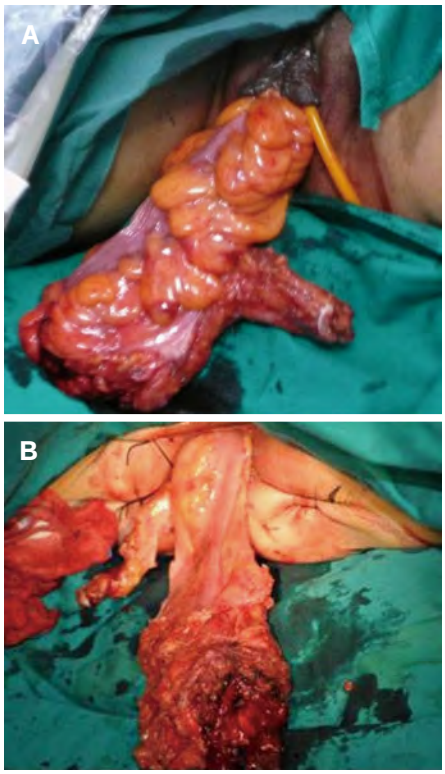


Figure 2 Natural orifice specimen extraction in laparoscopic total mesorectal excision surgery. A: Transvaginal; B: Transana specimen extraction.

the end of distal rectum was opened by ultrasonic scalpel and the specimen was extracted transanally with the specimen bag; (3) The anvil of circular end-to-end anastomosis stapler was introduced to peritoneal cavity transrectally and simultaneously put purse string suture by 2-0 prolene in the proximal colon stump; and (4) Finally, the head of the circular stapler was passed per anal, and a circular anastomosis was created (Figure 2B).

RESULTS

Within a 3-year time period, all 21 laparoscopic resections were performed successfully using a natural orifice removal approach. None of the patients were converted to open operation. The median age was 62 years (range, 50-80 years). Two of 14 patients (14%) had undergone abdominal surgery previously. The mean body mass index was 23.6 kg/m^2 (range, 18-30 kg/m^2). Median operating time was 185 min (range, 122-260 min) and the estimated blood loss was 48 mL. All patients underwent a same postoperative protocol: patient-controlled analgesia for the first 24 to 48 h. The diet was started as tolerated. The mean length of hospital stay was 7.5 d (range, 2-11 d). One patient developed postoperative ileus and had an extended hospital stay. The patient complained of minimal pain. There were no postoperative complications like rupture of the rectus, bleeding or leakage of the anastomosis, anastomosis stenosis or surgery-associated death. All margins in the resected specimens were macroscopically and microscopically free of any tumor. The mean size of the lesion was 2.8 cm (range, 1.8-6.0 cm), and the mean number of lymph nodes harvested was 18.7 (range, 8-27). Patients' demographics and pathologic details are shown in Table 1. At a mean follow-up of 20.6 mo (range, 10-37 mo), no patient experienced pain, drainage from the vaginal extraction site, and dyspareunia, and there was no incisional hernia (Figure 3).

Table 1 Clinical data and outcomes of 21 rectal cancer cases

	Transvaginal	Transanal	*P* value
Patients (*n*)	*n* = 5	*n* = 16	
Sex			> 0.05
Male	0	4	
Female	5	12	
Age, yr (median)	61	62	
Site of tumor [distance to the edge of the anus (cm)]			> 0.05
5-10	4	9	
10-15	1	7	
Operative time (min)	195 ± 35	187 ± 35	> 0.05
Operative bleeding (mL)	36 ± 15	45 ± 20	> 0.05
Post-op flatus (d)	2.0 ± 1.5	1.0 ± 0.8	> 0.05
Post-op hospitalization (d)	7.0 ± 1.2	6.5 ± 1.5	> 0.05
Intra-abdominal infection	0	0	> 0.05
Anastomosis leak	0	0	
Ileus	0	1	
Number of removal lymph node	16 ± 3	18 ± 2	> 0.05
Differentiation			> 0.05
Well	2	4	
Moderate	2	10	
Poor	1	2	
TNM stage			> 0.05
Stage Ⅰ	1	3	
Stage Ⅱ	3	11	
Stage Ⅲ	1	2	
Stage Ⅳ	0	0	

DISCUSSION

At present, despite the advantages of laparoscopic colorectal surgery, during the specimen retrieval through substantial incisions, there is an increased postoperative pain, wound infections, and incisional hernias. Along with the development of the minimally invasive surgery, abdominal wall scarless surgery becomes the new goal for people who pursue laparoscopic surgery[13,14]. In order to leave no scar on the abdominal wall, to achieve fast recovery from surgery using a more minimally invasive technique, and better cosmetic appearance, the idea of the scarless surgery started from NOTES[15,16]. In the era of NOTES, incisionless transrectal or transvaginal approaches for colorectal resections have been investigated with promising results. Transanal retrieval of specimen in laparoscopic TME has been described but not widely adopted[17-21].

Because the NOTES requires advanced technique and equipment, and requests the surgeon to have a higher strain capacity of the anatomy and the techniques. So, at present, it is still at the initial step in many countries. Recently, we have performed fast track rehabilitation in laparoscopic colorectal resection for elderly patients[22].



Figure 3 Postoperative scars.

In this article, we described two different ways to retrieve the specimen based on the technique of the traditional laparoscopic radical surgery for rectal cancer, and experienced no additional difficulty during surgery, no needs of specific equipment.

Some additional hints which are helpful during above procedures as follows: First, if the tumor is a medium-lower rectal cancer, large in size or in the fat patient, mesocolon is generally fleshy and contracted, making it shorter and difficult when transanal retrieval of specimen is done. Secondly, before transanal retrieval of specimen, when the upper and lower margin tie off, it is very important to repeat transanal irrigation by povidone iodine solution and saline. This can reduce the chances of infection and tumor cell implantation in the pelvis. Finally, it is important to put the sample in the plastic bag in the abdominal cavity, which will reduce the chances of infection and helps in smooth extraction of specimens[23,24].

In conclusion, the present study showed that NOSE is safe and feasible to perform L-TME for rectal cancer and extract the specimen without scar on abdominal wall. NOSE is an applicable option for patients requiring L-TME and appears to be associated with little incisional pain and rapid recovery. Future randomized controlled trials are necessary to show the superiority of this approach with regard to postoperative pain and morbidity, hospital stay, recovery, function and cosmesis. NOSE constitutes a stepping stone in the transition to future incisionless NOTES colectomy[25,26].

COMMENTS

Background

Despite the advantages of laparoscopic total mesorectal excision rectal surgery, the need for an incision in the abdominal wall to remove the surgical specimen is a morbid factor.

Research frontiers

The development of natural orifice translumenal endoscopic surgery and natural orifice specimen extraction (NOSE) appears to be the next major frontier in minimally invasive surgery.

Innovations and breakthroughs

Present research showed that laparoscopic total mesorectal excision (L-TME) with transvaginal or transanal extraction is a safe and effective procedure. This technique is feasible and simple to perform, avoids the abdominal wall incision and its potential complications.

Applications

Natural orifice specimen extraction may provide both an attractive way to reduce abdominal wall morbidity and a bridge to pure natural orifice transluminal endoscopic surgery for rectal surgery.

Terminology

NOSE in colorectal surgery prevents the need for an enlarged port site or minilaparotomy to extract the surgical specimen. The current trend to develop less invasive laparoscopic techniques by reducing the number and size of abdominal incisions has spurred new interest in practice.

Peer review

The authors performed laparoscopic total mesorectal excision with NOSE, which is associated with rapid recovery. They conclude that NOSE is technically feasible for L-TME. It could be better if the authors could provide data from prospective control study showing that the recovery parameters of this approach is superior to those of L-TME with trans-abdominal incision.

REFERENCES

1 **González QH**, Rodríguez-Zentner HA, Moreno-Berber JM, Vergara-Fernández O, Tapia-Cid de León H, Jonguitud LA, Ramos R, Moreno-López JA. Laparoscopic versus open total mesorectal excision: a nonrandomized comparative prospective trial in a tertiary center in Mexico City. *Am Surg* 2009; **75**: 33-38 [PMID: 19213394]

2 **Xiong B**, Ma L, Zhang C. Laparoscopic versus open total mesorectal excision for middle and low rectal cancer: a meta-analysis of results of randomized controlled trials. *J Laparoendosc Adv Surg Tech A* 2012; **22**: 674-684 [PMID: 22881123 DOI: 10.1089/lap.2012.0143]

3 **Kellokumpu IH**, Kairaluoma MI, Nuorva KP, Kautiainen HJ, Jantunen IT. Short- and long-term outcome following laparoscopic versus open resection for carcinoma of the rectum in the multimodal setting. *Dis Colon Rectum* 2012; **55**: 854-863 [PMID: 22810470 DOI: 10.1097/DCR.0b013e31825b9052.]

4 **DeSouza A**, Domajnko B, Park J, Marecik S, Prasad L, Abcarian H. Incisional hernia, midline versus low transverse incision: what is the ideal incision for specimen extraction and hand-assisted laparoscopy? *Surg Endosc* 2011; **25**: 1031-1036 [PMID: 20737171 DOI: 10.1007/s00464-010-1309-2.]

5 **Singh R**, Omiccioli A, Hegge S, McKinley C. Does the extraction-site location in laparoscopic colorectal surgery have an impact on incisional hernia rates? *Surg Endosc* 2008; **22**: 2596-2600 [PMID: 18347858 DOI: 10.1007/s00464-008-9845-8.]

6 **Winslow ER**, Fleshman JW, Birnbaum EH, Brunt LM. Wound complications of laparoscopic vs open colectomy. *Surg Endosc* 2002; **16**: 1420-1425 [PMID: 12085142 DOI: 10.1007/s00464-002-8837-3]

7 **Auyang ED**, Santos BF, Enter DH, Hungness ES, Soper NJ. Natural orifice translumenal endoscopic surgery (NOTES(®)): a technical review. *Surg Endosc* 2011; **25**: 3135-3148 [PMID: 21553172 DOI: 10.1007/s00464-011-1718-x.]

8 **Santos BF**, Hungness ES. Natural orifice translumenal endoscopic surgery: progress in humans since white paper. *World J Gastroenterol* 2011; **17**: 1655-1665 [PMID: 21483624 DOI: 10.3748/wjg.v17.i13.1655.]

9 **Brinkmann L**, Lorenz D. [Minilaparoscopic surgery: alternative or supplement to single-port surgery?]. *Chirurg* 2011; **82**: 419-424 [PMID: 21424297 DOI: 10.1007/s00104-010-2007-2.]

10 **Zaghiyan KN**, Murrell Z, Fleshner PR. Scarless single-incision laparoscopic loop ileostomy: a novel technique. *Dis Colon Rectum* 2011; **54**: 1542-1546 [PMID: 22067183 DOI: 10.1097/DCR.0b013e31822b71eb]

11 **Palanivelu C**, Rangarajan M, Jategaonkar PA, Anand NV. An innovative technique for colorectal specimen retrieval: a new era of "natural orifice specimen extraction" (N.O.S.E). *Dis Colon Rectum* 2008; **51**: 1120-1124 [PMID: 18481149 DOI: 10.1007/s10350-008-9316-2.]

12 **Benhidjeb T**, Stark M. An innovative technique for colorectal specimen retrieval: a new era of "Natural Orifice

Specimen Extraction" (N.O.S.E.). *Dis Colon Rectum* 2010; **53**: 502-503; author reply 503 [PMID: 20305453 DOI: 10.1007/DCR.0b013e3181ca7dd7]
13 **Dostalík J**, Gunková P, Martínek L, Mazur M, Gunka I, Richter V, Tulinský L. [NOSE (Natural Orifice Specimen Extraction) in laparoscopic colorectal surgery]. *Rozhl Chir* 2012; **91**: 141-145 [PMID: 22881078]
14 **Karahasanoglu T**, Hamzaoglu I, Aytac E, Baca B. Transvaginal assisted totally laparoscopic single-port right colectomy. *J Laparoendosc Adv Surg Tech A* 2011; **21**: 255-257 [PMID: 21457116 DOI: 10.1089/lap.2010.0438]
15 **Torres RA**, Orban RD, Tocaimaza L, Vallejos Pereira G, Arévalo JR. Transvaginal specimen extraction after laparoscopic colectomy. *World J Surg* 2012; **36**: 1699-1702 [PMID: 22374544 DOI: 10.1007/s00268-012-1528-x]
16 **Park JS**, Choi GS, Lim KH, Jang YS, Kim HJ, Park SY, Jun SH. Clinical outcome of laparoscopic right hemicolectomy with transvaginal resection, anastomosis, and retrieval of specimen. *Dis Colon Rectum* 2010; **53**: 1473-1479 [PMID: 20940594 DOI: 10.1007/DCR.0b013e3181f1cc17]
17 **Awad ZT**. Laparoscopic subtotal colectomy with transrectal extraction of the colon and ileorectal anastomosis. *Surg Endosc* 2012; **26**: 869-871 [PMID: 21938571 DOI: 10.1007/s00464-011-1926-4.]
18 **Diana M**, Perretta S, Wall J, Costantino FA, Leroy J, Demartines N, Marescaux J. Transvaginal specimen extraction in colorectal surgery: current state of the art. *Colorectal Dis* 2011; **13**: e104-e111 [PMID: 21564461 DOI: 10.1111/j.1463-1318.2011.02599.x.]
19 **Costantino FA**, Diana M, Wall J, Leroy J, Mutter D, Marescaux J. Prospective evaluation of peritoneal fluid contamination following transabdominal vs. transanal specimen extraction in laparoscopic left-sided colorectal resections. *Surg Endosc* 2012; **26**: 1495-1500 [PMID: 22179455 DOI: 10.1007/s00464-011-2066-6.]
20 **Hara M**, Takayama S, Sato M, Imafuji H, Takahashi H, Takeyama H. Laparoscopic anterior resection for colorectal cancer without minilaparotomy using transanal bowel reversing retrieval. *Surg Laparosc Endosc Percutan Tech* 2011; **21**: e235-e238 [PMID: 22002283 DOI: 10.1097/SLE.0b013e3182297667]
21 **Park JS**, Choi GS, Kim HJ, Park SY, Jun SH. Natural orifice specimen extraction versus conventional laparoscopically assisted right hemicolectomy. *Br J Surg* 2011; **98**: 710-715 [PMID: 21305535 DOI: 10.1002/bjs.7419]
22 **Wang Q**, Suo J, Jiang J, Wang C, Zhao YQ, Cao X. Effectiveness of fast-track rehabilitation vs conventional care in laparoscopic colorectal resection for elderly patients: a randomized trial. *Colorectal Dis* 2012; **14**: 1009-1013 [PMID: 21985126 DOI: 10.1111/j.1463-1318.2011.02855.x.]
23 **Lamadé W**, Hochberger J, Ulmer C, Matthes K, Thon KP. Triluminal hybrid NOS as a novel approach for colonic resection with colorectal anastomosis. *Surg Innov* 2010; **17**: 28-35 [PMID: 20181546 DOI: 10.1177/1553350609359920]
24 **Makris KI**, Rieder E, Kastenmeier AS, Swanström LL. Video. Transanal specimen retrieval using the transanal endoscopic microsurgery (TEM) system in minimally invasive colon resection. *Surg Endosc* 2012; **26**: 1161-1162 [PMID: 22052426 DOI: 10.1007/s00464-011-2021-6]
25 **Takayama S**, Hara M, Sato M, Takeyama H. Hybrid natural orifice transluminal endoscopic surgery for ileocecal resection. *World J Gastrointest Surg* 2012; **4**: 41-44 [PMID: 22408718 DOI: 10.4240/wjgs.v4.i2.41.]
26 **Wolthuis AM**, Van Geluwe B, Fieuws S, Penninckx F, D' Hoore A. Laparoscopic sigmoid resection with transrectal specimen extraction: a systematic review. *Colorectal Dis* 2012; **14**: 1183-1188 [PMID: 22022977 DOI: 10.1111/j.1463-1318.2011.02869.x.]

P- Reviewers Lohsiriwat V, Marinis A **S- Editor** Gou SX
L- Editor Ma JY **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i5.755

World J Gastroenterol 2013 February 7; 19(5): 755-760
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Laparoscopy-assisted total gastrectomy with trans-orally inserted anvil (OrVil™): A single institution experience

Guan-Qun Liao, Xiao-Wei Ou, Shi-Qiang Liu, Shao-Rui Zhang, Wen Huang

Guan-Qun Liao, Xiao-Wei Ou, Shi-Qiang Liu, Shao-Rui Zhang, Wen Huang, Department of General Surgery, Foshan Affiliated Hospital of South Medical University, Foshan 528000, Guangdong Province, China

Author contributions: Liao GQ and Ou XW conceived the study; Liao GQ, Liu SQ, Zhang SR acquired and interpreted the data; Liao GQ drafted the manuscript; all authors were involved in patient treatment and approved the final version of the paper.

Correspondence to: Xiao-Wei Ou, Chief, Professor, Department of General Surgery, Foshan Affiliated Hospital of South Medical University, Foshan 528000, Guangdong Province, China. ouxiaoweidf@126.com

Telephone: +86-757-88032138 Fax: +86-757-88032137
Received: October 18, 2012 Revised: December 6, 2012
Accepted: December 15, 2012
Published online: February 7, 2013

Abstract

AIM: To investigate the feasibility of laparoscopy-assisted total gastrectomy (LATG) using trans-orally inserted anvil (OrVil™) in terms of operative characteristics and short term outcomes.

RESULTS: Characteristics of 27 patients with gastric cancer who underwent LATG from October 2009 to October 2012 in the Foshan Affiliated Hospital of South Medical University were retrospectively reviewed. Among these patients, six were reconstructed by mini-laparotomy and 21 by OrVil™. The clinicopathological characteristics, total operation time, total blood loss, abdominal incision and complications of anastomosis including stenosis and leakage, were compared between the groups undergoing LATG with OrVil™ and the group undergoing mini-laparotomy.

RESULTS: The operations were successfully performed on all the patients without intraoperative complications or conversion to open surgery. Two (10%) patients received palliative procedure under laparoscope who were prepared for LATG preoperatively. One case had hepatic metastatic carcinoma and 1 case had tumor recurrence near the anastomosis 8 mo after surgery. The mean follow-up duration was 10 mo (range, 2-24 mo). Operation time was significantly reduced by the use of OrVil™ (198.42 ± 30.28 min *vs* 240.83 ± 8.23 min). The postoperative course with regard to occurrence of stenosis and leakage was not different between the two groups. There were no significant differences in estimated blood loss. The upper abdominal incision was smaller in OrVil™ group than in mini-laparotomy group (4.31 ± 0.45 cm *vs* 6.43 ± 0.38 cm).

CONCLUSION: LATG using OrVil™ is a technically feasible surgical procedure with sufficient lymph node dissection, less operation time and acceptable morbidity.

© 2013 Baishideng. All rights reserved.

Key words: Total gastrectomy; Esophagojejunostomy; Laparoscopy-assisted total gastrectomy; Reconstruction; OrVil™

Liao GQ, Ou XW, Liu SQ, Zhang SR, Huang W. Laparoscopy-assisted total gastrectomy with trans-orally inserted anvil (OrVil™): A single institution experience. *World J Gastroenterol* 2013; 19(5): 755-760 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i5/755.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i5.755

INTRODUCTION

Laparoscopic gastrectomy (LG) including laparoscopy-assisted gastrectomy (LAG) and totally LG with regional lymph node dissection as an alternative surgical treatment for gastric cancer has become increasingly common worldwide, especially in Asia[1-3]. However, laparoscopy-assisted total gastrectomy (LATG) has not been

accepted as widely as other LGs because of the low incidence of gastric cancer requiring LATG and because it is particularly difficult even for some experienced surgeons to perform[4,5]. Nevertheless, comparing with the conventional open total gastrectomy, laparoscopic surgery as an advanced procedure offers the advantages of less invasiveness and the same curability if surgeons are adroit at performing LATG[6,7].

Since 2009, our institution has adopted laparoscopic modalities for both the early and advanced stage gastric cancer patients, including 27 cases of LATG. Herein, we review our experience with LATG and analyze the results of LATG in terms of operative characteristics and short-term outcomes.

MATERIALS AND METHODS

Patients

We retrospectively reviewed a series of 27 patients who underwent LATG between October 2009 and October 2012. Twenty-one of them were reconstructed by transorally inserted anvil (OrVil™) and six by mini-laparotomy. The mini-laparotomy was performed in relatively early period and set for the comparisons of short-term outcomes.

Preoperative tumor node metastasis (TNM) stage was determined in all the patients according to the International Union Against Cancer (UICC, 7th edition) and based on endoscopic biopsy and abdominal computed tomography. The indication for LATG in gastric cancer was limited to preoperative stage $T_{1-4}N_{0-3}M_0$. Patients whose NRS 2002 score was more than 4 received more than 1-wk nutrition therapy before operation. Patients suitable for endoscopic submucosal dissection or had surgical contraindications were excluded. Written informed consent was signed by each patient who agreed to undergo LATG.

Surgical procedure

All patients were placed in relaxed dorsal lithotomy position. The surgeon usually stood on the left side of the patient, the first assistant surgeon on the right, and the second assistant surgeon holding the camera stood between the patient's legs. At the beginning, five trocars were introduced into the right upper quadrant (5 mm), right middle quadrant (5 mm), subumbilical (10 mm; camera port), left middle quadrant (5 mm), and left upper quadrant (12 mm) regions of the abdomen. The intraperitoneal pressure was maintained as 12 mmHg with carbon dioxide.

Total gastrectomy with complete omentectomy and extended lymphadenectomy (D2) was performed in all the patients. After sufficient mobilization of the duodenum near the pylorus ring and abdominal esophagus, the duodenum and esophagus were transected using EndoGIA™ Universal stapler (60 mm; Covidien). The stomach was bagged in an isolation pocket and pulled out extracorporeally through a 4-6 cm upper midline incision. In the next step, the gastrointestinal continuity was restored in a Roux-en-Y mode extracorporeally through the incision.

The OrVil™ orogastric tube was transorally introduced into the esophagus. The orogastric tube was then used to make a small hole on the middle of the abdominal esophageal stump. The tube was pulled out into the abdominal cavity through the hole until the anvil reached the esophageal stump. The orogastric tube was disconnected from the anvil and taken out of the esophagus. Subsequently, intracorporeal stapling esophagojejunostomy was performed and the jejunal stump was intracorporeally sutured with EndoGIA™ Universal (Figure 1). The intraperitoneal chemohyperthermia was performed and two drains were placed around the esophagojejunal anastomosis and pelvic cavity, respectively.

Postoperative course

The theory of fast track surgery is prevalent in our institution, but we adopt a conservative approach for the LATG patients postoperatively. The preoperatively inserted nasogastric tube for air decompression was removed at the end of surgery. A soft diet commenced orally on postoperative day (POD) 4, and abdominal drain tube was removed after 1 or 2 d when the drainage was less than 30 mL per 24 h. After a meglumine diatrizoate meal examination of esophago-intestinal tract was performed to evaluate anastomotic leakage and stenosis on PODs 8 to 10, patients were discharged on PODs 10 to 13.

Statistical analysis

Data were analyzed by the SPSS statistical software (SPSS 13.0). Quantitative variables were compared using the Student's *t* test and were expressed as means ± SD. *P* values were considered to be statistically significant at 0.05.

RESULTS

Patient characteristics

Patient characteristics including age, gender, body mass index (kg/m^2), history of abdominal surgery, NRS 2002 score and comorbidities are listed in Table 1. The operations were successfully performed in all the patients, without intraoperative complications or conversion to open surgery. Two (10%) cases received palliative procedure under laparoscope who were prepared for LATG preoperatively. One case developed hepatic metastatic carcinoma and 1 case had tumor recurrence near the anastomosis 8 mo after surgery. Mean follow-up duration was 10 mo (range, 2-24 mo).

Surgical procedure

Table 2 shows the surgical outcomes and postoperative complications. All patients underwent LATG with antecolic type Roux-en-Y esophagojejunostomy and D2

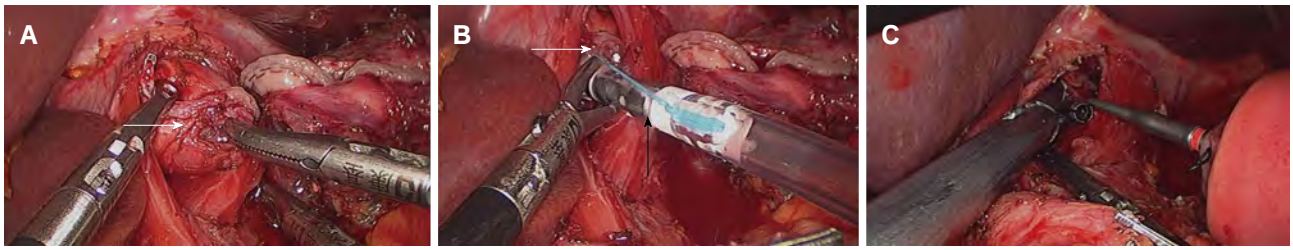


Figure 1 Esophagojejunostomy with trans-orally inserted anvil. A: The trans-orally inserted anvil orogastric tube was transorally introduced into the esophagus (white arrow); B: The tube was pulled out into the abdominal cavity through the hole. The black arrow shows the place to be separated; C: The orogastric tube was disconnected from the anvil and intracorporeal stapling esophagojejunostomy was performed.

Table 1 Characteristics of patients

Case	Gender	Age (yr)	NRS score	BMI	Previous abdominal operation	Comorbidity
1	Female	46	5	27.2	No	Urinary lithiasis
2	Male	77	7	24.1	No	No
3	Male	65	8	21.4	Appendectomy	No
4	Male	61	4	26.4	No	No
5	Male	62	5	22.1	Cholecystotomy	No
6	Female	71	8	20.9	No	Gout; high blood pressure
7	Male	48	5	24.4	No	No
9	Male	62	5	23.7	Cesarean section	Hepatic cyst; high blood pressure; cholecystolithiasis
10	Male	60	2	23.8	No	No
11	Female	55	2	23.2	No	No
12	Female	52	8	17.4	Cesarean section	No
13	Male	70	2	21.5	No	No
14	Male	54	3	20.5	No	Urinary lithiasis; urinary infection
15	Male	42	7	18.8	Gastrectomy	
16	Male	72	3	24.8	No	
17	Male	61	6	22.1	No	High blood pressure
18	Female	63	2	20.8	Appendectomy	
19	Male	66	5	19.2	No	
mean	NA				NA	NA

BMI: Body mass index; NRS: Nutritional risk screening (2002); NA: Not available.

lymph node dissection. One case received combined spleen and pancreatic tail resection. Operation time was significantly reduced by the use of OrVil™ (198.42 ± 30.28 min *vs* 240.83 ± 8.23 min, $P < 0.05$). The postoperative course with regard to stenosis and leakage did not differ between the two groups. There were no significant differences in estimated blood loss (130.57 ± 65.17 mL *vs* 140.83 ± 78.41 mL, $P > 0.05$). The upper abdominal incision was smaller in OrVil™ group than in mini-laparoscopy group (4.31 ± 0.45 cm *vs* 6.43b ± 0.38 cm, $P < 0.05$).

Postoperative course

The mean time to first oral intake and postoperative hospital stay were 3.2 d (range, 2-5 d) and 12.5 d (range, 10-19 d). Anastomotic stenosis and major leakage occurred in one case, respectively. All the patients were evaluated at over stage I and received adjuvant chemotherapy.

Tumor characteristics

Histologically, 13 patients had poorly differentiated carcinoma and 3 patients had signet ring cell carcinoma. The mean tumor size was 4.5 cm (range, 3.2-7 cm). The location of the tumor was the upper body in 7 patients and the mid body in 11 patients. Esophageal invasion was detected in 1 patient and double lesions were detected in 1 patient who had a mid-body cancer. The mean length of proximal resection margin was 4.7 cm (range, 2.2-6.1 cm) and the distal one was 6.2 cm (range, 3.1-9 cm). TNM staging according to the 7th UICC identified stage ⅡA in 2, stage ⅡB in 7, stage ⅢA in 6, stage ⅢB in 5 and stage ⅢC in 1 patient. The mean number of retrieved lymph nodes was 22.4 (range, 16-42). Multiple lymph node metastases were detected, 1-2 lymph nodes in 2 patients, 3-6 in 8 patients and more than 7 in 11 patients.

DISCUSSION

Since the first report of LG in 1992[8], LAG has been carried out not only in distal and proximal gastrectomy, but also in total gastrectomy which was more often used in advanced gastric cancer[9-11]. Although performance of LATG for gastric cancer has been increasing worldwide, especially in Asia, it remains controversial if laparoscopic

Table 2 Comparisons of characteristics between trans-orally inserted anvil group and mini-laparotomy group

	OrVil™ group (n = 21)	Mini-laparotomy group (n = 6)	P value
Total operation time (min)	198 (180-320)	240 (230-290)	0.018
Total blood loss (mL)	130 (100-400)	140 (100-300)	0.211
Abdominal incision (cm)	4.3 (4-6)	6.4 (5.5-7.0)	0.022
Complications of anastomosis	2	0	1.000
Stenosis	1	0	1.000
Leakage	1	0	1.000

In square brackets: Range. OrVil™: Trans-orally inserted anvil.

D2 dissection is equivalent to open surgery for advanced gastric cancer (AGC). In our cases, the dissection of more than 15 lymph nodes was performed and the final cutting edge negative rate was 100%. Some recent studies focus on the outcome of D2 lymph node dissection in LAG and open surgery for gastric cancer[11-13]. Du *et al*[11] evaluated 82 patients with AGC who underwent LATG with D2 dissection compared with 94 patients who received open surgery; a similar number of harvested lymph nodes (HLNs) was obtained in both groups. Cui *et al*[13] retrospectively analyzed 131 cases including a single LATG group, and found that laparoscopic D2 dissection is equivalent to open gastrectomy in the number of HLNs, regardless of tumor location.

The mean operation time for LATG with OrVil™ was 198 min, which was significantly shortened compared with the traditional mini-laparotomy group (240 min), and the mean operation time for LATG was also significantly shorter than for mini-laparotomy (180 min *vs* 406 min) in the previous studies[9,10,13]. It takes a longer time to perform esophagojejunal anastomosis through a narrow mini-laparotomy in LATG, which can be avoided by the use of OrVil™. The same conclusion is confirmed by other operative team and with OrVil™ their mean operation time was 152-243 min which mainly affected by tumor stage[3,5,9,14].

The incidence of postoperative complications in patients who underwent LATG has been reported to be 9.4%-39.4%, and common complications include anastomotic leakage, anastomotic stenosis, and pancreatic fistula[9,2,14,15]. Some studies revealed that the incidence of complications in the LATG group was similar to that in the open total gastrectomy group; however, other studies showed a lower or higher rate of complications in the LATG group[15-17]. In this study, 1 case developed anastomotic leakage and 1 case had anastomotic stenosis. The complication rate was 27%, being slightly lower compared with those from previous studies[15,18,19]. The high frequency of anastomotic complications in patients who underwent LATG might result from the excessive traction of the distal esophagus and the extensive mobilization of the jejunal limb. In our series, the rates of complications associated with anastomosis were not statitistically different between the LATG with OrVil™ and traditional mini-laparoscopy groups. However, it should be mentioned that the number of the mini-laparotomy group was small which may produce statistics bias. The same procedure was performed postoperatively in these two groups, so the comparison of mean time to first oral intake and postoperative hospital stays was meaningless.

There are some reconstructive methods used after LATG, such as Roux-en-Y esophagojejunostomy, and extracorporeal or intracorporeal anastomosis using a hand-sewn, circular stapler, or side-to-side linear stapler[1,9,20,21]. Roux-en-Y esophagojejunostomy by extracorporeal anastomosis through a small skin incision is the most common approach. However, it is difficult to perform through a mini-laparotomy, particularly on obese patients, and too larger laparotomy makes it similar to conventional open surgery[22]. OrVil™ as an intracorporeal circular stapling esophagojejunostomy can simplify the reconstruction procedure after total gastrectomy[23]. This device requires no purse-string sutures and offers wide intracorporeal operating views[24,25]. In this study, compared with control group, the smaller body incision and less operation time were observed. Moreover, two respective studies concluded that this technique was simple, safe, and efficient for performing gastrojejunostomy, and additionally less expensive and accelerated the surgical learning curve[23,26,27]. However, the earlier studies reported postoperative infection and recommended oral gargling with hexamidine solution and abdominal irrigation after anvil insertion[9,28]. No postoperative abdominal infection occurred in our series.

There were some limitations in this study. First, this retrospective analysis might have selection bias as a result of comparison of these nonrandomized groups with a retrospective profile. Second, there was no survival data. Thus, long-term oncological outcomes of LATG with OrVil™ need to be evaluated by future studies. Third, the sample size of the mini-laparotomy group is small and the operation was performed in relatively early period which cause the learning curve effect.

In conclusion, LATG using OrVil™ for gastric cancer may be a technically feasible surgical procedure with advantages of sufficient lymph node dissection, less operation time and acceptable morbidity. However, the number of patients is small in this study. It will be necessary to confirm these results by a large cohort study in the validity of LATG with OrVil™.

COMMENTS

Background

Laparoscopic gastrectomy (LG) including laparoscopy-assisted gastrectomy (LAG) and totally LG with regional lymph node dissection as an alternative surgical treatment for gastric cancer has become increasingly common worldwide, especially in Asia.

Research frontiers

Since the first report of LG in 1992, laparoscopy-assisted gastrectomy has been carried out not only in distal and proximal gastrectomy, but also in total gastrectomy which was more often used in advanced gastric cancer. Although performance of laparoscopy-assisted total gastrectomy for gastric cancer has

been increasing worldwide, especially in Asia, it remains controversial if laparoscopic D2 dissection is equivalent to open surgery for advanced gastric cancer.

Innovations and breakthroughs

LATG using orally inserted anvil (OrVil™) for gastric cancer may be a technically feasible surgical procedure with advantages of sufficient lymph node dissection, less operation time and acceptable morbidity. However, the number of patients is small in this study. It will be necessary to confirm these results by a large cohort study in the validity of LATG with OrVil™.

Peer review

This is an interesting manuscript on LG with trans-orally anastomosis. Since little is known about this technique, many readers would be interested to learn this experience.

REFERENCES

1 **Lee JH**, Han HS, Lee JH. A prospective randomized study comparing open vs laparoscopy-assisted distal gastrectomy in early gastric cancer: early results. *Surg Endosc* 2005; **19**: 168-173 [PMID: 15580441 DOI: 10.1007/s00464-004-8808-y]

2 **Kinoshita T**, Shibasaki H, Oshiro T, Ooshiro M, Okazumi S, Katoh R. Comparison of laparoscopy-assisted and total laparoscopic Billroth-I gastrectomy for gastric cancer: a report of short-term outcomes. *Surg Endosc* 2011; **25**: 1395-1401 [PMID: 20972584 DOI: 10.1007/s00464-010-1402-6]

3 **Ahn SH**, Lee JH, Park DJ, Kim HH. Comparative study of clinical outcomes between laparoscopy-assisted proximal gastrectomy (LAPG) and laparoscopy-assisted total gastrectomy (LATG) for proximal gastric cancer. *Gastric Cancer* 2012; Epub ahead of print [PMID: 22821182 DOI: 10.1007/s10120-012-0178-x]

4 **Cho HJ**, Kim W, Hur H, Jeon HM. Laparoscopy-assisted completion total gastrectomy for gastric cancer in remnant stomach: report of 2 cases. *Surg Laparosc Endosc Percutan Tech* 2009; **19**: e57-e60 [PMID: 19390266 DOI: 10.1097/SLE.0b013e31819cd62c]

5 **Kawamura H**, Yokota R, Homma S, Kondo Y. Comparison of invasiveness between laparoscopy-assisted total gastrectomy and open total gastrectomy. *World J Surg* 2009; **33**: 2389-2395 [PMID: 19760315 DOI: 10.1007/s00268-009-0208-y]

6 **Eom BW**, Kim YW, Lee SE, Ryu KW, Lee JH, Yoon HM, Cho SJ, Kook MC, Kim SJ. Survival and surgical outcomes after laparoscopy-assisted total gastrectomy for gastric cancer: case-control study. *Surg Endosc* 2012; **26**: 3273-3281 [PMID: 22648107 DOI: 10.1007/s00464-012-2338-9]

7 **Kodera Y**. Introduction of laparoscopy-assisted distal gastrectomy: a tale of two cities. *Gastric Cancer* 2012; Epub ahead of print [PMID: 22569738 DOI: 10.1007/s10120-012-0161-6]

8 **Goh P**, Tekant Y, Isaac J, Kum CK, Ngoi SS. The technique of laparoscopic Billroth II gastrectomy. *Surg Laparosc Endosc* 1992; **2**: 258-260 [PMID: 1341542]

9 **Jeong GA**, Cho GS, Kim HH, Lee HJ, Ryu SW, Song KY. Laparoscopy-assisted total gastrectomy for gastric cancer: a multicenter retrospective analysis. *Surgery* 2009; **146**: 469-474 [PMID: 19715803 DOI: 10.1016/j.surg.2009.03.023]

10 **Lee SW**, Nomura E, Bouras G, Tokuhara T, Tsunemi S, Tanigawa N. Long-term oncologic outcomes from laparoscopic gastrectomy for gastric cancer: a single-center experience of 601 consecutive resections. *J Am Coll Surg* 2010; **211**: 33-40 [PMID: 20610246 DOI: 10.1016/j.jamcollsurg.2010.03.018]

11 **Du J**, Zheng J, Li Y, Li J, Ji G, Dong G, Yang Z, Wang W, Gao Z. Laparoscopy-assisted total gastrectomy with extended lymph node resection for advanced gastric cancer--reports of 82 cases. *Hepatogastroenterology* 2010; **57**: 1589-1594 [PMID: 21443126]

12 **Huang JL**, Wei HB, Zheng ZH, Wei B, Chen TF, Huang Y, Guo WP, Hu B. Laparoscopy-assisted D2 radical distal gastrectomy for advanced gastric cancer. *Dig Surg* 2010; **27**: 291-296 [PMID: 20689290 DOI: 10.1159/000281818]

13 **Cui M**, Xing JD, Yang W, Ma YY, Yao ZD, Zhang N, Su XQ. D2 dissection in laparoscopic and open gastrectomy for gastric cancer. *World J Gastroenterol* 2012; **18**: 833-839 [PMID: 22371644 DOI: 10.3748/wjg.v18.i8.833]

14 **Bracale U**, Marzano E, Nastro P, Barone M, Cuccurullo D, Cutini G, Corcione F, Pignata G. Side-to-side esophagojejunostomy during totally laparoscopic total gastrectomy for malignant disease: a multicenter study. *Surg Endosc* 2010; **24**: 2475-2479 [PMID: 20396906 DOI: 10.1007/s00464-010-0988-z]

15 **Sakuramoto S**, Kikuchi S, Futawatari N, Katada N, Moriya H, Hirai K, Yamashita K, Watanabe M. Laparoscopy-assisted pancreas- and spleen-preserving total gastrectomy for gastric cancer as compared with open total gastrectomy. *Surg Endosc* 2009; **23**: 2416-2423 [PMID: 19266232 DOI: 10.1007/s00464-009-0371-0]

16 **Hamabe A**, Omori T, Tanaka K, Nishida T. Comparison of long-term results between laparoscopy-assisted gastrectomy and open gastrectomy with D2 lymph node dissection for advanced gastric cancer. *Surg Endosc* 2012; **26**: 1702-1709 [PMID: 22207307 DOI: 10.1007/s00464-011-2096-0]

17 **Gordon AC**, Kojima K, Inokuchi M, Kato K, Sugihara K. Long-term comparison of laparoscopy-assisted distal gastrectomy and open distal gastrectomy in advanced gastric cancer. *Surg Endosc* 2012; Epub ahead of print [PMID: 22890478 DOI: 10.1007/s00464-012-2459-1]

18 **Inaba K**, Satoh S, Ishida Y, Taniguchi K, Isogaki J, Kanaya S, Uyama I. Overlap method: novel intracorporeal esophagojejunostomy after laparoscopic total gastrectomy. *J Am Coll Surg* 2010; **211**: e25-e29 [PMID: 21036074 DOI: j.jamcollsurg.2010.09.005]

19 **Lee SE**, Ryu KW, Nam BH, Lee JH, Kim YW, Yu JS, Cho SJ, Lee JY, Kim CG, Choi IJ, Kook MC, Park SR, Kim MJ, Lee JS. Technical feasibility and safety of laparoscopy-assisted total gastrectomy in gastric cancer: a comparative study with laparoscopy-assisted distal gastrectomy. *J Surg Oncol* 2009; **100**: 392-395 [PMID: 19598150]

20 **Gould JC**, Garren M, Boll V, Starling J. The impact of circular stapler diameter on the incidence of gastrojejunostomy stenosis and weight loss following laparoscopic Roux-en-Y gastric bypass. *Surg Endosc* 2006; **20**: 1017-1020 [PMID: 16763928 DOI: 10.1007/s00464-005-0207-5]

21 **Usui S**, Yoshida T, Ito K, Hiranuma S, Kudo SE, Iwai T. Laparoscopy-assisted total gastrectomy for early gastric cancer: comparison with conventional open total gastrectomy. *Surg Laparosc Endosc Percutan Tech* 2005; **15**: 309-314 [PMID: 16340559]

22 **Omori T**, Oyama T, Mizutani S, Tori M, Nakajima K, Akamatsu H, Nakahara M, Nishida T. A simple and safe technique for esophagojejunostomy using the hemidouble stapling technique in laparoscopy-assisted total gastrectomy. *Am J Surg* 2009; **197**: e13-e17 [PMID: 19101245 DOI: 10.1016/j.amjsurg.2008.04.019]

23 **Jeong O**, Park YK. Intracorporeal circular stapling esophagojejunostomy using the transorally inserted anvil (OrVil) after laparoscopic total gastrectomy. *Surg Endosc* 2009; **23**: 2624-2630 [PMID: 19343421 DOI: 10.1007/s00464-009-0461-z]

24 **Chavarriaga LF**, Cook MW, White B, Jeansonne L, Gletsu N, Parker CB, Sweeney J, Davis SS, Lin E. Transoral technique for gastrojejunostomy in laparoscopic Roux-en-Y gastric bypass (LRYGBP) can accelerate learning curve and reduce cost. *Obes Surg* 2010; **20**: 846-850 [PMID: 19015931 DOI: 10.1007/s11695-008-9761-y]

25 **Hirahara N**, Monma H, Shimojo Y, Matsubara T, Hyakudomi R, Yano S, Tanaka T. Reconstruction of the esophagojejunostomy by double stapling method using EEA™ OrVil

™ in laparoscopic total gastrectomy and proximal gastrectomy. *World J Surg Oncol* 2011; **9**: 55 [PMID: 21599911 DOI: 10.1186/1477-7819-9-55]

26 **Kunisaki C**, Makino H, Oshima T, Fujii S, Kimura J, Takagawa R, Kosaka T, Akiyama H, Morita S, Endo I. Application of the transorally inserted anvil (OrVil) after laparoscopy-assisted total gastrectomy. *Surg Endosc* 2011; **25**: 1300-1305 [PMID: 20953884 DOI: 10.1007/s00464-010-1367-5]

27 **Park do J**, Han SU, Hyung WJ, Kim MC, Kim W, Ryu SY, Ryu SW, Song KY, Lee HJ, Cho GS, Kim HH. Long-term outcomes after laparoscopy-assisted gastrectomy for advanced gastric cancer: a large-scale multicenter retrospective study. *Surg Endosc* 2012; **26**: 1548-1553 [PMID: 22170319 DOI: 10.1007/s00464-011-2065-7]

28 **Kim KH**, Kim MC, Jung GJ, Choi HJ, Jang JS, Kwon HC. Comparative analysis of five-year survival results of laparoscopy-assisted gastrectomy versus open gastrectomy for advanced gastric cancer: a case-control study using a propensity score method. *Dig Surg* 2012; **29**: 165-171 [PMID: 22614362 DOI: 10.1159/000338088]

P- Reviewers Du JJ, Pogorelic Z, Milone M **S- Editor** Gou SX
L- Editor A **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i5.761

World J Gastroenterol 2013 February 7; 19(5): 761-768
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

META-ANALYSIS

Preoperative administration of bevacizumab is safe for patients with colorectal liver metastases

De-Bang Li, Feng Ye, Xiu-Rong Wu, Lu-Peng Wu, Jing-Xi Chen, Bin Li, Yan-Ming Zhou

De-Bang Li, First Department of General Surgery, First Hospital of Lanzhou University, Lanzhou 730000, Gansu Province, China
Feng Ye, Xiu-Rong Wu, Lu-Peng Wu, Jing-Xi Chen, Bin Li, Yan-Ming Zhou, Department of Hepatobiliary and Pancreatovascular Surgery, First Affiliated Hospital of Xiamen University, Oncologic Center of Xiamen, Xiamen 361003, Fujian Province, China
Author contributions: Li DB, Ye F, and Wu XR participated in the design and coordination of the study, carried out the critical appraisal of studies and wrote the manuscript; Wu LP, Chen JX, and Li B developed the literature search, carried out the extraction of data, assisted in the critical appraisal of included studies and assisted in writing the manuscript; Zhou YM interpreted data, corrected and approve the manuscript; all authors read and approved the final manuscript.
Correspondence to: Yan-Ming Zhou, MD, Department of Hepatobiliary and Pancreatovascular Surgery, First Affiliated Hospital of Xiamen University, Oncologic Center of Xiamen, 55 Zhenhai Road, Xiamen 361003, Fujian Province, China. zhouyms@yahoo.com.cn
Telephone: +86-592-2139708 Fax: +86-592-2137289
Received: August 5, 2012 Revised: November 20, 2012
Accepted: December 25, 2012
Published online: February 7, 2013

Abstract

AIM: To assess the impact of preoperative neoadjuvant bevacizumab (Bev) on the outcome of patients undergoing resection for colorectal liver metastases (CLM).

METHODS: Eligible trials were identified from Medline, Embase, Ovid, and the Cochrane database. The data were analyzed with fixed-effects or random-effects models using Review Manager version 5.0.

RESULTS: Thirteen nonrandomized studies with a total of 1431 participants were suitable for meta-analysis. There was no difference in overall morbidity and severe complications between the Bev + group and Bev - group (43.3% *vs* 36.8%, P = 0.06; 17.1% *vs* 11.4%, P = 0.07, respectively). Bev-related complications including wound and thromboembolic/bleeding events were also similar in the Bev + and Bev - groups (14.4% *vs* 8.1%, P = 0.21; 4.1% *vs* 3.8%, P = 0.98, respectively). The incidence and severity of sinusoidal dilation were lower in patients treated with Bev than in patients treated without Bev (43.3% *vs* 63.7%, P < 0.001; 16.8% *vs* 46.5%, P < 0.00, respectively).

CONCLUSION: Bev can be safely administered before hepatic resection in patients with CLM, and has a protective effect against hepatic injury in patients treated with oxaliplatin chemotherapy.

© 2013 Baishideng. All rights reserved.

Key words: Colorectal cancer; Liver metastases; Bevacizumab; Postoperative complication; Sinusoidal dilatation

Li DB, Ye F, Wu XR, Wu LP, Chen JX, Li B, Zhou YM. Preoperative administration of bevacizumab is safe for patients with colorectal liver metastases. *World J Gastroenterol* 2013; 19(5): 761-768 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i5/761.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i5.761

INTRODUCTION

The liver is the most common metastatic site of colorectal cancer (CC). Approximately 50% of CC patients develop colorectal liver metastases (CLM) over the course of the disease[1]. Hepatectomy is the most important modality in the treatment of CLM, offering a 5-year survival rate of approximately 30%-65%[2]. However, the long-term survival of CLM patients remains poor due to the high rate of recurrence and metastases after surgery. It is reported that neoadjuvant chemotherapy before hepa-

tectomy is associated with improved survival of CLM patients[3,4].

Bevacizumab (Bev) is a monoclonal antibody against vascular endothelial growth factor (VEGF) and can inhibit the growth of human tumor xenografts. In addition to inhibiting the antiangiogenic effect, Bev may also improve the delivery of chemotherapy by altering the tumor vasculature and decreasing the elevated interstitial pressure in tumors[5]. Randomized clinical trials[6,7] have demonstrated that the addition of Bev to fluorouracil-based combination chemotherapy can improve overall survival, the response rate and duration of response, and prolong time to disease progression in CLM patients with metastatic colorectal cancer. For this reason, some researchers advocate the use of Bev in the neoadjuvant setting before surgery for resectable CLM[8-11]. However, Bev is known to be associated with bleeding, thrombosis, gastrointestinal perforation, impaired wound healing and liver regeneration[8], thus alerts surgeons to the safe use of Bev at the time of hepatic surgery.

Chemotherapy-specific liver injuries have been more frequently reported as a disadvantage with the use of oxaliplatin in particular, because it is associated with the occurrence of sinusoidal dilation, a distinctive type of hepatic and vascular injury characteristic of hepatic veno-occlusive disease[12]. Nakano *et al*[13] reported that such injury was associated with increased morbidity and mortality after hepatectomy in CLM patients. Although the etiology of sinusoidal dilation remains unclear, it has been documented that VEGF is one of the causative cytokines for the development of sinusoidal dilation in patients undergoing bone marrow transplantation[14]. VEGF blockade by Bev may therefore attenuate sinusoidal injury[15].

Although several recent studies[8-10,15-24] have commented on the impact of preoperative Bev administration on the safety and/or oxaliplatin-associated liver injury after CLM resection, none of these studies were randomized controlled trials. A meta-analysis is therefore required to provide an improved level of evidence on this subject.

MATERIALS AND METHODS

Study selection

A Medline, Embase, Ovid and Cochrane database search was performed to identify all studies published up to March 2012 that assessed the influence of preoperative Bev administration on the outcome after CLM resection. The following Mesh search headings were used: "colorectal cancer", "liver metastases", "bevacizumab", "hepatic resection", "hepatectomy" and "neoadjuvant chemotherapy". A manual search of the reference lists of relevant papers was also carried out to identify additional trials.

Data extraction

Two reviewers (Li B and Wu LP) independently appraised each article and extracted the following parameters: first author, year of publication, study population characteristics, study design, number of patients in each arm, sex, age, inclusion and exclusion criteria, and outcomes of interest. All relevant text, tables and figures were reviewed for data extraction. Discrepancies between the reviewers were resolved through discussion and consensus.

Morbidity and mortality were defined as those events occurring within 30 or 90 d after surgery. Severe complications were defined as events requiring intensive care management or surgical, endoscopic, or radiologic interventions[25].

Criteria for inclusion and exclusion

Only trials that compared postoperative outcomes after hepatectomy between CLM patients treated with neoadjuvant chemotherapy with and without Bev administration before surgery were included. Abstracts, letters, proceedings from scientific meetings, editorials and expert opinions, reviews without original data, case reports, studies lacking control groups, repetitive data, non-English language papers and animal studies were excluded. To avoid drug interactions, studies involving other targeted molecular therapies were also excluded.

Statistical analysis

The meta-analysis was performed using the Review Manager (RevMan) software version 5.0 (The Cochrane Collaboration, Software Update, Oxford, United Kingdom). The Mantel-Haenszel method was used to combine odds ratio with 95%CI for the outcomes of interest, including overall morbidity, major complications, Bev-related complications, general complications, mortality, and incidence and severity of sinusoidal dilation. Heterogeneity between trials was assessed by calculating the Q and I^2 statistic. If $I^2 > 10\%$, a random-effects approach instead of a fixed-effects analysis was undertaken. Publication bias was assessed visually using a funnel plot. Statistical significance was defined as $P < 0.05$.

RESULTS

Eligible studies

Thirteen nonrandomized studies published between 2007 and 2012 met the inclusion criteria and were suitable for meta-analysis[8-10,15-24]. The characteristics of the included studies are summarized in Table 1. Sample size ranged from 31 to 274, with a total of 1431 participants.

Meta-analysis

Results from overall meta-analysis are outlined in Table 2.

Postoperative morbidity

Ten studies reported on overall morbidity, which was found to be comparable in the Bev + group and Bev - group (43.3% *vs* 36.8%, $P = 0.06$) (Figure 1A). Similarly,

Table 1 Study population characteristics of included trials

Ref.	Country	Group	No. of patients	M/F	Age (yr)	No. of lesions	Interval between Bev-treatment and surgery	MR
D'Angelica *et al*[8]	United States	Bev –	32	9/23	51 (34-77)[1]	1 (1-11)[1]	-	17
		Bev +	16	-	-	-	6.9 (3-15) wk[1]	-
Reddy *et al*[9]	United States	Bev –	57	37/20	60 (51-68)[1]	2 (1-4)[1]	-	39
		Bev +	39	25/14	55 (49-64)[1]	2 (1-3)[1]	10 (8-13) wk[1]	27
Mahfud *et al*[10]	Multicenter	Bev –	45	19/26	62 (59-65)[1]	6 (3-8)[1]	-	25
		Bev +	45	31/14	58 (54-61)[1]	4 (3-5)[1]	9 wk[1]	19
Ribero *et al*[15]	United States	Bev –	43	26/17	57 (26-80)[1]	2 (1-8)[1]	-	-
		Bev +	62	36/26	53.5 (34-85)[1]	2 (1-21)[1]	≥ 6 wk	-
Kesmodel *et al*[16]	United States	Bev –	44	30/14	58 (31-80)[1]	2 (1-9)[1]	-	30
		Bev +	81	48/33	57 (29-84)[1]	3 (1-31)[1]	58 (31-117) d[1]	47
Zorzi *et al*[17]	United States	Bev –	13	-	-	-	-	-
		Bev +	19	-	-	-	-	-
Aussilhou *et al*[18]	France	Bev –	20	-	-	-	-	20
		Bev +	11	-	-	-	-	9
Klinger *et al*[19]	Austria	Bev –	50	34/16	62.4[1]	-	-	-
		Bev +	56	32/24	63.0[1]	-	2-5 wk	-
Pessaux *et al*[20]	France	Bev –	21	13/8	63.3 ± 11.7[2]	3 ± 3[2]	-	4
		Bev +	21	10/11	65 ± 8.2[2]	3.8 ± 2.5[2]	11.7 ± 4.7 wk[2]	5
Rubbia-Brandt *et al*[21]	Multicenter	Bev –	204	-	-	-	-	-
		Bev +	70	-	-	-	-	-
Tamandl *et al*[22]	Austria	Bev –	112	35/77	63.6 (28.9-84.2)[1]	2 (1-10)[1]	-	17
		Bev +	102	39/63	63.3 (31.4-81.6)[1]	2 (1-10)[1]	34 (17-99) d[1]	28
Wicherts *et al*[23]	Multicenter	Bev –	97	61/36	62 ± 11[2]	4.5 ± 4.6[2]	-	38
		Bev +	67	42/25	58 ± 11[2]	5.6 ± 4.5[2]	8 (3-19) wk[1]	31
van der Pool *et al*[24]	The Netherlands	Bev –	53	33/20	62 (41-79)[1]	3 (1-7)[1]	-	14
		Bev +	51	29/22	64 (41-77)[1]	2 (1-8)[1]	11 (5-38) wk[1]	9

M: Male; F: Female; MR: Major resections (≥ segments). [1]Median (range); [2]Mean.

Table 2 Results of meta-analysis

Outcome of interest	No. of studies	No. of patients	OR	95%CI	*P*-value	I^2 (%)
Overall morbidity	10[8-10,16-18,20,22-24]	Bev + = 452, Bev - = 494	1.17	1.00, 1.37	0.06	0
Severe complication	8[8-10,16,17,20,22,23]	Bev + = 390, Bev - = 421	1.57	0.97, 2.53	0.07	18
Wound complication	6[8-10,16,22,23]	Bev + = 269, Bev - = 296	1.43	0.82, 2.50	0.21	0
Bleeding/thromboembolic complication	5[8-10,23,24]	Bev + = 218, Bev - = 284	0.99	0.41, 2.38	0.98	0
Cardiovascular complication	2[16,23]	Bev + = 148, Bev - = 141	1.14	0.23, 5.56	0.88	0
Pulmonary complication	6[8,16,18,20,23,24]	Bev + = 247, Bev - = 267	1.10	0.60, 2.02	0.77	0
Renal or urinary complication	6[8,16,18,20,23,24]	Bev + = 247, Bev - = 267	0.73	0.24, 2.23	0.58	0
Hepatic dysfunction	5[16-18,20,23]	Bev + = 223, Bev - = 219	0.48	0.22, 1.05	0.07	0
Mortality	10[8-10,16-18,20,22-24]	Bev + = 452, Bev - = 494	0.63	0.16, 2.56	0.52	0
Overall sinusoidal dilation	6[15,19-21,23,24]	Bev + = 265, Bev - = 395	0.53	0.38, 0.75	< 0.001	0
Moderate or severe sinusoidal dilation	6[15,18-21,24]	Bev + = 267,Bev - = 387	0.34	0.19,0.64	< 0.001	46

OR: Odds ratio; Bev: Bevacizumab.

there was no significant difference in severe complications between the Bev + and Bev - groups (17.1% *vs* 11.4%, $P = 0.07$) (Figure 1B). Nor was there a significant difference in cardiovascular, pulmonary and renal or urinary complications between the Bev + and Bev - groups (2.7% *vs* 2.1%, $P = 0.88$; 10.1% *vs* 9.3%, $P = 0.67$; 1.2% *vs* 2.2%, $P = 0.58$, respectively).

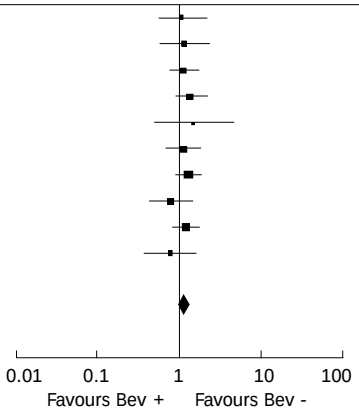
Bev-related complications including wound and thromboembolic/bleeding events were also similar in the Bev + and Bev - groups (14.4% *vs* 8.1%, $P = 0.21$; 4.1% *vs* 3.8%, $P = 0.98$, respectively). Four studies reported other types of Bev-related complications[16,22-24]. Kesmodel *et al*[16] reported hypertension in nine patients and proteinuria in two patients. van der Pool *et al*[24] reported hypertension in one patient. In one study, mild arterial hypertension occurred before surgery in one patient, necessitating dose reduction and treatment with beta-blocker therapy. No bowel perforations occurred in 13 patients with primary colorectal tumor *in situ* who received Bev. Anastomotic leakage with localized peritonitis occurred in one of seven patients who underwent synchronous colorectal and hepatic resections[23]. In another report by Tamandl *et al*[22], one patient developed anastomotic dehiscence after combined hepatic surgery and right colectomy.

Five studies reported on hepatic dysfunction, which was nonsignificantly less frequent in the Bev + group as compared with the Bev - group (5.3% *vs* 9.5%, $P = 0.07$).

A

Study or sub-category	Bev + Events	Bev + Total	Bev - Events	Bev - Total	Weight (%)	Risk ratio M-H, fixed, 95%CI
Aussilhou 2009	6	11	10	20	4.2	1.09 (0.54, 2.19)
D'Angelica 2007	7	16	12	32	4.7	1.17 (0.57, 2.38)
Kesmodel 2008	40	81	19	44	14.5	1.14 (0.76, 1.71)
Mahfud 2010	25	45	18	45	10.6	1.39 (0.89, 2.16)
Pessaux 2010	6	21	4	21	2.4	1.50 (0.49, 4.56)
Reddy 2008	17	39	22	57	10.6	1.13 (0.70, 1.83)
Tamandl 2010	45	102	38	112	21.4	1.30 (0.93, 1.82)
van der Pool 2012	13	51	17	53	9.8	0.79 (0.43, 1.46)
Wicherts 2011	29	67	35	97	16.9	1.20 (0.82, 1.76)
Zorzi 2008	8	19	7	13	4.9	0.78 (0.38, 1.62)
Total (95%CI)		452		494	100.0	1.17 (1.00, 1.37)
Total events	196		182			

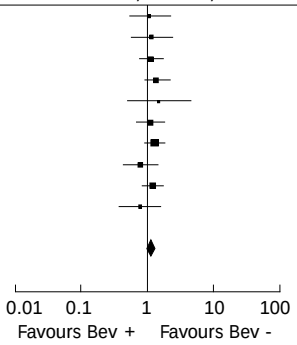
Heterogeneity: χ^2 = 3.94, df = 9 (P = 0.92), I^2 = 0%
Test for overall effect: Z = 1.92 (P = 0.06)



B

Study or sub-category	Bev + Events	Bev + Total	Bev - Events	Bev - Total	Weight (%)	Odds ratio M-H, random, 95%CI
D'Angelica 2007	1	16	3	32	4.0	0.64 (0.06, 6.74)
Kesmodel 2008	16	81	5	44	15.8	1.92 (0.65, 5.65)
Mahfud 2010	14	45	8	45	18.1	2.09 (0.78, 5.63)
Pessaux 2010	0	21	0	21		Not estimable
Reddy 2008	11	39	14	57	20.2	1.21 (0.48, 3.03)
Tamandl 2010	11	102	8	112	19.2	1.57 (0.61, 4.08)
Wicherts 2011	11	67	5	97	15.2	3.61 (1.19, 10.95)
Zorzi 2008	3	19	5	13	7.5	0.30 (0.06, 1.58)
Total (95%CI)		390		421	100.0	1.57 (0.97, 2.53)
Total events	67		48			

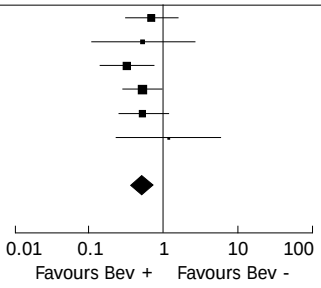
Heterogeneity: Tau^2 = 0.07; χ^2 = 7.29, df = 6 (P = 0.29), I^2 = 18%
Test for overall effect: Z = 1.84 (P = 0.07)



C

Study or sub-category	Bev + Events	Bev + Total	Bev - Events	Bev - Total	Weight (%)	Odds ratio M-H, fixed, 95%CI
Klinger 2009	22	52	24	47	16.7	0.70 (0.32, 1.55)
Pessaux 2010	3	21	5	21	4.9	0.53 (0.11, 2.59)
Ribero 2007	17	62	23	43	22.6	0.33 (0.14, 0.74)
Rubbia-Brandt 2010	45	70	158	204	33.1	0.52 (0.29, 0.94)
van der Pool 2012	25	51	34	53	19.5	0.54 (0.25, 1.18)
Wicherts 2011	3	9	8	27	3.1	1.19 (0.24, 5.96)
Total (95%CI)		265		395	100.0	0.53 (0.38, 0.75)
Total events	115		252			

Heterogeneity: χ^2 = 2.76, df = 5 (P = 0.74), I^2 = 0%
Test for overall effect: Z = 3.58 (P = 0.0003)



D

Study or sub-category	Bev + Events	Bev + Total	Bev - Events	Bev - Total	Weight (%)	Odds ratio M-H, random, 95%CI
Aussilhou 2009	6	11	6	20	11.4	2.80 (0.61, 12.86)
Klinger 2009	5	52	16	46	17.2	0.20 (0.07, 0.60)
Pessaux 2010	2	21	4	21	8.8	0.45 (0.07, 2.76)
Ribero 2007	5	62	12	43	16.7	0.23 (0.07, 0.70)
Rubbia-Brandt 2010	22	70	127	204	28.7	0.28 (0.16, 0.50)
van der Pool 2012	5	51	15	53	17.2	0.28 (0.09, 0.83)
Total (95%CI)		267		387	100.0	0.34 (0.19, 0.64)
Total events	45		180			

Heterogeneity: Tau^2 = 0.26; χ^2 = 9.26, df = 5 (P = 0.10), I^2 = 46%
Test for overall effect: Z = 3.40 (P = 0.0007)

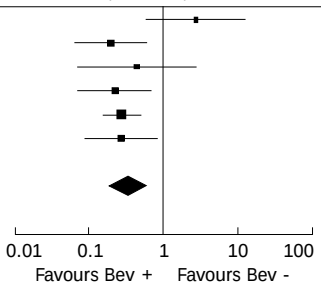


Figure 1 Forest plot. A: Overall morbidity; B: Severe complications; C: Overall sinusoidal dilation; D: Moderate or severe sinusoidal dilation. Bev: Bevacizumab.

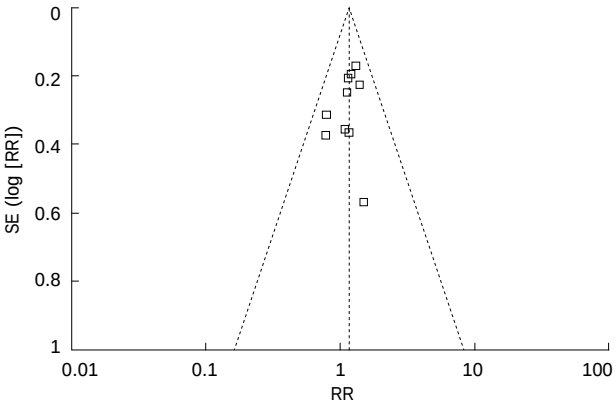


Figure 2 Funnel plot of the results obtained from studies comparing overall morbidity. RR: Risk ratio.

Postoperative mortality

Ten studies reported on postoperative mortality. There were 3 (0.6%) deaths in the Bev + group, which was similar to that in the Bev - group (5 deaths, 1.0%).

Nontumorous liver histology

Seven studies evaluated the effect of Bev for CLM on nontumorous liver histology, and one study reported a significant difference in neoadjuvant treatment regimens between patient groups. To ensure homogeneity within groups, only patients treated with oxaliplatin were included for analysis[23]. Pooled analysis showed that Bev significantly reduced the incidence (Bev + 43.3% *vs* Bev - 63.7%, $P < 0.001$) and severity (Bev + 16.8% *vs* Bev - 46.5%, $P < 0.001$) of sinusoidal dilation (Figure 1C, D).

Publication bias

A funnel plot of the studies included in the meta-analysis reporting on overall morbidity is shown in Figure 2. None of the studies lay outside the limits of the 95%CI, and there was no evidence of publication bias.

DISCUSSION

Liver regeneration is an important component of the recovery process that occurs after various forms of hepatic injury, including partial hepatectomy (PH)[26]. Angiogenesis, the formation of new blood vessels, is a fundamental process in liver regeneration and repair. VEGF is considered a key regulator of normal and pathological angiogenesis. VEGF increases vascular dilatation and permeability, and induces the migration and proliferation of endothelial cells. These activities are mediated *via* two receptors for VEGF: kinase insert domain-containing receptor, and fms-like tyrosine kinase-1 receptor[27,28]. Endogenous expression of VEGF in hepatocytes and its receptors in endothelial cells has been shown to increase after PH[26].VEGF treatment protected the liver against chemically induced cytotoxicity, associated with a marked increase in the proliferation of hepatocytes and sinusoidal endothelial cells (SEC)[28,29]. In addition, exogenous VEGF administration promoted the increase of vessel density, vessel diameter, intrasinusoidal space, liver body weight ratio and hepatocyte proliferation after PH in the rat model. Conversely, these effects were completely suppressed by anti-VEGF treatment[30]. These results suggest that VEGF plays an important role in liver regeneration. Therefore, the safety of VEGF inhibitor administration at the time of hepatic surgery needs to be addressed.

The present meta-analysis shows that both overall and severe complications were not significantly different between the Bev + and Bev - groups. In addition, Bev treatment did not seem to increase the risk of Bev-related complications (wound and bleeding/thromboembolic events), hepatic dysfunction, and postoperative deaths. On the other hand, Wicherts *et al*[23] reported that liver functional recovery parameters including prothrombin time and serum total bilirubin level were equivalent between the Bev + and Bev - groups in the postoperative period, suggesting that Bev administration should be safe before hepatic resection.

To increase the safety of surgery, preoperative portal vein embolization (PVE) has been used for major hepatic resection in CLM patients, because PVE can induce homolateral atrophy and contralateral compensatory hypertrophy of the remnant liver, thus decreasing the risk of postoperative liver failure[31]. In a relatively large cohort study of 100 patients, Covey *et al*[32] found that the mean growth of non-embolized hemiliver was comparable in patients treated with and without neoadjuvant chemotherapy (22% ± 3% *vs* 26% ± 3%) with a similar number of patients with less than 5% growth of liver (4 *vs* 6) after PVE. Similar findings were also reported by other authors[33,34]. Zorzi *et al*[17] found that chemotherapy with Bev did not alter non-embolized liver hypertrophy. In contrast, a study conducted by Aussilhou *et al*[18] demonstrated that the hypertrophy of the future liver remnant after PVE was impaired in patients treated with Bev. These inconsistent results may be attributed to different durations of chemotherapy used in the two studies. It was reported that postoperative morbidity was correlated with the number of cycles of chemotherapy before surgery. Karoui *et al*[35] reported that morbidity in patients who received six or more cycles of chemotherapy was significantly higher that that in patients who received less than six cycles (54% *vs* 19%, $P = 0.047$). Indeed, almost 80% of the patients in the series by Aussilhou *et al*[18] received six or more cycles of chemotherapy compared with 53% in the study by Zorzi *et al*[17].

Although oxaliplatin is often utilized as a chemotherapeutic agent in the treatment of CLM, it can exert adverse effects on the liver. Oxaliplatin-based chemotherapy has been shown to cause hepatic sinusoidal dilation[12]. Our study has shown that Bev can significantly reduce the incidence and severity of sinusoidal dilation,

thus supporting the use of Bev for CLM. An explanation for the above protective effect remains unclear. Increased expression of matrix metalloproteinase (MMP)-9 and MMP-2 by SEC was found to play an important role in sinusoidal dilation development in the monocrotaline-induced rat model[36]. An *in vitro* study[37] suggested that VEGF could up-regulate MMP-9 expression. It is postulated that VEGF blockade by Bev may attenuate sinusoidal injury by down-regulating MMP-9[15].

There is no consensus on the optimal time interval between discontinuation of Bev and hepatic surgery. D' Angelica *et al*[8] found that postoperative complications were more common in patients who received Bev within 8 wk. Similarly, Reddy *et al*[9] noted that patients who underwent hepatectomy within 8 wk of Bev treatment may be at a higher risk of overall, severe and hepatic complications after surgery. Whereas Kesmodel *et al*[16] failed to confirm that the time interval from discontinuation of Bev ($\geq$ 60 *vs* < 60 d) to surgery was associated with an increased likelihood of developing complications. In addition, a subgroup analysis of patients who received Bev also demonstrated that there was no significant difference in complication rates between patients who received Bev 31-45 d, 46-60 d and greater than 60 d before surgery (P = 0.21). In another report, Mahfud *et al*[10] showed that the occurrence of postoperative complications was similar in patients who had received Bev for < 6 wk and in those who had taken Bev $\geq$ 6 wk before liver resection. Based on the evidence that the median half-life of Bev in humans is approximately 21 d (range 11-50 d), some authors recommend waiting at least 6-8 wk from discontinuation of Bev to surgery[8,9,16].

The main limitation of this meta-analysis was that all evidence came from nonrandomized trials which could introduce potential bias in data collection and analysis. However, there is evidence that nonrandomized studies may generally give valid results[38].

In conclusion, our meta-analysis has shown that Bev can be safely administered before hepatic resection in CLM patients, knowing that it has a protective effect against oxaliplatin-related liver injury. The issue of optimal timing of hepatectomy in patients treated with Bev should be addressed in future prospective multicenter trials.

COMMENTS

Background

Bevacizumab (Bev), a monoclonal antibody that targets vascular endothelial growth factor (VEGF) A, has recently been added to standard neoadjuvant chemotherapy in patients with colorectal liver metastases (CLM). However, Bev is reportedly associated with bleeding, thrombosis, gastrointestinal perforation, impaired wound healing and liver regeneration. Therefore, the safety of Bev at the time of hepatic surgery needs to be addressed.

Research frontiers

The study evaluated the impact of preoperative administration of neoadjuvant Bev on the outcome of patients undergoing resection for CLM using a meta-analysis of all relevant studies.

Innovations and breakthroughs

Findings from this meta-analysis suggest that preoperative Bev does not seem to increase postoperative morbidity after hepatic surgery of CLM and has a protective effect against hepatic injury in patients treated with oxaliplatin chemotherapy.

Applications

Bev can be safely administered in CLM patients before hepatic resection.

Terminology

Bev, a monoclonal antibody against VEFG, can inhibit the growth of human tumor xenografts.

Peer review

The topic is interesting and the methodology of the meta-analysis is appropriate.

REFERENCES

1 **Mohammad WM**, Balaa FK. Surgical management of colorectal liver metastases. *Clin Colon Rectal Surg* 2009; **22**: 225-232 [PMID: 21037813 DOI: 10.1055/s-0029-1242462.]

2 **Liu JH**, Hsieh YY, Chen WS, Hsu YN, Chau GY, Teng HW, King KL, Lin TC, Tzeng CH, Lin JK. Adjuvant oxaliplatin- or irinotecan-containing chemotherapy improves overall survival following resection of metachronous colorectal liver metastases. *Int J Colorectal Dis* 2010; **25**: 1243-1249 [PMID: 20574727 DOI: 10.1007/s00384-010-0996-4]

3 **Tanaka K**, Adam R, Shimada H, Azoulay D, Lévi F, Bismuth H. Role of neoadjuvant chemotherapy in the treatment of multiple colorectal metastases to the liver. *Br J Surg* 2003; **90**: 963-969 [PMID: 12905549 DOI: 10.1002/bjs.4160]

4 **Benoist S**, Nordlinger B. The role of preoperative chemotherapy in patients with resectable colorectal liver metastases. *Ann Surg Oncol* 2009; **16**: 2385-2390 [PMID: 19554377 DOI: 10.1245/s10434-009-0492-7]

5 **Willett CG**, Boucher Y, di Tomaso E, Duda DG, Munn LL, Tong RT, Chung DC, Sahani DV, Kalva SP, Kozin SV, Mino M, Cohen KS, Scadden DT, Hartford AC, Fischman AJ, Clark JW, Ryan DP, Zhu AX, Blaszkowsky LS, Chen HX, Shellito PC, Lauwers GY, Jain RK. Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. *Nat Med* 2004; **10**: 145-147 [PMID: 14745444 DOI: 10.1038/nm988]

6 **Hurwitz H**, Fehrenbacher L, Novotny W, Cartwright T, Hainsworth J, Heim W, Berlin J, Baron A, Griffing S, Holmgren E, Ferrara N, Fyfe G, Rogers B, Ross R, Kabbinavar F. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. *N Engl J Med* 2004; **350**: 2335-2342 [PMID: 15175435]

7 **Kabbinavar FF**, Schulz J, McCleod M, Patel T, Hamm JT, Hecht JR, Mass R, Perrou B, Nelson B, Novotny WF. Addition of bevacizumab to bolus fluorouracil and leucovorin in first-line metastatic colorectal cancer: results of a randomized phase II trial. *J Clin Oncol* 2005; **23**: 3697-3705 [PMID: 15738537 DOI: 10.1200/JCO.2005.05.112]

8 **D'Angelica M**, Kornprat P, Gonen M, Chung KY, Jarnagin WR, DeMatteo RP, Fong Y, Kemeny N, Blumgart LH, Saltz LB. Lack of evidence for increased operative morbidity after hepatectomy with perioperative use of bevacizumab: a matched case-control study. *Ann Surg Oncol* 2007; **14**: 759-765 [PMID: 17103075 DOI: 10.1245/s10434-006-9074-0]

9 **Reddy SK**, Morse MA, Hurwitz HI, Bendell JC, Gan TJ, Hill SE, Clary BM. Addition of bevacizumab to irinotecan- and oxaliplatin-based preoperative chemotherapy regimens does not increase morbidity after resection of colorectal liver metastases. *J Am Coll Surg* 2008; **206**: 96-106 [PMID: 18155574 DOI: 10.1016/j.jamcollsurg.2007.06.290]

10 **Mahfud M**, Breitenstein S, El-Badry AM, Puhan M, Rickenbacher A, Samaras P, Pessaux P, Lopez-Ben S, Jaeck D,

Figueras J, Alain-Clavien P. Impact of preoperative bevacizumab on complications after resection of colorectal liver metastases: case-matched control study. *World J Surg* 2010; **34**: 92-100 [PMID: 19838754 DOI: 10.1007/s00268-009-0251-8]

11 **Neeff HP**, Drognitz O, Klock A, Illerhaus G, Opitz OG, Hopt UT, Makowiec F. Impact of preoperative targeted therapy on postoperative complications after resection of colorectal liver metastases. *Int J Colorectal Dis* 2012; **27**: 635-645 [PMID: 22139030 DOI: 10.1007/s00384-011-1360-z]

12 **Rubbia-Brandt L**, Audard V, Sartoretti P, Roth AD, Brezault C, Le Charpentier M, Dousset B, Morel P, Soubrane O, Chaussade S, Mentha G, Terris B. Severe hepatic sinusoidal obstruction associated with oxaliplatin-based chemotherapy in patients with metastatic colorectal cancer. *Ann Oncol* 2004; **15**: 460-466 [PMID: 14998849 DOI: 10.1093/annonc/mdh095]

13 **Nakano H**, Oussoultzoglou E, Rosso E, Casnedi S, Chenard-Neu MP, Dufour P, Bachellier P, Jaeck D. Sinusoidal injury increases morbidity after major hepatectomy in patients with colorectal liver metastases receiving preoperative chemotherapy. *Ann Surg* 2008; **247**: 118-124 [PMID: 18156931 DOI: 10.1097/SLA.0b013e31815774de]

14 **Iguchi A**, Kobayashi R, Yoshida M, Kobayashi K, Matsuo K, Kitajima I, Maruyama I. Vascular endothelial growth factor (VEGF) is one of the cytokines causative and predictive of hepatic veno-occlusive disease (VOD) in stem cell transplantation. *Bone Marrow Transplant* 2001; **27**: 1173-1180 [PMID: 11551028]

15 **Ribero D**, Wang H, Donadon M, Zorzi D, Thomas MB, Eng C, Chang DZ, Curley SA, Abdalla EK, Ellis LM, Vauthey JN. Bevacizumab improves pathologic response and protects against hepatic injury in patients treated with oxaliplatin-based chemotherapy for colorectal liver metastases. *Cancer* 2007; **110**: 2761-2767 [PMID: 17960603 DOI: 10.1002/cncr.23099]

16 **Kesmodel SB**, Ellis LM, Lin E, Chang GJ, Abdalla EK, Kopetz S, Vauthey JN, Rodriguez-Bigas MA, Curley SA, Feig BW. Preoperative bevacizumab does not significantly increase postoperative complication rates in patients undergoing hepatic surgery for colorectal cancer liver metastases. *J Clin Oncol* 2008; **26**: 5254-5260 [PMID: 18854565 DOI: 10.1200/JCO.2008.17.7857]

17 **Zorzi D**, Chun YS, Madoff DC, Abdalla EK, Vauthey JN. Chemotherapy with bevacizumab does not affect liver regeneration after portal vein embolization in the treatment of colorectal liver metastases. *Ann Surg Oncol* 2008; **15**: 2765-2772 [PMID: 18636296 DOI: 10.1245/s10434-008-0035-7]

18 **Aussilhou B**, Dokmak S, Faivre S, Paradis V, Vilgrain V, Belghiti J. Preoperative liver hypertrophy induced by portal flow occlusion before major hepatic resection for colorectal metastases can be impaired by bevacizumab. *Ann Surg Oncol* 2009; **16**: 1553-1559 [PMID: 19363584 DOI: 10.1245/s10434-009-0447-z]

19 **Klinger M**, Eipeldauer S, Hacker S, Herberger B, Tamandl D, Dorfmeister M, Koelblinger C, Gruenberger B, Gruenberger T. Bevacizumab protects against sinusoidal obstruction syndrome and does not increase response rate in neoadjuvant XELOX/FOLFOX therapy of colorectal cancer liver metastases. *Eur J Surg Oncol* 2009; **35**: 515-520 [PMID: 19200687 DOI: 10.1016/j.ejso.2008.12.013]

20 **Pessaux P**, Panaro F, Casnedi S, Zeca I, Marzano E, Bachellier P, Jaeck D, Chenard MP. Targeted molecular therapies (cetuximab and bevacizumab) do not induce additional hepatotoxicity: preliminary results of a case-control study. *Eur J Surg Oncol* 2010; **36**: 575-582 [PMID: 20452168 DOI: 10.1016/j.ejso.2010.04.010]

21 **Rubbia-Brandt L**, Lauwers GY, Wang H, Majno PE, Tanabe K, Zhu AX, Brezault C, Soubrane O, Abdalla EK, Vauthey JN, Mentha G, Terris B. Sinusoidal obstruction syndrome and nodular regenerative hyperplasia are frequent oxaliplatin-associated liver lesions and partially prevented by bevacizumab in patients with hepatic colorectal metastasis. *Histopathology* 2010; **56**: 430-439 [PMID: 20459550 DOI: 10.1111/j.1365-2559.2010.03511.x]

22 **Tamandl D**, Gruenberger B, Klinger M, Herberger B, Kaczirek K, Fleischmann E, Gruenberger T. Liver resection remains a safe procedure after neoadjuvant chemotherapy including bevacizumab: a case-controlled study. *Ann Surg* 2010; **252**: 124-130 [PMID: 20562613 DOI: 10.1097/SLA.0b013e3181deb67f]

23 **Wicherts DA**, de Haas RJ, Sebagh M, Saenz Corrales E, Gorden DL, Lévi F, Paule B, Azoulay D, Castaing D, Adam R. Impact of bevacizumab on functional recovery and histology of the liver after resection of colorectal metastases. *Br J Surg* 2011; **98**: 399-407 [PMID: 21254017 DOI: 10.1002/bjs.7368.]

24 **van der Pool AE**, Marsman HA, Verheij J, Ten Kate FJ, Eggermont AM, Ijzermans JN, Verhoef C. Effect of bevacizumab added preoperatively to oxaliplatin on liver injury and complications after resection of colorectal liver metastases. *J Surg Oncol* 2012; **106**: 892-897 [PMID: 22552819 DOI: 10.1002/jso.23142.]

25 **Dindo D**, Demartines N, Clavien PA. Classification of surgical complications: a new proposal with evaluation in a cohort of 6336 patients and results of a survey. *Ann Surg* 2004; **240**: 205-213 [PMID: 15273542 DOI: 10.1097/01.sla.0000133083.54934.ae]

26 **Assy N**, Spira G, Paizi M, Shenkar L, Kraizer Y, Cohen T, Neufeld G, Dabbah B, Enat R, Baruch Y. Effect of vascular endothelial growth factor on hepatic regenerative activity following partial hepatectomy in rats. *J Hepatol* 1999; **30**: 911-915 [PMID: 10365820]

27 **Endo D**, Kogure K, Hasegawa Y, Maku-uchi M, Kojima I. Activin A augments vascular endothelial growth factor activity in promoting branching tubulogenesis in hepatic sinusoidal endothelial cells. *J Hepatol* 2004; **40**: 399-404 [PMID: 15123352 DOI: 10.1016/j.jhep.2003.11.019]

28 **Marino G**, Piazzese E, Gruttadauria S, Nicotra G, Guarnaccia M, Emmanuele G, Bartoloni G, Messina A, Travali S, Famulari C, Gruttadauria G. New model of liver regeneration induced through use of vascular endothelial growth factor. *Transplant Proc* 2006; **38**: 1193-1194 [PMID: 16757304 DOI: 10.1016/j.transproceed.2006.02.022]

29 **Namisaki T**, Yoshiji H, Kojima H, Yoshii J, Ikenaka Y, Noguchi R, Sakurai S, Yanase K, Kitade M, Yamazaki M, Asada K, Uemura M, Nakamura M, Fukui H. Salvage effect of the vascular endothelial growth factor on chemically induced acute severe liver injury in rats. *J Hepatol* 2006; **44**: 568-575 [PMID: 16310882 DOI: 10.1016/j.jhep.2005.08.018]

30 **Bockhorn M**, Goralski M, Prokofiev D, Dammann P, Grünewald P, Trippler M, Biglarnia A, Kamler M, Niehues EM, Frilling A, Broelsch CE, Schlaak JF. VEGF is important for early liver regeneration after partial hepatectomy. *J Surg Res* 2007; **138**: 291-299 [PMID: 17275844 DOI: 10.1016/j.jss.2006.07.027]

31 **Pamecha V**, Glantzounis G, Davies N, Fusai G, Sharma D, Davidson B. Long-term survival and disease recurrence following portal vein embolisation prior to major hepatectomy for colorectal metastases. *Ann Surg Oncol* 2009; **16**: 1202-1207 [PMID: 19130138 DOI: 10.1245/s10434-008-0269-4]

32 **Covey AM**, Brown KT, Jarnagin WR, Brody LA, Schwartz L, Tuorto S, Sofocleous CT, D'Angelica M, Getrajdman GI, DeMatteo R, Kemeny NE, Fong Y. Combined portal vein embolization and neoadjuvant chemotherapy as a treatment strategy for resectable hepatic colorectal metastases. *Ann Surg* 2008; **247**: 451-455 [PMID: 18376189 DOI: 10.1097/SLA.0b013e31815ed693]

33 **Goéré D**, Farges O, Leporrier J, Sauvanet A, Vilgrain V,

Belghiti J. Chemotherapy does not impair hypertrophy of the left liver after right portal vein obstruction. *J Gastrointest Surg* 2006; **10**: 365-370 [PMID: 16504881 DOI: 10.1016/j.gassur.2005.09.001]

34 **Nafidi O**, Désy D, Létourneau R, Côté J, Plasse M, Vandenbroucke F, Roy A, Dagenais M, Lapointe RW. Hypertrophy of the non-embolized liver after chemotherapy. *HPB (Oxford)* 2009; **11**: 103-107 [PMID: 19590632 DOI: 10.1111/j.1477-2574.2009.00004.x]

35 **Karoui M**, Penna C, Amin-Hashem M, Mitry E, Benoist S, Franc B, Rougier P, Nordlinger B. Influence of preoperative chemotherapy on the risk of major hepatectomy for colorectal liver metastases. *Ann Surg* 2006; **243**: 1-7 [PMID: 16371728 DOI: 10.1097/01.sla.0000193603.26265.c3]

36 **Deleve LD**, Wang X, Tsai J, Kanel G, Strasberg S, Tokes ZA. Sinusoidal obstruction syndrome (veno-occlusive disease) in the rat is prevented by matrix metalloproteinase inhibition. *Gastroenterology* 2003; **125**: 882-890 [PMID: 12949732 DOI: 10.1016/S0016-5085(03)01056-4]

37 **Wang H**, Keiser JA. Vascular endothelial growth factor upregulates the expression of matrix metalloproteinases in vascular smooth muscle cells: role of flt-1. *Circ Res* 1998; **83**: 832-840 [PMID: 9776730 DOI: 10.1161/01.RES.83.8.832]

38 **Benson K**, Hartz AJ. A comparison of observational studies and randomized, controlled trials. *N Engl J Med* 2000; **342**: 1878-1886 [PMID: 10861324]

P- Reviewer Krivokapic Z **S- Editor** Wen LL
L- Editor Webster JR **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i5.769

World J Gastroenterol 2013 February 7; 19(5): 769-772
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Degarelix as a new antiangiogenic agent for metastatic colon cancer?

Francois Ghiringhelli, Nicolas Isambert, Sylvain Ladoire

Francois Ghiringhelli, Nicolas Isambert, Sylvain Ladoire, Unite Mixte de Recherche 866, Institut National de la Sante et de la Recherche Medicale/Universite de Bourgogne, 21000 Dijon, France
Francois Ghiringhelli, Nicolas Isambert, Sylvain Ladoire, Departement of Oncologie Medicale, Centre Georges Francois Leclerc, 21000 Dijon, France
Author contributions: Ghiringhelli F, Isambert N and Ladoire S contributed to write and revise the paper.
Correspondence to: Francois Ghiringhelli, MD, PhD, Departement of Oncologie Medicale, Centre Georges Francois Leclerc, 1 rue du Professeur Marion, 21000 Dijon, France. fghiringhelli@cgfl.fr
Telephone: +33-3-8393353 Fax: +33-3-8393434
Received: August 31, 2012 Revised: December 18, 2012
Accepted: December 22, 2012
Published online: February 7, 2013

Abstract

Recently, follicle stimulating hormone receptor was found to be selectively expressed by endothelial cells on tumor-associated blood vessels in a wide range of human cancers. In this context, we hypothesized that degarelix, a new gonadotropin-releasing hormone receptor antagonist developed for patients with prostate cancer, may have antiangiogenic effects *via* its capacity to block follicle stimulating hormone (FSH) production. We report the case of a patient with metastatic colon cancer exhibiting tumor progression after failure of all conventional chemotherapeutic regimens. The addition of degarelix to the last chemotherapeutic regimen was proposed as compassionate treatment. Degarelix induced a rapid decrease in FSH level. This treatment induced radiological stabilization and carcinoembryonic antigen stabilization during 1 year. Contrast-enhanced ultrasonography demonstrated reduction of tumor vasculature. This case represents the first report of an antitumoral effect of degarelix in metastatic colon cancer and suggests an antiangiogenic property of this drug.

© 2013 Baishideng. All rights reserved.

Key words: Colon cancer; Degarelix; Chemotherapy; Angiogenesis; Antiangiogenic agents

Ghiringhelli F, Isambert N, Ladoire S. Degarelix as a new antiangiogenic agent for metastatic colon cancer? *World J Gastroenterol* 2013; 19(5): 769-772 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i5/769.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i5.769

INTRODUCTION

In adult humans, the follicle stimulating hormone receptor (FSHR) is expressed only in the testicular sertoli cells and the ovarian granulosa cells. Recently, FSHR was also found to be selectively expressed by endothelial cells on tumor-associated blood vessels in a wide range of human cancers, compared to blood vessels from normal tissue[1]. This leads to the hypothesis that follicle stimulating hormone (FSH) and FSHR could be involved in endothelial cell survival or proliferation, especially during tumor-associated angiogenesis. Thus, the FSH pathway could be a new target for cancer therapy, notably in cancer types in which tumor angiogenesis is critical for cancer progression. Degarelix is a new gonadotropin-releasing hormone (GnRH) receptor antagonist, developed for patients with prostate cancer requiring androgen-deprivation therapy. Degarelix blocks the GnRH receptors in the anterior pituitary gland, resulting in a rapid decrease in the secretion of both luteinising hormone (LH) and FSH[2]. In this context, we hypothesized that degarelix may have antiangiogenic effects *via* its capacity to block FSH production, thereby interrupting the FSH-FSHR pathway on tumor-associated blood vessels.

We report here on a patient with metastatic colon cancer exhibiting tumor progression after failure of all conventional chemotherapeutic regimens, and who ex-

perienced long term disease stabilization associated with tumor devascularization under degarelix treatment.

CASE REPORT

A 69 year-old woman was referred to our center for sideropenic anemia in March 2008. A tumor of the cecum was discovered and the patient underwent right colectomy. The tumor was staged T3 N2, and non-resectable peritoneal carcinomatosis was discovered. The tumor had a K-RAS mutation status. The patient was initially treated starting in July 2008 by FOLFIRI + bevacizumab, and subsequently by LV5FU2 + bevacizumab because of hematological toxicity and grade 3 diarrhea. During treatment, the tumor marker carcinoembryonic antigen (CEA) decreased from 15 ng/mL, to 6 ng/mL in July 2009. Disease progression occurred in December 2009, with a CEA level of 14 ng/mL. The patient received FOLFOX + bevacizumab from December 2009 to September 2010, at which time CEA level increased to 42 ng/mL, and the patient presented an occlusive syndrome with abdominal pain requiring morphine treatment. On compassionate grounds, we proposed the addition of degarelix to LV5FU2 + bevacizumab. Degarelix was initiated subcutaneously at 240 mg for 1 mo, followed by monthly maintenance doses of 80 mg. FSH level was 42.5 IU/L before the first injection. A peritoneal nodule of 135 mm was selected as a target lesion for radiological evaluation by computed tomography (CT) scan and contrast-enhanced ultrasonography. Fifteen days after the first injection, the FSH serum level was < 2 IU/L (Figure 1A), and remained at this level during the entire treatment period. Contrast-enhanced ultrasonography was performed on day 0 and day 15. Radiological evaluation was performed by the same 2 independent radiologists, who were blinded to the patient's clinical, biological and treatment data. A single target tumor was studied and selected on the basis of size (> 2 cm), and site (tumors with a good acoustic window that enabled data acquisition for longer than 3 min). A dynamic study was conducted after a single intravenous bolus injection of 4.8 mL of a contrast agent consisting of sulfur hexafluoride-filled microbubbles (SonoVue; Bracco, Milan, Italy). The investigation recordings and timing were triggered as soon as the contrast agent was injected. A total of 720 images were acquired during each 3-min investigative examination. Three semi-quantitative perfusion parameters were extracted from the time-intensity curves, namely peak intensity, time to peak intensity, total area under the time-intensity curve (AUC)[3]. Before degarelix initiation, AUC of the peritoneal nodule was 1650 mm^2. Fifteen days after treatment initiation, a dramatic reduction of almost 80% in the vascularized surface was observed, at 351 mm^2 (Figure 1B, C) (The area of interest is marked by a dotted white line). Moreover, resolution of the occlusive syndrome was achieved 5 wk after degarelix initiation, and morphine treatment was stopped without residual abdominal pain. Tolerance was excellent, without side effects using NCI-CTCAE v3 grade. Patient was previously treated by amlodipine for bevacizumab related and blood pressure was not modified by degarelix. The patient was menopause at the beginning of the treatment with degarelix so no side affect related to gonad suppression were observed. CT scans performed 2 mo after the beginning of the treatment showed stable disease according to the RECIST criteria with a stable target lesion and a regression of small none target lesion of peritoneal carcinomatosis. Successive CT scans performed between September 2010 and August 2011 confirmed stable disease according to the RECIST criteria (version 1.1) (target lesion evaluated between 137 and 138 mm). CEA levels remained stable during this period (Figure 1A). Unfortunately, occlusive syndrome re-appeared in October 2011. CT scan showed peritoneal progression, and only best supportive care was maintained. The patient died 6 wk later.

DISCUSSION

To the best of our knowledge, this case represents the first report of an antitumoral effect of degarelix in metastatic colon cancer.

The FSH receptor was recently recognized as a specific marker of endothelial cells in blood vessels from different tumor types[1]. Biological data have shown that the binding of FSH to FSH receptor in granulosa cells induces increased production of the hypoxia-inducible factor 1α[4]. This transcriptional factor is commonly induced by hypoxic conditions and drives the up-regulation of vascular endothelial growth factor (VEGF), one of the most important pro-angiogenic factors secreted during cancer growth. Thus, we hypothesized that triggering the FSH-receptor could induce VEGF production and thereby promote tumor angiogenesis. FSH signaling is also known to generate activated Gq protein[5]. Gq protein has been shown to induce VEGFR-2 signaling in human endothelial cells, even in the absence of VEGF[6]. This effect may enhance the proliferation and migration of endothelial cells in cancer, independently of VEGF availability. Thus, blocking FSH-receptor signaling may represent a new antiangiogenic strategy. In this report, we present the first clinical observation that degarelix, a GnRH antagonist used for the treatment of castrate-sensitive prostate cancer, could considerably decrease FSH production in a post-menopausal cancer-bearing woman. Contrast-enhanced ultrasonography seemed to indicate that this period of 12 mo with disease stabilization in a previously chemorefractory patient could be related to an antiangiogenic effect of degarelix, suggesting that this treatment could overcome resistance to anti-VEGF therapies, such as bevacizumab. However, we cannot provide specific immunohistochemical analysis of FSHR expression on tumor-associated blood vessels from the patient's peritoneal tumors. This constitutes a

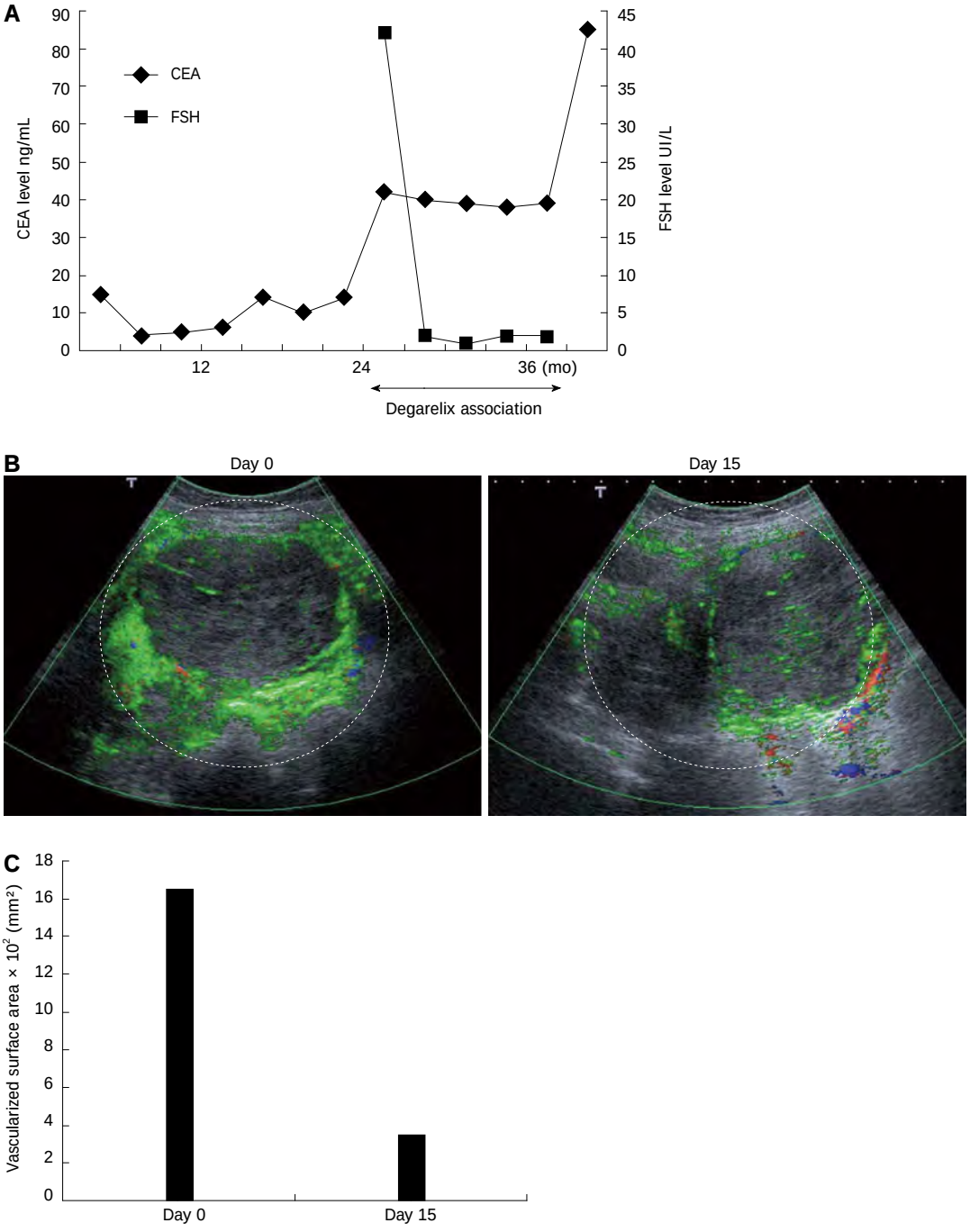


Figure 1 A tumor of the cecum. A: Kinetic of carcinoembryonic antigen (CEA) and follicle stimulating hormone (FSH) serum level; B: Representative images of contrast-enhanced ultrasonography performed before (left panel) and 15 d after (right panel) the beginning of the treatment by degarelix; C: Evaluation of vascularized surface area on contrast-enhanced ultrasonography performed before and 15 d after the beginning of the treatment by degarelix.

limitation of this report, but it should be noted that in the initial report by Radu *et al*[1], consistent expression of FSHR by endothelial cells was detected in all the colorectal cancers analyzed. As we do not test the efficacy of degarelix monotherapy was could not determine if only combination of 5 FU/leucovorin bevacizumab and degarelix or degarelix monotherapy alone could be proposed in further clinical trial. Nonetheless, further clinical trials guided by FSHR expression on tumor vasculature are warranted to investigate the antiangiogenic effect of degarelix in cancer patients.

REFERENCES

1 **Radu A**, Pichon C, Camparo P, Antoine M, Allory Y, Couvelard A, Fromont G, Hai MT, Ghinea N. Expression of follicle-stimulating hormone receptor in tumor blood vessels. *N Engl J Med* 2010; **363**: 1621-1630 [PMID: 20961245 DOI: 10.1056/NEJMoa1001283]

2 **Crawford ED**, Tombal B, Miller K, Boccon-Gibod L, Schröder F, Shore N, Moul JW, Jensen JK, Olesen TK, Persson BE. A phase III extension trial with a 1-arm crossover from leuprolide to degarelix: comparison of gonadotropin-releasing hormone agonist and antagonist effect on prostate cancer. *J Urol* 2011; **186**: 889-897 [PMID: 21788033 DOI:

10.1016/j.juro.2011.04.083]
3 **Schirin-Sokhan R**, Winograd R, Roderburg C, Bubenzer J, do Ó NC, Guggenberger D, Hecker H, Trautwein C, Tischendorf JJ. Response evaluation of chemotherapy in metastatic colorectal cancer by contrast enhanced ultrasound. *World J Gastroenterol* 2012; **18**: 541-545 [PMID: 22363120 DOI: 10.3748/wjg.v18.i6.541]
4 **Alam H**, Weck J, Maizels E, Park Y, Lee EJ, Ashcroft M, Hunzicker-Dunn M. Role of the phosphatidylinositol-3-kinase and extracellular regulated kinase pathways in the induction of hypoxia-inducible factor (HIF)-1 activity and the HIF-1 target vascular endothelial growth factor in ovarian granulosa cells in response to follicle-stimulating hormone. *Endocrinology* 2009; **150**: 915-928 [PMID: 18845636 DOI: 10.1210/en.2008-0850]
5 **Castro-Fernández C**, Maya-Núñez G, Méndez JP. Regulation of follicle-stimulating and luteinizing hormone receptor signaling by. *Endocrine* 2004; **25**: 49-54 [PMID: 15545706]
6 **Zeng H**, Zhao D, Yang S, Datta K, Mukhopadhyay D. Heterotrimeric G alpha q/G alpha 11 proteins function upstream of vascular endothelial growth factor (VEGF) receptor-2 (KDR) phosphorylation in vascular permeability factor/VEGF signaling. *J Biol Chem* 2003; **278**: 20738-20745 [PMID: 12670961]

P- Reviewers Kozono D, Takahashi M **S- Editor** Song XX
L- Editor A **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i5.773

World J Gastroenterol 2013 February 7; 19(5): 773-775
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Internal carotid thrombus in patients with inflammatory bowel disease: Two cases

Sébastien Richard, Gioia Mione, Julien Perrin, Marie Toussaint-Hacquard, Jean-Christophe Lacour, Xavier Ducrocq

Sébastien Richard, Gioia Mione, Jean-Christophe Lacour, Xavier Ducrocq, Department of Neurology, CHU Nancy, Hospital Central, 54035 Nancy Cedex, France
Julien Perrin, Marie Toussaint-Hacquard, Department of Biological Hematology, CHU Nancy, hôpitaux de Brabois, rue du Morvan, 54511 Vandoeuvre-les-Nancy Cedex, France
Author contributions: Richard S and Mione G contributed equally to this work; Richard S, Mione G, Perrin J, Toussaint-Hacquard M, Lacour JC and Ducrocq X designed and performed research; Richard S, Mione G, Perrin J and Toussaint Hacquard M analyzed data; Richard S and Mione G wrote the paper.
Correspondence to: Sébastien Richard, PhD, Department of Neurology, CHU Nancy, Hospital Central, 29 avenue du Marechal de Lattre de Tassigny-CO n° 34, 54035 Nancy Cedex, France. s.richard@chu-nancy.fr
Telephone: +33-3-83851656 Fax: +33-3-83851159
Received: October 4, 2012 Revised: October 25, 2012
Accepted: November 6, 2012
Published online: February 7, 2013

Abstract

Increased ischemic stroke risk is observed in patients with inflammatory bowel disease (IBD). Causes and physiopathological aspects of cerebral infarct, in this specific population, are less often described. There is little information to provide guidelines for the best curative and preventive treatment. We report 2 cases of ischemic strokes due to internal carotid thrombus in patients during active phase of IBD. Ulceration of early atherosclerotic plaques activated by a hypercoagulation state may cause a thrombus. A combined therapy with heparin and corticosteroids was used for both our patients. Lysis of the thrombus was obtained after several days without surgical treatment and shown by ultrasonography. These cases highlight an aetiology of stroke in patients with IBD and use of a synergic treatment to respond to hypercoagulability in link with IBD. Benefits and safety of this therapy should be confirmed with clinical studies.

© 2013 Baishideng. All rights reserved.

Key words: Inflammatory bowel disease; Ischemic stroke; Carotid thrombus; Hypercoagulable state; Atherosclerosis

Richard S, Mione G, Perrin J, Toussaint-Hacquard M, Lacour JC, Ducrocq X. Internal carotid thrombus in patients with inflammatory bowel disease: Two cases. *World J Gastroenterol* 2013; 19(5): 773-775 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i5/773.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i5.773

INTRODUCTION

Patients with inflammatory bowel diseases (IBD) have a higher risk of arterial cerebral infarction even if they are young with few cardio-vascular risk factors[1]. Causes and physiopathological aspects are less known. We describe 2 cases of IBD patients who presented ischemic strokes due to homolateral internal carotid thrombus.

CASE REPORT

Case report 1

A 32 year-old man, with Crohn's disease (CD) without immunosuppressive treatment for several years, was admitted for sudden right hemiplegia. He was a light smoker without any other vascular risk factor like hypertension, dyslipidemia, diabetes, and had no family history of vascular disease. At admission the National Institute of Health Stroke Scale (NIHSS) was 13, and blood pressure was 110/70 mm Hg. Brain magnetic resonance imaging (MRI) revealed ischemic stroke with thrombosis of the initial part of the left middle cerebral artery (MCA). Intravenous thrombolysis with plasminogen tissue activator was administered. Cervical ultrasonography (US) revealed occlusion of the left internal carotid artery (Figure

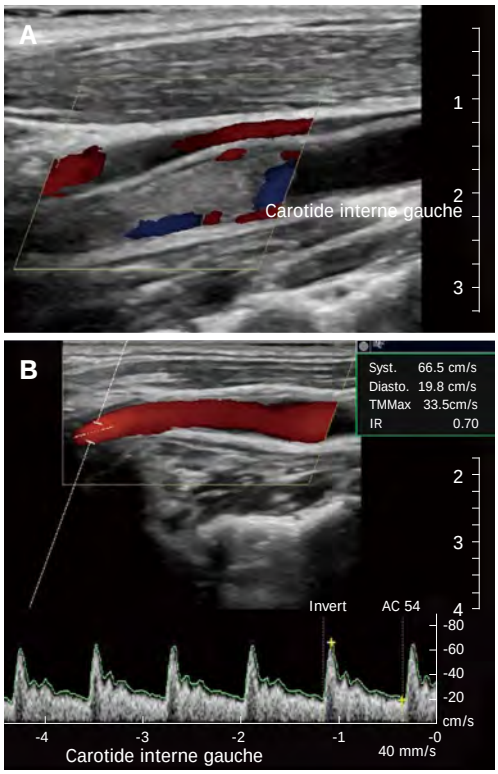


Figure 1 Cervical ultrasonographies in case 1, showing occlusion of the left internal carotid (A), due to a thrombus, and repermeabilization of the vessel with tiny plaque (B) after 15 d of treatment.

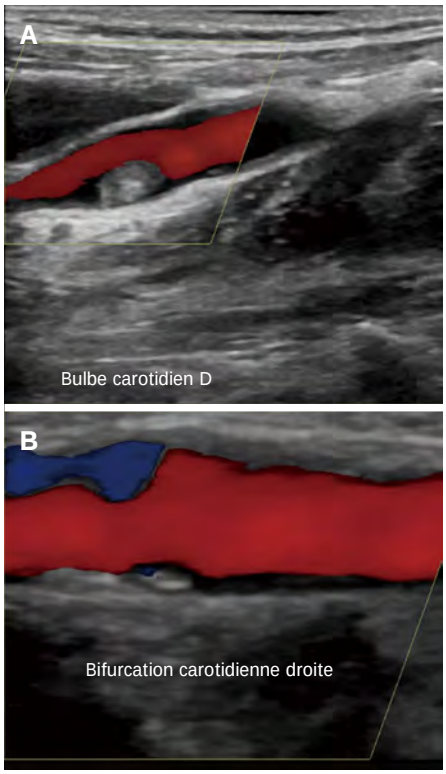


Figure 2 Cervical ultrasonographies in case 2, showing thrombus in the right internal carotid (A) and resorption of this with tiny plaque (B) after 1 wk of treatment.

1A), due to a thrombus, confirmed by computed tomography (CT) angiography. Blood tests were performed: the platelet count was 342 G/L, hyperhomocysteinemia 137 μmol/L; C-reactive protein (CRP) 15.4 mg/L; low-density lipoprotein (LDL)-cholesterol 0.87g/L; preprandial blood glucose level 1.01g/L, and the erythrocyte sedimentation rate (ESR) was elevated at 40 mm. Digestive endoscopy found signs of active CD. Unfractionated heparin and corticosteroid treatment (prednisolone 0.5 mg/kg per day) were initiated and 15 d later, there was total resorption of the thrombus, apart from a tiny plaque as controlled by cervical US (Figure 1B) and CT angiography. One month later, the NIHSS was 6, an overlap treatment of vitamin K antagonist was initiated and the patient was transferred to a rehabilitation center with a daily dose of prednisolone and folic acid. Proctectomy with terminal ileostomy was performed 4 mo later.

Case report 2

A non-smoker 42 year-old woman with ulcerative colitis diagnosed 3 years previously and treated with corticosteroids and mesalazine, was hospitalised for sudden right hemiplegia. She had no personal medical history of diabetes, hypertension and dyslipidemia and no family history of vascular disease. She had already presented several ischemic strokes, revealing intra-cardiac thrombus, but had refused any oral anticoagulant treatment. At admission, the NIHSS was 19 and blood pressure was 120/80 mmHg. A brain MRI showed a recent ischemic stroke with occlusion of the origin of the right MCA. Cerebral angiography with thrombectomy was performed and complicated by hemorrhagic transformation of the cerebral infarct. Severe bloody diarrhoea was controlled by a high dose of corticosteroids (methylprednisolone 1 mg/kg for 10 d before progressive decrease). Cervical US showed a thrombus in the right internal carotid (Figure 2A). There were biological signs of inflammation with an elevated ESR at 64 mm and CRP at 14.4 mg/L, slight hyperhomocysteinemia (17.9 μmol/L), a low level of LDL-cholesterol at 0.80 g/L, a normal preprandial blood glucose level at 0.90 g/L, and a platelet count of 462 G/L. Ten days later, after partial resorption of cerebral haemorrhage, low molecular weight heparin (LWMH), enoxaparine, was introduced at a dose of 100 UI/kg twice a day. After one week of treatment, cervical US revealed regression of the thrombus with only a tiny plaque on the right internal carotid wall (Figure 2B). LWMH was switched for aspirin as the patient rejected any oral anticoagulant. She was finally transferred to a rehabilitation center with a NIHSS of 6.

DISCUSSION

These observations suggest that 2 factors can contribute to carotid thrombus: abnormalities of the coagulation system and disease of arterial wall[2].

Hypercoagulability is almost always observed during the active phase of IBD and can lead to an arterial or

intracardiac thrombus, as described in the second case. Elevated platelets (with hyperactivation), fibrinogen, factor V, Ⅷ, plasminogen activator inhibitor and decreased antithrombin and tissue plasminogen activator have been described. Vitamin K malabsorption causes not only short partial thromboplastin times but also protein C and S deficiency[3]. There may also be a higher prevalence of antiphospholipid antibodies in IBD patients[4]. Common carotid artery intima-media thickness is significantly higher in IBD patients compared with controls with a significant association with homocysteine levels and age[5]. This is due to modifications of the vessel-wall induced by chronic inflammation: cytokines activate endothelium with conversion to a pro-inflammatory thrombogenic phenotype and increased von Willebrand factor[6]. Accelerated atherosclerosis is found in systemic inflammatory disorders[7], and atherosclerotic plaques were found after resorption of carotid thrombus in both the cases described here, as well as in a similar case described by Lafitte *et al*[8]. In IBD patients, an inflammatory state may also lead to endothelial damage and early atherosclerotic lesions. In the cases we describe, the formation of carotid thrombus may have been due to ulceration of plaques which have activated a pro-thrombotic coagulation state. For both our patients, lysis of the thrombus was obtained after several days of heparin treatment with double impact: fibrinolysis and the still debated anti-inflammatory action[9]. Nevertheless, this approach has only been described in a few cases and should be evaluated with clinical studies, especially as it may provoke mucosal[10] and cerebral bleeding in case of extensive stroke. We also administered corticosteroids to both our patients to decrease the inflammation, hypercoagulation state, to promote mucosal regeneration and then, to potentiate the benefits of heparin.

Internal carotid thrombus is a cause of ischemic stroke in IBD patients, and is a consequence of the combination of early atherosclerotic lesions and hypercoagulation state. Combining heparin and anti-inflammatory therapy may be considered as a synergic treatment but, further work is required to establish efficacy and safety.

REFERENCES

1 **Andersohn F**, Waring M, Garbe E. Risk of ischemic stroke in patients with Crohn's disease: a population-based nested case-control study. *Inflamm Bowel Dis* 2010; **16**: 1387-1392 [PMID: 20014016]

2 **Yoshida H**, Granger DN. Inflammatory bowel disease: a paradigm for the link between coagulation and inflammation. *Inflamm Bowel Dis* 2009; **15**: 1245-1255 [PMID: 19253306 DOI: 10.1002/ibd.20896]

3 **Conlan MG**, Haire WD, Burnett DA. Prothrombotic abnormalities in inflammatory bowel disease. *Dig Dis Sci* 1989; **34**: 1089-1093 [PMID: 2743849 DOI: 10.1007/BF01536380]

4 **Twig G**, Zandman-Goddard G, Szyper-Kravitz M, Shoenfeld Y. Systemic thromboembolism in inflammatory bowel disease: mechanisms and clinical applications. *Ann N Y Acad Sci* 2005; **1051**: 166-173 [PMID: 16126956 DOI: 10.1196/annals.1361.058]

5 **Papa A**, Santoliquido A, Danese S, Covino M, Di Campli C, Urgesi R, Grillo A, Guglielmo S, Tondi P, Guidi L, De Vitis I, Fedeli G, Gasbarrini G, Gasbarrini A. Increased carotid intima-media thickness in patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2005; **22**: 839-846 [PMID: 16225493 DOI: 10.1111/j.1365-2036.2005.02657.x]

6 **Nachman RL**, Silverstein R. Hypercoagulable states. *Ann Intern Med* 1993; **119**: 819-827 [PMID: 8379603 DOI: 10.1007/s10620-012-2148-x]

7 **Kayahan H**, Sari I, Cullu N, Yuksel F, Demir S, Akarsu M, Goktay Y, Unsal B, Akpinar H. Evaluation of early atherosclerosis in patients with inflammatory bowel disease. *Dig Dis Sci* 2012; **57**: 2137-2143 [PMID: 22466100]

8 **Lafitte M**, Debruxelles S, Sibon I, Rouanet F, Couffinhal T. Cerebral embolism from subclinical carotid atherosclerotic lesion in a young woman with inflammatory Crohn disease. *Eur Heart J* 2009; **30**: 575 [PMID: 18996955 DOI: 10.1093/eurheartj/ehn496]

9 **Chande N**, MacDonald JK, Wang JJ, McDonald JW. Unfractionated or low molecular weight heparin for induction of remission in ulcerative colitis: a Cochrane inflammatory bowel disease and functional bowel disorders systematic review of randomized trials. *Inflamm Bowel Dis* 2011; **17**: 1979-1986 [PMID: 21618363 DOI: 10.1002/ibd.21776]

10 **Nogami H**, Iiai T, Maruyama S, Tani T, Hatakeyama K. Common carotid arterial thrombosis associated with ulcerative colitis. *World J Gastroenterol* 2007; **13**: 1755-1757 [PMID: 17461484]

P- Reviewer Mowat C **S- Editor** Song XX **L- Editor** A **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i5.776

World J Gastroenterol 2013 February 7; 19(5): 776-777
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Strangulated ileal trans-coloanal-anastomotic hernia: A complication of Altemeier's procedure previously never reported

Maria Di Lena, Emanuele Angarano, Ivana Giannini, Altomarino Guglielmi, Donato Francesco Altomare

Maria Di Lena, Emanuele Angarano, Ivana Giannini, Altomarino Guglielmi, Donato Francesco Altomare, Department of Emergency and Organ Transplantation, University "Aldo Moro" of Bari, 70124 Bari, Italy
Author contributions: Di Lena M gave substantial contribution to conception and design of the data, draft the paper and approved the version to be published; Angarano E gave substantial contribution to conception and acquisition of the data, revised the paper and approved the version to be published; Giannini I substantial contribution to analysis and interpretation of the data and approved the version to be published; Guglielmi A revised the paper for important intellectual content and approved the version to be published; Altomare DF draft and revised the article for important intellectual content and approved the version to be published.
Correspondence to: Altomare Donato Francesco, MD, Department of Emergency and Organ Transplantation, University "Aldo Moro"of Bari, Policlinico, piazza G Cesare 11, 70124 Bari, Italy. donatofrancesco.altomare@uniba.it
Telephone: +39-80-5478735 Fax: +39-80-5592107
Received: October 17, 2012 Revised: November 12, 2012
Accepted: November 24, 2012
Published online: February 7, 2013

Abstract

A postoperative complication after Altemeier operation, so far never reported, is described in a 42 years old mentally disabled patient with external full thickness rectal prolapse who usually had prolonged straining at defecation. After 6 d from perineal rectosigmoidectomy, the patient, was discharged free of complications. Four days later he was readmitted in emergency for strangulated perineal trans-anastomotic ileal hernia that occurred at home during efforts to defecate. The clinical feature required an emergency operation for repositioning the ileal loops into the abdomen, resection of the necrotic ileum, and end colostomy. The outcome of the second operation was free of complication and the patient was discharged on the 6th postoperative day. In conclusion, after Altemeier operation prolonged straining at defecation should be carefully avoided

© 2013 Baishideng. All rights reserved.

Key words: Rectal prolapsed; Perineal rectosigmoidectomy; Altemeier's procedure; Complication; Hernia

Di Lena M, Angarano E, Giannini I, Guglielmi A, Altomare DF. Strangulated ileal trans-coloanal-anastomotic hernia: A complication of Altemeier's procedure previously never reported. *World J Gastroenterol* 2013; 19(5): 776-777 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i5/776.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i5.776

INTRODUCTION

Full thickness rectal prolapse is an uncommon but very disabling anorectal disease affecting any age and in particular mentally disabled patients. Several surgical approaches have been proposed to treat this disorder including abdominal rectopexy and perineal mucosal (Delorme) or full thickness rectal resection (Altemeier). The Altemeier's procedure is usually preferred (1) in elderly, high-risk, frail patients unsuitable for abdominal operation; (2) for emergency incarcerated external prolapse[1] and for gangrenous rectal prolapse[2]; and (3) in mentally disabled patients because of its short, painless postoperative course and low complication rate.

CASE REPORT

A 42-year-old male patient affected by congenital hypothyroidism with a psychomotor retardation, anorexia and bulimia was admitted to our Colorectal Unit because of irreducible full thickness rectal prolapse. From several years the patient used to have prolonged and forced



Figure 1 Strangulated ileal trans-coloanal-anastomotic perineal hernia showing ischaemia of the herniated ileum.

straining at defecation (about 30 min) with exteriorization of the prolapse at every defecation. However, in the last 2 mo the rectal prolapse became irreducible. A perineal rectosigmoidectomy with levatorplasty (Altemeier's operation) was performed under general anesthesia with the patient in jack-knife position. Twelve cm of prolapsed rectum was resected and a hand-sewn interrupted coloanal anastomosis was performed. The patient was discharged on the 6th postoperative day free of complication and oral laxatives and antithrombotic prophylaxis were prescribed. Four days later the patient was re-admitted in emergency since a strangulated ileal trans-coloanal-anastomotic hernia occurred after a prolonged and strain defecation (Figure 1). Laparotomy was urgently performed and no signs of intrabdominal infection or ascites were found. However, the distal part of the ileum (about 2 m) was herniated outside the pelvis through a partial posterior dehiscence of the colo-anal anastomosis with torsion of intestinal loops around the vascular stalk. The large portion of small intestine herniated was reduced with difficulty into the abdomen with the help of a second operator from the perineal side. Once the ileum was replaced into the abdomen, the coloanal anastomosis was fully opened and the anus temporarily closed with continue resorbable suture. The vitality of the herniated ileum (up to 5 cm from the ileo-cecal valve) was poor after one hour and, therefore, it was resected. Then, a hand-sewn ileo-cecal anastomosis and a diverting sigmoid end colostomy were performed. The second postoperative course was uneventful and the patient was discharged on the 6th postoperative day. The patient was re-evaluated at follow-up for 4 mo and he is now waiting for a refashioning of the colo-anal anastomosis.

DISCUSSION

Altemeier's procedure is usually recommended in mentally disabled patients with full thickness rectal prolapse because of its short and painless postoperative course. Postoperative complications are uncommon (less than 10%[3,4] of cases), nevertheless they usually occur in the early postoperative days. The complication we herein described, to our knowledge for the first time, was unexpected since it occurred several days after the patient was discharged in good clinical condition and with normal bowel movements.

We suggest that anamnestic data of the behavior of prolonged straining at defecation in mentally disabled patients should have been considered, and adequate advise given to the relatives to assist patients during defecation in order to prevent strain. Finally, the choice of creating a diverting sigmoidostomy instead of trying to repair the coloanal anastomosis seems more prudent, due to the presence of edema in traumatized anastomotic leakage.

REFERENCES

1 **Zuo ZG**, Song HY, Xu C, Li J, Ni SC, Zhou ZH, Chen SQ. [Application of Altemeier procedure in the emergent management of acute incarcerated rectal prolapse]. *Zhonghua Wei Chang Wai Ke Zazhi* 2010; **13**: 427-429 [PMID: 20577921]

2 **Voulimeneas I**, Antonopoulos C, Alifierakis E, Ioannides P. Perineal rectosigmoidectomy for gangrenous rectal prolapse. *World J Gastroenterol* 2010; **16**: 2689-2691 [PMID: 20518093 DOI: 10.3748/wjg.v16.i21.2689]

3 **Pescatori M**, Zbar AP. Tailored surgery for internal and external rectal prolapse: functional results of 268 patients operated upon by a single surgeon over a 21-year period*. *Colorectal Dis* 2009; **11**: 410-419 [PMID: 18637923 DOI: 10.1111/j.1463-1318.2008.01626.x]

4 **Altomare DF**, Binda G, Ganio E, De Nardi P, Giamundo P, Pescatori M. Long-term outcome of Altemeier's procedure for rectal prolapse. *Dis Colon Rectum* 2009; **52**: 698-703 [PMID: 19404077 DOI: 10.1007/DCR.0b013e31819ecffe]

P- Reviewer Kim NK **S- Editor** Gou SX **L- Editor** A **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i5.778

World J Gastroenterol 2013 February 7; 19(5): 778-780
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Ectopic adrenal cortical adenoma in the gastric wall: Case report

Pei-Tu Ren, Hong Fu, Xiao-Wen He

Pei-Tu Ren, Hong Fu, Xiao-Wen He, Department of General Surgery, Shaoxing People's Hospital, Shaoxing Hospital of Zhejiang University, Shaoxing 312000, Zhejiang Province, China
Author contributions: Ren PT, Fu H, and He XW contributed equally to manuscript writing and revision.
Correspondence to: Xiao-Wen He, PhD, Department of General Surgery, Shaoxing People's Hospital, Shaoxing Hospital of Zhejiang University, Shaoxing 312000, Zhejiang Province, China. hexiaowen.2006@yahoo.com.cn
Telephone: +86-575-88229452 Fax: +86-575-88229452
Received: October 2, 2012 Revised: November 14, 2012
Accepted: December 20, 2012
Published online: February 7, 2013

Abstract

Ectopic adrenal cortical neoplasms are extremely rare. Ectopic adrenocortical tissue can be found in locations such as the celiac axis, the broad ligament, the adnexa of the testes, and the spermatic cord; however, they rarely involve the stomach. We report an unusual case of a patient with an ectopic adrenal cortical adenoma in the gastric wall. The patient was a 72-year old female admitted to our hospital with upper abdominal discomfort. Physical examination revealed tenderness below the xiphoid process. Both computed tomography and fibergastroscopy revealed a mass on the lesser curvature side of the gastric antrum; it was initially diagnosed as a gastric stromal tumor. After adequate preparation, the patient underwent surgery. During the procedure, we found a 30 mm × 30 mm mass with medium density in the lesser curvature near the gastric antrum within the serosa. Following immunohistochemistry examination, we corrected the diagnosis to an ectopic adrenal cortical adenoma; the tumor was nonfunctional.

© 2013 Baishideng. All rights reserved.

Key words: Ectopic adrenal cortical neoplasms; Adrenal adenoma; Stomach; Adult; Nonfunctional adenoma

Ren PT, Fu H, He XW. Ectopic adrenal cortical adenoma in the gastric wall: Case report. *World J Gastroenterol* 2013; 19(5): 778-780 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i5/778.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i5.778

INTRODUCTION

The adrenal gland arises from primordial mesenchyme in the wall of the dorsal coelom adjacent to the dorsal mesentery and urogenital structures. Therefore, most ectopic adrenocortical tissue is found along the path of embryonic migration within the urogenital tract. Ectopic adrenocortical tissue is found in such locations as the celiac axis, the broad ligament, the adnexa of the testes, and the spermatic cord[1]. However, an ectopic adrenal cortical adenoma rarely involves the stomach. We report an unusual case of a patient with an ectopic adrenal cortical adenoma in the gastric wall and review the literature.

CASE REPORT

The patient was a 72-year old female. She was admitted on 17th November, 2011 with upper abdominal discomfort for 4 d, accompanied by postprandial nausea and vomiting. She denied chills, fever, or diarrhea. Her medical and family history were noncontributory.

Physical examination: temperature 37.5 ℃, pulse 90 bpm, respiration 20 bpm, blood pressure 172/96 mmHg. The heart and lungs were normal. The abdomen was flat and soft, with tenderness below the xiphoid process. No enlarged liver, spleen, or mass was palpable. Murphy's sign was negative. Laboratory and radiology findings: Routine blood work showed white blood cell 12.7 × 10^9/L, neutrophils 85.3%. Liver function tests were in normal range. Fasting blood glucose was 8.88 mmol/L. Adrenocorticotropic hormone was in normal range. On B-ultrasonography, the gallbladder was 64 mm × 37 mm. The gallbladder wall was thickened, with a stone incarcerated



Figure 1 Enhanced image of nodule located in the lesser gastric curvature.

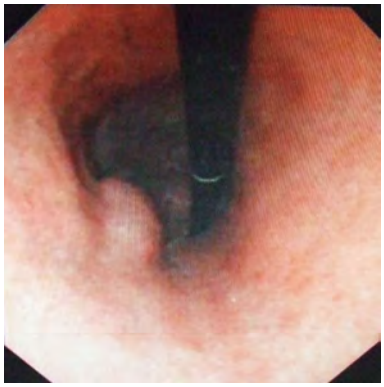


Figure 2 Elevated nodule on the lesser curvature side of the gastric antrum.

in the neck. Computed tomography (CT) scan showed a 15 mm × 25 mm abnormal enhanced nodule located in the submucosa of the lesser curvature of the stomach with a CT value of 150 HU. The size, morphology, and location of the kidneys and adrenal glands were normal bilaterally, and there were multiple cysts in both kidneys. The radiological diagnoses were multiple renal cysts and a gastric stromal tumor (Figure 1). Fibergastroscopy showed no hyperemia in the gastric mucosa. A raised nodule about 30 mm × 25 mm in size was found on the lesser curvature side of the gastric antrum; it was firm with soft mucosa. The endoscopic diagnosis was also a gastric stromal tumor (Figure 2). After adequate preparation, the patient underwent surgery. During the operation, we found a distended gallbladder with a stone inside. There was a 30 mm × 30 mm mass with medium density in the lesser curvature near the gastric antrum and the mass located in the serosa layer. The liver, spleen, pancreas, kidney, and adrenal glands were normal. We resected the gallbladder, ligated the vessels of lesser gastric curvature, and opened the gastric wall 3 cm from the margin of the mass. We found that the gastric mucosa above the mass was integrated, and we performed a simple resection of the mass. On gross examination, the mass was purplish-red in color. It was a 20 mm × 30 mm ellipse with soft margins and a medium texture (Figure 3A). On microscopic exam, the tumor cells were rich in cytoplasm and eosinophilic, and numerous sinusoid capillaries could be seen. The cytoplasm contained melanin, which was confirmed by decolorization. The tumor cells were arranged in cords or gobbets with a low ratio of nucleus to cytoplasm, and rare mitotic figures (Figure 3B, C). Immunohistochemistry: S-100 basement cell negative (Figure 4A), melan-A positive (Figure 4B), P63 basement cell negative, sinusoidal endothelial CD34 positive (Figure 4C). The pathologic diagnosis was of an ectopic adrenal cortical adenoma in the gastric wall.

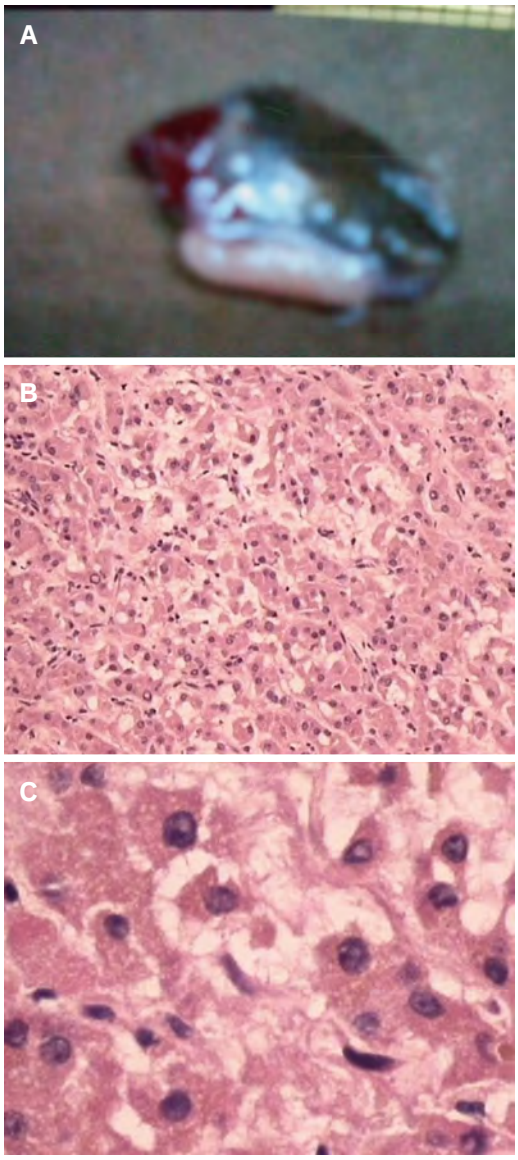


Figure 3 Analysis of tumor cells. A: Gastric wall mass, approximately 20 mm × 30 mm in size; B: Tumor cells under low power magnification; C: Tumor cells under high power magnification.

DISCUSSION

Ectopic adrenal tissue is mostly found in children. According to Anderson *et al*[2], accessory adrenal tissue is found in 50% of post-mortem examinations in neonates

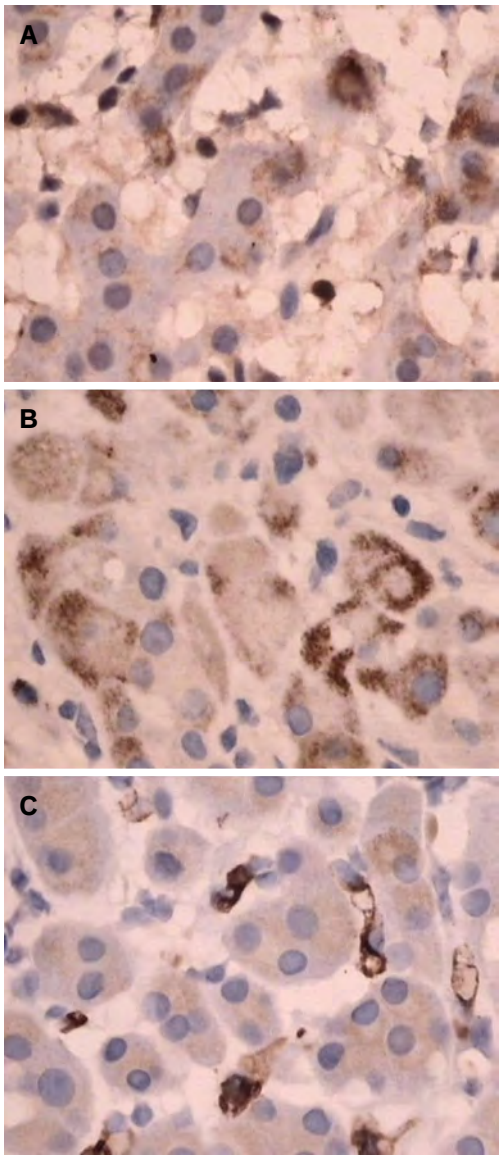


Figure 4 Immunohistochemistry. A: S-100 basement cell negative; B: Melan-A positive; C: CD34 positive.

and children. Usually, maturity leads to atrophy of the ectopic adrenal tissue, so that such tissue is found in only 1% of adults. The adrenal cells have a double embryological origin. The cortex arises from the coelomic mesothelium and the medulla from neural crest ectoderm. At approximately 7 ± 8 wk of pregnancy, the medulla components start moving towards the cortical elements, forming the adrenal gland. During migration of the medulla, fragments of tissue, most frequently the cortex, can be separated, forming accessory adrenal glands. Most ectopic adrenals remain in the vicinity of the adrenal gland, but they are also found to be closely related to the sex organs because of the spatial relationship between the adrenal primordium and the genital ridge in early embryogenesis. Accessory adrenal tissue can also be incorporated into adjacent organs due to incomplete separation of cortical adrenal cells from the coelomic mesothelium. Therefore, most ectopic adrenocortical tissue is found along the path of embryonic migration within the urogenital tract. The most common sites include the fat tissue of the posterior peritoneum near the adrenal glands, the celiac axis, the broad ligament, the adnexa of the testis, and the spermatic cord. There are also rare reported cases of ectopic adrenal cortical adenoma in the lung, spinal region, and brain[3-8].

However, there is no previous report of ectopic adrenal cortical adenoma of the gastric wall in the literature. In the case reported here, the patient presented with abdominal discomfort and the mass was found by CT. It was initially diagnosed as a gastric stromal tumor, which was corrected to ectopic adrenal cortical adenoma by pathology; the tumor was nonfunctional. The patient had no history of operation on adrenal tissue, so we believe the ectopic adrenal cortical adenoma in the gastric wall was due to the malposition or self-differentiation of mesothelial cells during the embryonic period.

REFERENCES

1 **Harrison LA**, McMillan JH, Batnitzky S, Kepes JJ. MR appearance of an ectopic intraspinal adrenal cortical adenoma. *AJNR Am J Neuroradiol* 1990; **11**: 1185-1187 [PMID: 2124054]
2 **Anderson JR**, Ross AH. Ectopic adrenal tissue in adults. *Postgrad Med J* 1980; **56**: 806-808 [PMID: 7267489 DOI: 10.1136/pgmj.56.661.806]
3 **Barwick TD**, Malhotra A, Webb JA, Savage MO, Reznek RH. Embryology of the adrenal glands and its relevance to diagnostic imaging. *Clin Radiol* 2005; **60**: 953-959 [PMID: 16124976 DOI: 10.1016/j.crad.2005.04.006]
4 **Dahl EV**, Bahn RC. Aberrant adrenal contical tissue near the testis in human infants. *Am J Pathol* 1962; **40**: 587-598 [PMID: 13883088]
5 **Graham LS**. Celiac accessory adrenal glands. *Cancer* 1953; **6**: 149-152
6 **Mitchell A**, Scheithauer BW, Sasano H, Hubbard EW, Ebersold MJ. Symptomatic intradural adrenal adenoma of the spinal nerve root: report of two cases. *Neurosurgery* 1993; **32**: 658-661; discussion 661-662 [PMID: 8474656 DOI: 10.1227/00006123-199304000-00024]
7 **Vestfrid MA**. Ectopic adrenal cortex in neonatal liver. *Histopathology* 1980; **4**: 669-672 [PMID: 7439895 DOI: 10.1111/j.1365-2559.1980.tb02963.x]
8 **Cassarino DS**, Santi M, Arruda A, Patrocinio R, Tsokos M, Ghatak N, Quezado M. Spinal adrenal cortical adenoma with oncocytic features: report of the first intramedullary case and review of the literature. *Int J Surg Pathol* 2004; **12**: 259-264 [PMID: 15306940 DOI: 10.1177/106689690401200309]

P- Reviewers Zubarik R, Yen HH **S- Editor** Song XX
L- Editor Rutherford A **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i5.781

World J Gastroenterol 2013 February 7; 19(5): 781-783
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Splenic lymphangioma that manifested as a solid-cystic mass: A case report

Fan Yang, Wei-Xia Chen

Fan Yang, Wei-Xia Chen, Department of Radiology, West China Hospital of Sichuan University, Chengdu 610041, Sichuan Province, China
Author contributions: Yang F designed the study, analyzed the data and wrote the paper; Chen WX revised the manuscript.
Correspondence to: Wei-Xia Chen, Professor, Department of Radiology, West China Hospital of Sichuan University, No. 37, Guoxue Alley, Chengdu 610041, Sichuan Province, China. wxchen25@126.com
Telephone: +86-28-85423139 Fax: +86-28-85422746
Received: November 26, 2012 Revised: December 15, 2012
Accepted: December 22, 2012
Published online: February 7, 2013

Abstract

Lymphangioma, a congenital malformation of the lymphatic system, is usually found in children, and generally occurs in the neck and mediastinum. It is rarely found in the spleen. The clinical features of splenic lymphangioma typically include abdominal pain, nausea, and abdominal distention. Frequently, however, this condition is asymptomatic and is incidentally detected by abdominal ultrasonography or by an abdominal computed tomography (CT) scan. In this paper, we retrospectively describe a case of incidentally detected splenic lymphangioma in a 30-year-old woman with special abdominal contrast material-enhanced CT findings, which was accurately diagnosed by histopathology. The clinical and physical examinations related to the mass were negative. A few cases of splenic lymphangioma have been reported previously; however, the presentation of the mass and the enhancement pattern in the contrast medium-enhanced CT images were quite extraordinary. These findings had misled our abdominal radiologists to consider it as other neoplastic diseases of the spleen.

© 2013 Baishideng. All rights reserved.

Key words: Lymphangioma; Benign tumor; Spleen; Splenic neoplasia; Computed tomography

Yang F, Chen WX. Splenic lymphangioma that manifested as a solid-cystic mass: A case report. *World J Gastroenterol* 2013; 19(5): 781-783 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i5/781.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i5.781

INTRODUCTION

Splenic lymphangiomas are usually benign tumors that predominantly affect children, whereas only a few cases have been reported in adults[1-3]. Previous case reports revealed no particular findings in the image analysis, which uniformly indicated solitary cysts, or multiple cysts without contrast enhancement or only with slight enhancement of the thin septa[1,3-5], except for one case of a solid mass with diffuse and prolonged enhancement[2]. Here we present a histopathologically proven case in which a solid-cystic mass of the spleen in the computed tomography (CT) images showed no enhancement of the cysts and prominent enhancement of the solid components. To the best of our knowledge, these special contrast-enhanced CT findings are relatively rare.

CASE REPORT

A 30-year year-old woman was admitted to our hospital with a splenic mass detected by abdominal ultrasonography, which was performed for recurrent epigastric pain at another hospital. The epigastric pain was later attributed to gall bladder lithiasis. The patient's past and familial medical histories indicated no abnormalities. There were no positive symptoms related to the splenic mass. Physical examinations detected no mass or splenomegaly in the left upper quadrant of the abdomen. Peripheral blood count, coagulation studies, and liver and kidney function tests were all within normal limits.

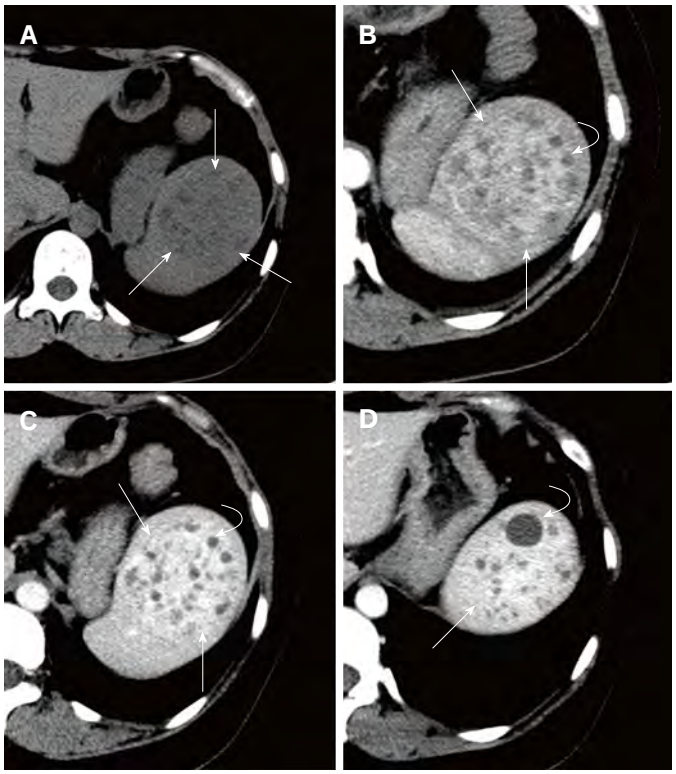


Figure 1 Computed tomography findings of the splenic lymphangioma. A: A heterogeneous solid-cystic mass (straight arrow) with a well-defined margin in the spleen, as shown in the plain image. The solid components show slight hypo-density; B: Arterial phase image shows the mass (straight arrow) with multiple non-enhanced cysts (curved arrow). The solid portions manifest as obvious enhancement; C: Parenchyma phase image shows obviously progressive enhancement of the solid portions; D: Parenchyma phase image of the upper location of the mass showing the same presentation and enhancement pattern.

CT showed a heterogeneous solid-cystic mass with a well-defined margin located in the normal sized spleen in the plain images. Within the mass, there were multiple small cysts of variable size. The cysts showed sharp borders with no enhancement at either the arterial or parenchymal phase, whereas the solid components presented with persistently progressive enhancement (Figure 1). At this point, our radiologists suspected a splenic tumor.

Splenectomy was eventually undertaken for the splenic mass. During the operation, a solid mass was found, which had no clear boundary with the peripheral splenic parenchyma. The final histopathological result was splenic cavernous lymphangioma. The findings revealed multiple lymphatic vesicles of variable size, and the blood sinuses in the red pulp within the mass that were dilated compared with the peripheral red pulp. The vesicles were lined by a single layer of flat endothelial cells, and some vesicles were filled with pink eosinophilic lymphatic fluid (Figure 2).

DISCUSSION

Lymphangiomas are generally considered to be congenital malformations of the lymphatic system, and they occur mostly in the neck, mediastinum and retroperitoneum[4]; they are seldom found in the spleen. Histologically, lymphangiomas are classified into three subtypes according to the congenital dilated lymphatic channels: capillary (super-microcystic), cavernous (microcystic), or cystic (macrocystic)[6]. Our case was ultimately diagnosed as the cavernous type. Cavernous lymphangiomas are composed of numerous irregular cavernous spaces containing amorphous eosinophilic fluid and few lymphocytes, lined by a single layer of flat endothelial cells[7]. Splenic lymphangiomas are mostly asymptomatic; therefore, the final diagnosis should be based on a combination of clinical, radiological, and histopathological findings.

In most cases, splenic lymphangiomas present with thin-walled cystic masses without enhancement or with only slight enhancement of the thin septa in the CT studies. We detailed a histologically confirmed case in which the mass manifested as a solid-cystic lesion. The multiple cysts showed no enhancement, as in previous cases[1,3-5]; however, the solid components showed obvious progressive enhancement. Histopathologically, however, there were no real solid components, and the solid, prominently enhanced portions in the CT images simply represented the dilated blood sinuses in the red pulp within the mass. The presence of reduced blood velocity and collecting blood in the dilated blood sinuses may have resulted in more contrast medium per voxel compared with the peripheral normal sinuses, and may have eventually led to obviously progressive enhancement.

Thus, lymphangioma presenting with this kind of solid-cystic mass in the image studies may be misdiagnosed. The differential diagnoses of splenic lymphangiomas often include other solid and cystic lesions of the spleen, such as hemangioma, chronic infection, lymphoma and metastasis. Hemangiomas may sometimes display solid and cystic portions[8]. Contrast-enhanced CT scans may show delayed enhancement within the solid portion. Punctate calcification can be seen in the central solid portion, and curvilinear calcification may be seen in the cystic areas[8]. Parasitic infection with *Echinococcus granulosus* is the main cause of cystic proliferations of the spleen[9]. The differential factors are the patients' history, calcifica-

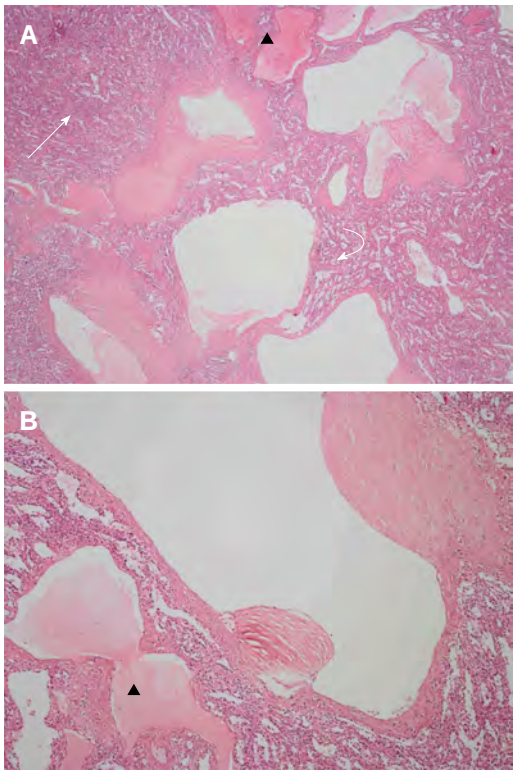


Figure 2 Histopathological findings of the splenic lymphangioma. A: Hematoxylin and eosin (HE) staining of the splenic lymphangioma, × 40, indicates the presence of numerous cystic lesions of variable size, and blood sinuses (curved arrow) in the red pulp within the mass that are dilated as compared with peripheral red pulp (straight arrow); B: HE staining, × 100, indicates that some vesicles are filled with pink eosinophilic lymph sacs (black triangle).

tion in the cystic walls, a cyst with daughter cysts or concomitant cystic lesions in the liver or other organs[10].

In conclusion, dynamic contrast-enhanced CT is a useful method for evaluating splenic lesions. We suggest that lymphangioma should be considered in the differential diagnosis of a splenic mass that manifests as a solid-cystic lesion in the image studies. In our case, the dilated blood sinuses in the red pulp within the mass might have contributed to the indication of solid components with evident enhancement.

ACKNOWLEDGMENTS

We thank Wen-Yan Zhang (Department of Pathology, West China Hospital of Sichuan University) for providing the histopathological resources.

REFERENCES

1 **Vezzoli M**, Ottini E, Montagna M, La Fianza A, Paulli M, Rosso R, Mazzone A. Lymphangioma of the spleen in an elderly patient. *Haematologica* 2000; **85**: 314-317 [PMID: 10702822]
2 **Chang WC**, Liou CH, Kao HW, Hsu CC, Chen CY, Yu CY. Solitary lymphangioma of the spleen: dynamic MR findings with pathological correlation. *Br J Radiol* 2007; **80**: e4-e6 [PMID: 17267469 DOI: 10.1259/bjr/93530580]
3 **Beltrán MA**, Barría C, Pujado B, Oliva J, Contreras MA, Wilson CS, Cruces KS. [Gigantic cystic splenic lymphangioma. Report of one case]. *Rev Med Chil* 2009; **137**: 1597-1601 [PMID: 20361136 DOI: 10.4067/S0034-98872009001200008]
4 **Abbott RM**, Levy AD, Aguilera NS, Gorospe L, Thompson WM. From the archives of the AFIP: primary vascular neoplasms of the spleen: radiologic-pathologic correlation. *Radiographics* 2004; **24**: 1137-1163 [PMID: 15256634 DOI: 10.1148/rg.244045006]
5 **Solomou EG**, Patriarheas GV, Mpadra FA, Karamouzis MV, Dimopoulos I. Asymptomatic adult cystic lymphangioma of the spleen: case report and review of the literature. *Magn Reson Imaging* 2003; **21**: 81-84 [PMID: 12620551 DOI: 10.1016/S0730-725X(02)00624-0]
6 **Lee WS**, Kim YH, Chee HK, Lee SA, Kim JD, Kim DC. Cavernous lymphangioma arising in the chest wall 19 years after excision of a cystic hygroma. *Korean J Thorac Cardiovasc Surg* 2011; **44**: 380-382 [PMID: 22263194 DOI: 10.5090/kjtcs.2011.44.5.380]
7 **Ogun GO**, Oyetunde O, Akang EE. Cavernous lymphangioma of the breast. *World J Surg Oncol* 2007; **5**: 69 [PMID: 17584918 DOI: 10.1186/1477-7819-5-69]
8 **Urrutia M**, Mergo PJ, Ros LH, Torres GM, Ros PR. Cystic masses of the spleen: radiologic-pathologic correlation. *Radiographics* 1996; **16**: 107-129 [PMID: 10946694]
9 **Adas G**, Karatepe O, Altiok M, Battal M, Bender O, Ozcan D, Karahan S. Diagnostic problems with parasitic and non-parasitic splenic cysts. *BMC Surg* 2009; **9**: 9 [PMID: 19476658 DOI: 10.1186/1471-2482-9-9]
10 **Polat P**, Kantarci M, Alper F, Suma S, Koruyucu MB, Okur A. Hydatid disease from head to toe. *Radiographics* 2003; **23**: 475-494; quiz 536-537 [PMID: 12640161 DOI: 10.1148/rg.232025704]

P- Reviewers Triantopoulou C, Albiin N **S- Editor** Gou SX
L- Editor Stewart GJ **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i5.784

World J Gastroenterol 2013 February 7; 19(5): 784-785
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

Severe gastrointestinal mucositis following high dose melphalan therapy for multiple myeloma

Sanjeev Kumar Sharma, Anil Handoo, Dharma Choudhary, Gaurav Dhamija, Nitin Gupta

Sanjeev Kumar Sharma, Anil Handoo, Dharma Choudhary, Gaurav Dhamija, Nitin Gupta, Department of Hemato-oncology and Bone Marrow Transplant, BLK Superspeciality Hospital, New Delhi 110005, India
Author contributions: Sharma SK, Handoo A, Choudhary D, Dhamija G, Gupta N designed and wrote the paper.
Correspondence to: Sanjeev Kumar Sharma, MD, DM, Department of Hemato-oncology and Bone Marrow Transplant, BLK Superspeciality Hospital, Pusa Road, New Delhi 110005, India. sksanjeev13@yahoo.com
Telephone: +91-11-30653790 Fax: +91-11-30653016
Received: October 15, 2012 Revised: November 10, 2012
Accepted: December 15, 2012
Published online: February 7, 2013

Abstract

Mucositis is a known complication following use of chemotherapy, but fatal mucositis is unusual and management of such cases may be challenging. Pathologically there is denudation of mucosa of gastrointestinal tract. Severe cases can develop ileus and even perforation of bowel wall. We report here a case of multiple myeloma who developed World Health Organization grade 4 gut mucositis following the use of high dose melphalan with the expulsion of "intestine-like" material.

© 2013 Baishideng. All rights reserved.

Key words: Gastrointestinal mucositis; Chemotherapy

Sharma SK, Handoo A, Choudhary D, Dhamija G, Gupta N. Severe gastrointestinal mucositis following high dose melphalan therapy for multiple myeloma. *World J Gastroenterol* 2013; 19(5): 784-785 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i5/784.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i5.784

TO THE EDITOR

Mucositis is the most common complication of chemotherapeutics agents. Melphalan when used in high doses for treatment for multiple myeloma can lead to severe mucositis[1,2]. Patient can develop gastrointestinal ulcers, vomiting and diarrhea with the use of high dose melphalan as a conditioning regimen for autologous transplant for multiple myeloma[3-5]. Pathologically there is denudation of mucosa of gastrointestinal tract. Severe cases can develop ileus and even perforation of bowel wall. We report here a case of multiple myeloma who developed World Health Organization (WHO) grade 4 gut mucositis following the use of high dose melphalan with the expulsion of "intestine-like" material.

A 62-years-old male was admitted for autologous stem cell transplant for multiple myeloma. He was in complete remission following 4 cycles of bortezomib and dexamethasone. Filgrastim (G-CSF) 10 mg/kg was given for 4 d for peripheral stem cell mobilization followed by melphalan 200 mg/m^2 as conditioning regimen. The CD34+ stem cell dose infused was 6×10^6/kg. Cephasol (supersaturated calcium phosphate rinse) was used six times daily to prevent oral mucositis. On day 3 patient developed vomitings and by day 5 he had WHO grade 4 gut mucositis with ileus. Patient also developed fever and was started on empirical antibiotics including meropenem (1 g three times daily, intravenously), metronidazole (500 mg three times daily, intravenously), and teicoplanin (400 mg once daily, intravenously) as per hospital policy, alongwith Ryle's tube aspiration and parenteral feeding. On day 7 he passed 25 cm × 1 cm pinkish, mucosa coated partly digested material mimicking segment of small intestine (Figure 1). Histopathological section of the segment revealed numerous neutrophils surrounding the partly digested material, without any muscle tissue (Figure 2). Blood culture was negative for bacteria and fungus. Viral serology tests for cytomegalovirus immunoglobulin M (IgM) and herpes simplex virus 1 and 2 IgM were negative. Stool examination did not reveal ova or cysts and was negative for Clostridial difficile toxin. Computed tomography of abdomen showed dilated bowel loops, with caecal wall thickness of 10 mm, there was no evidence of perforation or intussusception. Though he had neutrophil engraftment (absolute neutrophil count



Figure 1 Specimen showing the excreted, blood tinged, elongated material, measuring about 25 cm, resembling part of small intestine.

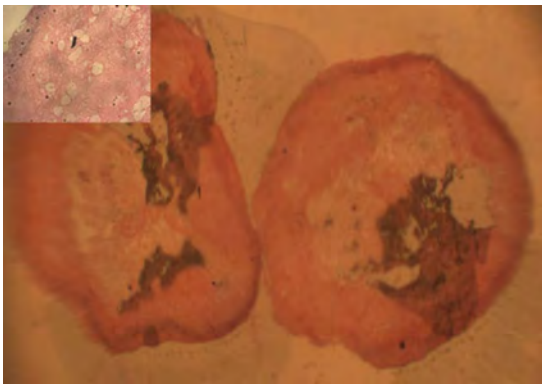


Figure 2 Cross section of the excreted material showing numerous neutrophils surrounding the partly digested material (inset, top left).

more than 0.5×10^9/L) on day 13, platelets remained below 10×10^9/L. Patient succumbed on day 20 because of enterocolitis and sepsis.

Oral and gastrointestinal mucositis due to cancer chemotherapy or radiotherapy continues to be an important clinical problem. Alimentary tract mucositis can involve any part of oral and gastrointestinal mucosa, from the mouth to the anus. The incidence of WHO grade 3 or 4 gastrointestinal mucositis can be as high as 20%-60% in patients undergoing hematopoietic stem cell transplantation, depending on the intensity of the conditioning regimen used[3,4]. In the study by Krishna *et al*[2], of the 47 patients who developed lower alimentary tract mucositis there was no mortality. Our patient developed severe gastrointestinal toxicity following high dose of melphalan, with excretion of a large mucosa coated segment mimicking part of the small intestine, and succumbed to the complications related to mucositis.

REFERENCES

1 **Peterson DE**, Bensadoun RJ, Roila F. Management of oral and gastrointestinal mucositis: ESMO Clinical Practice Guidelines. *Ann Oncol* 2011; **22** Suppl 6: vi78-vi84 [PMID: 21908510 DOI: 10.1093/annonc/mdr391]

2 **Krishna SG**, Zhao W, Grazziutti ML, Sanathkumar N, Barlogie B, Anaissie EJ. Incidence and risk factors for lower alimentary tract mucositis after 1529 courses of chemotherapy in a homogenous population of oncology patients: clinical and research implications. *Cancer* 2011; **117**: 648-655 [PMID: 20862749 DOI: 10.1002/cncr.25633]

3 **Vera-Llonch M**, Oster G, Ford CM, Lu J, Sonis S. Oral mucositis and outcomes of autologous hematopoietic stem-cell transplantation following high-dose melphalan conditioning for multiple myeloma. *J Support Oncol* 2007; **5**: 231-235 [PMID: 17564153]

4 **Tuncer HH**, Rana N, Milani C, Darko A, Al-Homsi SA. Gastrointestinal and hepatic complications of hematopoietic stem cell transplantation. *World J Gastroenterol* 2012; **18**: 1851-1860 [PMID: 22563164]

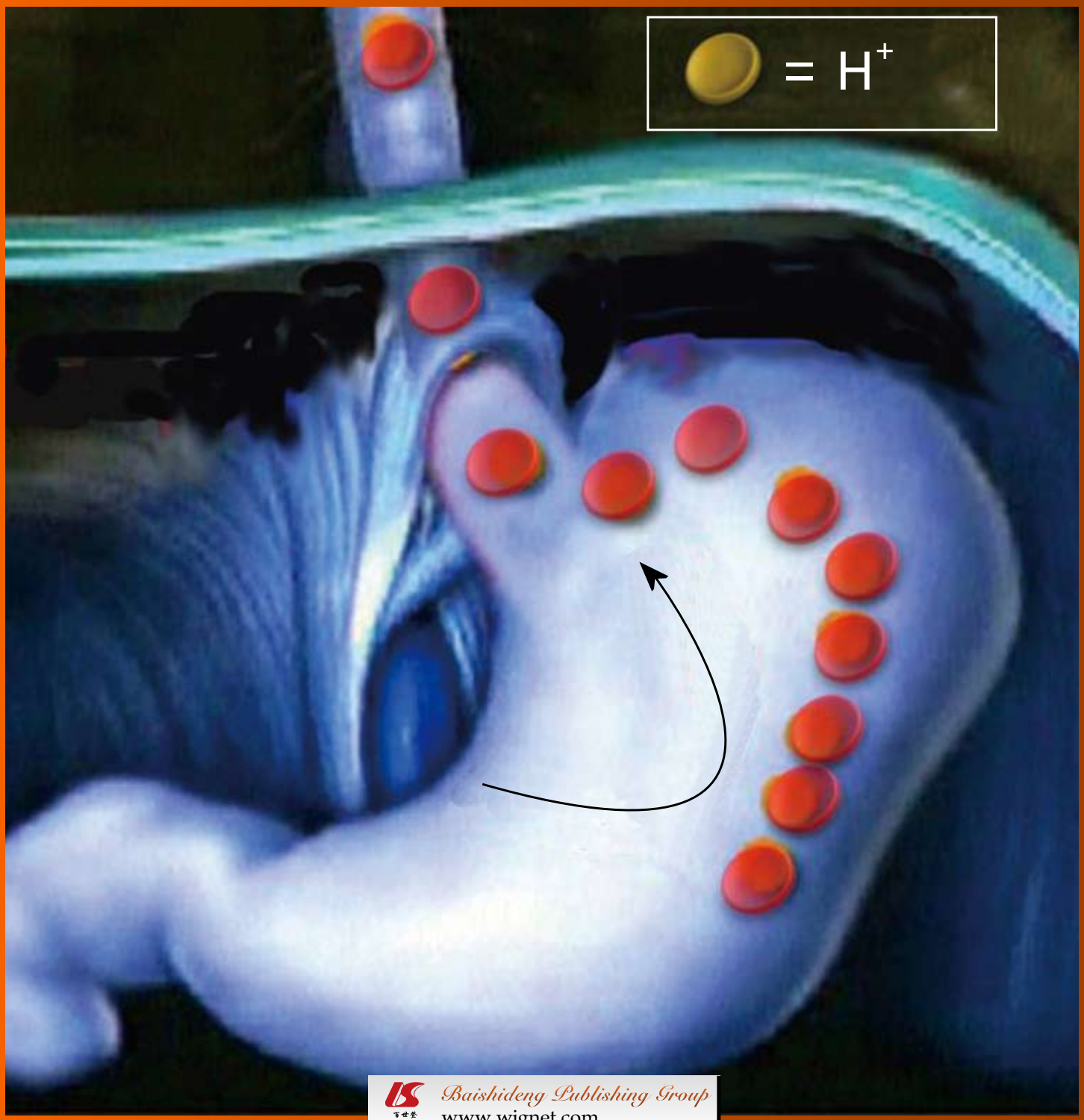
5 **Grazziutti ML**, Dong L, Miceli MH, Krishna SG, Kiwan E, Syed N, Fassas A, van Rhee F, Klaus H, Barlogie B, Anaissie EJ. Oral mucositis in myeloma patients undergoing melphalan-based autologous stem cell transplantation: incidence, risk factors and a severity predictive model. *Bone Marrow Transplant* 2006; **38**: 501-506 [PMID: 16980998 DOI: 10.1038/sj.bmt.1705471]

P- Reviewer Grant G **S- Editor** Gou SX **L- Editor** A **E- Editor** Zhang DN

ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

World J Gastroenterol 2013 February 14; 19(6): 786-967



Baishideng Publishing Group
www.wjgnet.com

World Journal of Gastroenterology

Contents

Weekly Volume 19 Number 6 February 14, 2013

EDITORIAL

786 *Helicobacter pylori* eradication and reflux disease onset: Did gastric acid get "crazy"?
Zullo A, Hassan C, Repici A, Bruzzese V

FRONTIER

790 Current hurdles in the management of eosinophilic oesophagitis: The next steps
Attwood SEA, Wilson MSJ

FIELD OF VISION

797 Hepatocellular carcinoma after ablation: The imaging follow-up scheme
Liu LN, Xu HX, Zhang YF, Xu JM

REVIEW

802 What is the role of adiponectin in obesity related non-alcoholic fatty liver disease?
Finelli C, Tarantino G

813 Current evidence for histone deacetylase inhibitors in pancreatic cancer
Koutsounas I, Giaginis C, Patsouris E, Theocharis S

829 Diagnosis and management of insulinoma
Okabayashi T, Shima Y, Sumiyoshi T, Kozuki A, Ito S, Ogawa Y, Kobayashi M, Hanazaki K

MINIREVIEWS

838 Hepatitis B vaccine in celiac disease: Yesterday, today and tomorrow
Vitaliti G, Praticò AD, Cimino C, Di Dio G, Lionetti E, La Rosa M, Leonardi S

ORIGINAL ARTICLE

846 Accommodation and peristalsis are functional responses to obstruction in rat hypertrophic ileum
Bertoni S, Saccani F, Gatti R, Rapalli A, Flammini L, Ballabeni V, Barocelli E

855 Role of contrast-enhanced ultrasound in follow-up assessment after ablation for hepatocellular carcinoma
Zheng SG, Xu HX, Lu MD, Xie XY, Xu ZF, Liu GJ, Liu LN

866 Interference of suppressor of cytokine signaling 3 promotes epithelial-mesenchymal transition in MHCC97H cells
Ji YY, Wang ZD, Li ZF, Li K

BRIEF ARTICLE

874 Intraductal ultrasound substantiates diagnostics of bile duct strictures of uncertain etiology
Meister T, Heinzow HS, Woestmeyer C, Lenz P, Menzel J, Kucharzik T, Domschke W, Domagk D

882 Chronic intestinal ischemia and splanchnic blood-flow: Reference values and correlation with body-composition
Zacho HD, Henriksen JH, Abrahamsen J

889 Mucosal healing effect of mesalazine granules in naproxen-induced small bowel enteropathy
Rácz I, Szalai M, Kovács V, Regőczi H, Kiss Gy, Horváth Z

897 Effect of *Gongronema latifolium* on gastric emptying in healthy dogs
Ogbu SO, Agwu KK, Asuzu IU

903 Ampulla dilation with different sized balloons to remove common bile duct stones
Li NP, Liu JQ, Zhou ZQ, Ji TY, Cai XY, Zhu QY

909 Novel *CDH1* germline mutations identified in Chinese gastric cancer patients
Chen QH, Deng W, Li XW, Liu XF, Wang JM, Wang LF, Xiao N, He Q, Wang YP, Fan YM

917 Effects of early enteral nutrition on immune function of severe acute pancreatitis patients
Sun JK, Mu XW, Li WQ, Tong ZH, Li J, Zheng SY

923 Testing for hepatitis B infection in prospective chemotherapy patients: A retrospective study
Wang Y, Luo XM, Yang D, Zhang J, Zhuo HY, Zhang J, Jiang Y

931 Quadruple therapy for eradication of *Helicobacter pylori*
Ma HJ, Wang JL

META-ANALYSIS

936 Pre-existing diabetes mellitus increases the risk of gastric cancer: A meta-analysis
Yoon JM, Son KY, Eom CS, Durrance D, Park SM

CASE REPORT

946 Dubin-Johnson syndrome coinciding with colon cancer and atherosclerosis
Sticova E, Elleder M, Hulkova H, Luksan O, Sauer M, Wunschova-Moudra I, Novotny J, Jirsa M

951 Successful balloon-occluded retrograde transvenous obliteration for bleeding duodenal varices using cyanoacrylate
Hashimoto R, Sofue K, Takeuchi Y, Shibamoto K, Arai Y

955 Rescue endoscopic band ligation of iatrogenic gastric perforations following failed endoclip closure

Han JH, Lee TH, Jung Y, Lee SH, Kim H, Han HS, Chae H, Park SM, Youn S

960 Misdiagnosis of left supraclavicular lymph node metastasis of hepatocellular carcinoma: A case report

Liu T, Gao JF, Yi YX, Ding H, Liu W

964 Severe hepatic necrosis of unknown causes following ABO-incompatible liver transplantation

Lu H, Zhang CY, Ding W, Lu YJ, Li GQ, Zhang F, Lu L

World Journal of Gastroenterology
Volume 19 Number 6 February 14, 2013

Contents

APPENDIX	I-VI	Instructions to authors
ABOUT COVER		Zullo A, Hassan C, Repici A, Bruzzese V. *Helicobacter pylori* eradication and reflux disease onset: Did gastric acid get "crazy"? *World Journal of Gastroenterology* 2013; 19(6): 786-789 http://www.wjgnet.com/1007-9327/full/v19/i6/786.htm

AIMS AND SCOPE

World Journal of Gastroenterology (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. *WJG* was established on October 1, 1995. It is published weekly on the 7th, 14th, 21st, and 28th each month. The *WJG* Editorial Board consists of 1352 experts in gastroenterology and hepatology from 64 countries.

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

INDEXING/ABSTRACTING

World Journal of Gastroenterology is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, Digital Object Identifier, and Directory of Open Access Journals. ISI, Journal Citation Reports®, Gastroenterology and Hepatology, 2011 Impact Factor: 2.471 (32/74); Total Cites: 16951 (7/74); Current Articles: 677 (1/74); and Eigenfactor® Score: 0.06035 (5/74).

FLYLEAF	I-IX	Editorial Board

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Shuai Ma*
Responsible Science Editor: *Xin-Zhen Huang*
Responsible Electronic Editor: *Jun-Yao Li*
Proofing Editorial Office Director: *Xiu-Xia Song*
Proofing Editor-in-Chief: *Lian-Sheng Ma*

NAME OF JOURNAL
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

EDITOR-IN-CHIEF
Ferruccio Bonino, MD, PhD, Professor of Gastroenterology, Director of Liver and Digestive Disease Division, Department of Internal Medicine, University of Pisa, Director of General Medicine 2 Unit University Hospital of Pisa, Via Roma 67, 56124 Pisa, Italy

Myung-Hwan Kim, MD, PhD, Professor, Head, Department of Gastroenterology, Director, Center for Biliary Diseases, University of Ulsan College of Medicine, Asan Medical Center, 388-1 Pungnap-2dong, Songpa-gu, Seoul 138-736, South Korea

Kjell Öberg, MD, PhD, Professor, Department of Endocrine Oncology, Uppsala University Hospital, SE-751 85 Uppsala, Sweden

Matt D Rutter, MBBS, MD, FRCP, Consultant Gastroenterologist, Senior Lecturer, Director, Tees Bowel Cancer Screening Centre, University Hospital of North Tees, Durham University, Stockton-on-Tees, Cleveland TS19 8PE, United Kingdom

Andrzej S Tarnawski, MD, PhD, DSc (Med), Professor of Medicine, Chief Gastroenterology, VA Long Beach Health Care System, University of California, Irvine, CA, 5901 E. Seventh Str., Long Beach, CA 90822, United States

EDITORIAL OFFICE
Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
World Journal of Gastroenterology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Co., Limited
Room 1701, 17/F, Henan Building,
No.90 Jaffe Road, Wanchai, Hong Kong, China
Fax: +852-31158812
Telephone: +852-58042046
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

PUBLICATION DATE
February 14, 2013

COPYRIGHT
© 2013 Baishideng. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT
All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i6.786

World J Gastroenterol 2013 February 14; 19(6): 786-789
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

EDITORIAL

Helicobacter pylori eradication and reflux disease onset: Did gastric acid get "crazy"?

Angelo Zullo, Cesare Hassan, Alessandro Repici, Vincenzo Bruzzese

Angelo Zullo, Cesare Hassan, Vincenzo Bruzzese, Gastroenterology and Digestive Endoscopy Unit, Nuovo Regina Margherita Hospital, 00153 Rome, Italy
Alessandro Repici, Digestive Endoscopy Unit, IRCCS Istituto Clinico Humanitas, 00100 Milan, Italy
Author contributions: Zullo A and Hassan C designed and edited the review; Repici A reviewed literature; Bruzzese V contributed with constructive criticisms.
Correspondence to: Dr. Angelo Zullo, MD, Gastroenterology and Digestive Endoscopy Unit, Nuovo Regina Margherita Hospital, Via E. Morosini 30, 00153 Roma, Italy. zullo66@yahoo.it
Telephone: +39-6-58446533 Fax: +39-6-58446608
Received: May 4, 2012 Revised: July 6, 2012
Accepted: July 18, 2012
Published online: February 14, 2013

Abstract

Gastroesophageal reflux disease (GORD) is highly prevalent in the general population. In the last decade, a potential relationship between *Helicobacter pylori* (*H. pylori*) eradication and GORD onset has been claimed. The main putative mechanism is the gastric acid hypersecretion that develops after bacterial cure in those patients with corpus-predominant gastritis. We performed a critical reappraisal of the intricate pathogenesis and clinical data available in this field. Oesophagitis onset after *H. pylori* eradication in duodenal ulcer patients has been ascribed to a gastric acid hypersecretion, which could develop following body gastritis healing. However, the absence of an acid hypersecretive status in these patients is documented by both pathophysiology and clinical studies. Indeed, duodenal ulcer recurrence is virtually abolished following *H. pylori* eradication. In addition, intra-oesophageal pH recording studies failed to demonstrated increased acid reflux following bacterial eradication. Moreover, oesophageal manometric studies suggest that *H. pylori* eradication would reduce - rather than favor - acid reflux into the oesophagus. Finally, data of clinical studies would suggest that *H. pylori* eradication is not significantly associated with either reflux symptoms or erosive oesophagitis onset, some data suggesting also an advantage in curing the infection when oesophagitis is already present. Therefore, the legend of "crazy acid" remains - as all the others - a fascinating, but imaginary tale.

© 2013 Baishideng. All rights reserved.

Key words: *Helicobacter pylori*; Oesophageal reflux; Oesophagitis; Eradication; Pathophysiology; Clinical studies

Zullo A, Hassan C, Repici A, Bruzzese V. *Helicobacter pylori* eradication and reflux disease onset: Did gastric acid get "crazy"? *World J Gastroenterol* 2013; 19(6): 786-789 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i6/786.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i6.786

INTRODUCTION

Although *Helicobacter pylori* (*H. pylori*) infection prevalence is declining in developed countries, such infection remains a worldwide spread disease with a definite morbidity and mortality. Indeed, *H. pylori* may cause non-ulcer dyspepsia, peptic ulcer disease, and gastric tumors, including both low-grade mucosa-associated lymphoid tissue lymphoma and adenocarcinoma[1-3]. In addition, an interaction between *H. pylori* with non-steroidal, anti-inflammatory drugs in damaging the gastroduodenal mucosa has been also recognized[4]. Similarly, the role of *H. pylori* in the pathogenesis of different extra-digestive diseases has also been claimed. However, an association has been consistently proven only between *H. pylori* infection and both idiopathic thrombocytopenic purpura and idiopathic iron deficiency anemia[5,6], whilst conflicting data exist for other diseases[7-9].

Gastroesophageal reflux disease (GORD) is a highly prevalent condition in the general population[10]. It is characterized by the reflux of gastric contents into the

oesophagus, leading to mucosal damage and/or typical and atypical symptoms[11]. Several factors may predispose patients to pathologic reflux, including hiatal hernia, lower esophageal sphincter (LES) hypotension, transient lower oesophageal sphincter relaxation, loss of esophageal peristaltic function, abdominal obesity, gastric hypersecretory states, and delayed gastric emptying[12].

In the last decade, a potential relationship between *H. pylori* infection and GORD has been claimed. In detail, it has been suggested that *H. pylori* eradication may cause both reflux symptoms and erosive oesophagitis. We performed a critical reappraisal of the intricate data available in such a topic.

DOES *H. PYLORI* ERADICATION CAUSE GORD?

The beginning

The first study suggesting a possible association between *H. pylori* and GORD was published by Labenz *et al*[13] on 1997. In this study, 25.8% of patients with duodenal ulcer (DU) cured for *H. pylori* infection developed erosive oesophagitis (Savary-Miller classification) at 3-year follow-up as compared to 12.9% of DU patients with ongoing infection ($P < 0.001$). Identified risk factors were male sex, severity of corpus gastritis, and weight gain. These results deserve some considerations. As shown in Figure 1, the incidence of erosive oesophagitis in *H. pylori* eradicated patients constantly increased at follow-up, so that at 3-year follow-up the curve did not still reach the plateau. This would probably suggest that an even higher oesophagitis incidence is expected at longer follow-up in these patients. However, this contrasts with data of a recent 7-year follow-up study which showed that reflux oesophagitis occurred in only 1.9% of DU patients following *H. pylori* eradication[14]. In addition, it should be noted that the curve of oesophagitis incidence in not eradicated DU patients showed a very similar slope as compared to that of eradicated patients (Figure 1). In detail, following a questionable delay of 1 year, reflux oesophagitis started to increase also in *H. pylori* infected patients, and the increase of incidence parallels with that of eradicated patients. A plausible explanation of such phenomenon could be that several not eradicated DU patients were taking anti-acid therapy, at least for the first year follow-up in this study. Indeed, this patient group included either control cases of *H. pylori* eradication trials (75 cases) or *H. pylori* eradication failure patients (141 cases) in whom DU symptoms most likely persisted[13]. Therefore, the observation of a distorted phenomenon could not be ruled out. On the other hand, it was observed that oesophagitis occurred more frequently in those *H. pylori* cured patients with weight gain > 2 kg [odds ratio (OR): 3.2; 95%CI: 1.2-9.4; $P < 0.05$] within 3-year follow-up. Unfortunately, both mean and range of weigh gain was not provided in this study. However, it is plausible that several patients changed their dietary and voluptuary habits following complete DU healing, with consequent body mass index

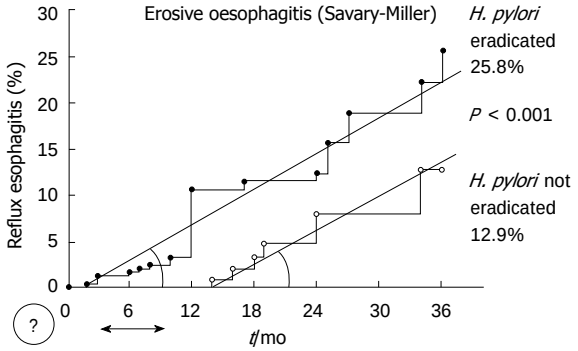


Figure 1 Incidence of erosive oesophagitis in *Helicobacter pylori* eradicated and not eradicated duodenal ulcer patients (Modified from reference 13). The comparable curve slopes would indicate a similar phenomenon. The final difference between patient groups depends on the 1-year delay between curve which remains unexplained. *H. pylori*: *Helicobacter pylori*.

(BMI) increase. The direct relationship between BMI and GORD is widely documented in literature, so that the "guilty" of erosive oesophagitis onset - at least in some patients - probably was the BMI increase rather than the *H. pylori* cure. Unfortunately, the lack of multivariate regression including *H. pylori* eradication as an independent risk factor for GORD does not allow to confirm the significant role observed solely at univariate analysis. Finally, the reason for which the risk of erosive oesophagitis incidence following *H. pylori* eradication was 3.6 (95%CI: 1.1-10.6) higher in males as compared to females remains unclear. This would contrast with the prevalence of GORD in the general population which equally occurs in both sexes[15].

The tale of a "crazy acid"

At least in theory, *H. pylori* eradication could cause GORD by: (1) recovering gastric acid secretion; (2) increasing the amount of reflux in the oesophagus; and (3) reducing the clearance of oesophageal acid exposure.

Regarding the first point, it has been observed that *H. pylori*-associated corpus-predominant gastritis is an independent risk factor (OR: 5.5; 95%CI: 2.8-13.6) for erosive oesophagitis development following *H. pylori* eradication in DU patients[13]. In this study, the body gastritis sum score before *H. pylori* eradication was 4.1 in those who developed oesophagitis as compared to 3.1 of those without oesophagitis onset. Therefore, it has been suggested that the higher degree of recovery leads into a higher acid secretion which, in turn, causes erosive oesophagitis[13]. However, by considering that the sum score (activity plus grade) of gastritis was ranging from 0 (absent) and 8 (severe), it is questionable that acid hypersecretion developed only in those patients in whom corpus gastritis score dropped from 4.1, but not from 3.1, to zero following bacterial cure. At this point, the crucial issue is to understand whether *H. pylori* eradication really causes gastric acid hypersecretion in DU patients. To our knowledge, neither pathophysiology nor clinical studies support such an event. It has been found that infected DU patients have a 3-fold increase of basal acid output (BAO)

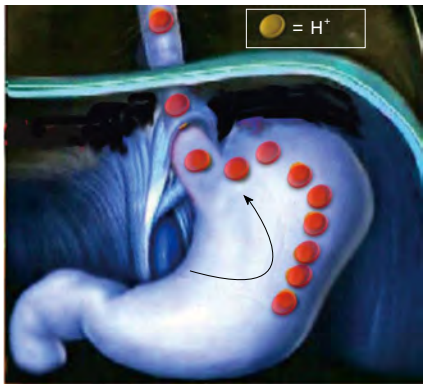


Figure 2 The "tale of a crazy acid". Following *Helicobacter pylori* gastric in duodenal ulcer patients the hypothesized acid hypersecretion would U-turn into the oesophagus instead of in the duodenal bulb.

and a 6-fold increase of stimulated maximal acid output (MAO) as compared to uninfected controls[16]. Of note, both these alterations normalize 1-year following *H. pylori* eradication, suggesting that DU patients cured for the infection completely restore normal acid secretion[16]. The absence of an acid hypersecretive status in these patients is further documented by the clinical observation that DU recurrence is virtually abolished following *H. pylori* eradication[17]. Therefore, it is unclear the reason for which the hypothesized hypersecretion in DU patients after bacterial cure should cause erosive oesophagitis onset, but not DU recurrence. To search for a plausible explication, one could hypothesize the "tale of a crazy acid". According to this theory, following the healing of corpus-predominant gastritis - a typical gastritis pattern of both gastric ulcer and cancer, but infrequent in DU[18] - acid hypersecretion occurs in some DU patients. In these patients the acid should invert its normal route - so failing to cause DU recurrence - and should turn into the oesophagus causing erosions (Figure 2). However, studies on 24-h intraoesophageal pH recording before and after *H. pylori* eradication showed that the percentage time of pH < 4.0 did not significantly change following eradication[19,20]. Other putative mechanisms by which *H. pylori* eradication could favor GORD are alterations of both LES pressure and oesophageal peristalsis. However, two studies found no difference in basal LES pressure between *H. pylori* infected and matched controls[21,22], whilst another study showed an even lower basal LES pressure and higher rate of ineffective oesophageal motility in infected patients[23]. Based on these manometric observations, it could be expected that *H. pylori* eradication would reduce - rather than favor - acid reflux into the oesophagus.

Clinical studies

Several clinical trials assessing the onset of both reflux symptoms and erosive oesophagitis have been performed. A recent meta-analysis evaluated data of 10 trials where data of patients treated for *H. pylori* infection were compared to those receiving placebo[24]. At 8-30 mo follow-up the incidence of reflux symptoms did not significantly differ between the two patient groups (17% *vs* 22.6%), with a trend even favoring bacterial eradication (OR: 0.81; 95%CI: 0.56-1.71). Likewise, erosive oesophagitis equally occurred in both groups (5% *vs* 5.1%; OR: 1.13; 95%CI: 0.72-1.78). Noteworthy, a study on 156 patients with both peptic ulcer and reflux oesophagitis found that the oesophageal lesions improved more frequently in *H. pylori* eradicated patients as compared to those with persistent infection[25]. Overall, these data would suggest that *H. pylori* eradication is not significantly associated with either reflux symptoms or erosive oesophagitis onset, some data suggesting also an advantage in curing the infection when oesophagitis is already present.

CONCLUSION

GORD is highly prevalent in the general population. Several altered mechanisms are involved in its pathogenesis, and different lifestyle and voluptuary habits have been identified as risk factors. In the last decade, *H. pylori* eradication has been charged to cause GORD. The main putative mechanism is that a gastric acid hypersecretion develops following bacterial cure in those patients with corpus-predominant gastritis. However, studies on acid secretion demonstrated that the hypersecretion status of both DU (BAO × 3; MAO × 6) and non-ulcer dyspeptic (MAO × 3) patients observed during *H. pylori* infection normalizes following bacterial eradication[16]. Indeed, DU does not recur following a successful *H. pylori* cure[17]. Therefore, the reported erosive oesophagitis onset after the infection cure in DU patients most likely depends on other factors rather gastric acid hypersecretion (Weight gain? Changes of voluptuary habits?). On the other hand, both oesophageal 24-h pH recording and manometry studies failed to demonstrate a significant role for *H. pylori* in GORD.

Gastric acid secretion may recover with *H. pylori* eradication in those patients with corpus-predominant (or atrophic) gastritis[26]. However, such a gastritis pattern is generally encountered in either gastric ulcer or cancer patients, and it is highly prevalent in Asian countries[18]. However, the reason for which such a hypersecretive status should cause GORD but not DU onset in these patients remains unclear.

The last but not the least, several, placebo-controlled, clinical trials have been recently summarized in a meta-analysis including near 4500 patients[25]. Data found that neither reflux symptoms nor erosive oesophagitis develop following *H. pylori* eradication. Therefore, the legend of "crazy acid" remains - as all the others - a fascinating, but imaginary tale!

REFERENCES

1 **Alakkari A**, Zullo A, O'Connor HJ. Helicobacter pylori and nonmalignant diseases. *Helicobacter* 2011; **16** Suppl 1: 33-37 [PMID: 21896083 DOI: 10.1111/j.1523-5378.2011.00878.x]

2 **Zullo A**, Hassan C, Cristofari F, Perri F, Morini S. Gastric low-grade mucosal-associated lymphoid tissue-lymphoma:

Helicobacter pylori and beyond. *World J Gastrointest Oncol* 2010; **2**: 181-186 [PMID: 21160595 DOI: 10.4251/wjgo.v2.i4.181]
3 **Fuccio L**, Eusebi LH, Bazzoli F. Gastric cancer, Helicobacter pylori infection and other risk factors. *World J Gastrointest Oncol* 2010; **2**: 342-347 [PMID: 21160805 DOI: 10.4251/wjgo.v2.i9.342]
4 **Zullo A**, Hassan C, Campo SM, Morini S. Bleeding peptic ulcer in the elderly: risk factors and prevention strategies. *Drugs Aging* 2007; **24**: 815-828 [PMID: 17896831]
5 **Qu XH**, Huang XL, Xiong P, Zhu CY, Huang YL, Lu LG, Sun X, Rong L, Zhong L, Sun DY, Lin H, Cai MC, Chen ZW, Hu B, Wu LM, Jiang YB, Yan WL. Does Helicobacter pylori infection play a role in iron deficiency anemia? A meta-analysis. *World J Gastroenterol* 2010; **16**: 886-896 [PMID: 20143469]
6 **Stasi R**, Sarpatwari A, Segal JB, Osborn J, Evangelista ML, Cooper N, Provan D, Newland A, Amadori S, Bussel JB. Effects of eradication of Helicobacter pylori infection in patients with immune thrombocytopenic purpura: a systematic review. *Blood* 2009; **113**: 1231-1240 [PMID: 18945961 DOI: 10.1182/blood-2008-07-167155]
7 **Zullo A**, Ridola L, Hassan C, Bruzzese V, Papini F, Vaira D. Glaucoma and Helicobacter pylori: eyes wide shut? *Dig Liver Dis* 2012; **44**: 627-628 [PMID: 22497902]
8 **Zullo A**, Hassan C, Morini S. Hepatic encephalopathy and Helicobacter pylori: a critical reappraisal. *J Clin Gastroenterol* 2003; **37**: 164-168 [PMID: 12869889]
9 **Sherman PM**, Lin FY. Extradigestive manifestation of Helicobacter pylori infection in children and adolescents. *Can J Gastroenterol* 2005; **19**: 421-424 [PMID: 16010304]
10 **Weber C**, Davis CS, Fisichella PM. Current applications of evolving methodologies in gastroesophageal reflux disease testing. *Dig Liver Dis* 2011; **43**: 353-357 [PMID: 21324762]
11 **Vakil N**, van Zanten SV, Kahrilas P, Dent J, Jones R. The Montreal definition and classification of gastroesophageal reflux disease: a global evidence-based consensus. *Am J Gastroenterol* 2006; **101**: 1900-1920; quiz 1943 [PMID: 16928254]
12 **Pandolfino JE**, Kwiatek MA, Kahrilas PJ. The pathophysiologic basis for epidemiologic trends in gastroesophageal reflux disease. *Gastroenterol Clin North Am* 2008; **37**: 827-843, viii [PMID: 19028320]
13 **Labenz J**, Blum AL, Bayerdörffer E, Meining A, Stolte M, Börsch G. Curing Helicobacter pylori infection in patients with duodenal ulcer may provoke reflux esophagitis. *Gastroenterology* 1997; **112**: 1442-1447 [PMID: 9136820]
14 **Maconi G**, Sainaghi M, Molteni M, Bosani M, Gallus S, Ricci G, Alvisi V, Porro GB. Predictors of long-term outcome of functional dyspepsia and duodenal ulcer after successful Helicobacter pylori eradication--a 7-year follow-up study. *Eur J Gastroenterol Hepatol* 2009; **21**: 387-393 [PMID: 19182682 DOI: 10.1097/MEG.0b013e3283069db0]
15 **Vakil N**. Disease definition, clinical manifestations, epidemiology and natural history of GERD. *Best Pract Res Clin Gastroenterol* 2010; **24**: 759-764 [PMID: 21126691 DOI: 10.1016/j.bpg.2010.09.009]
16 **el-Omar EM**, Penman ID, Ardill JE, Chittajallu RS, Howie C, McColl KE. Helicobacter pylori infection and abnormalities of acid secretion in patients with duodenal ulcer disease. *Gastroenterology* 1995; **109**: 681-691 [PMID: 7657096]
17 **Ford AC**, Delaney BC, Forman D, Moayyedi P. Eradication therapy for peptic ulcer disease in Helicobacter pylori positive patients. *Cochrane Database Syst Rev* 2006; (2): CD003840 [PMID: 16625592 DOI: 10.1002/14651858.CD003840.pub4]
18 **Malfertheiner P**. The intriguing relationship of Helicobacter pylori infection and acid secretion in peptic ulcer disease and gastric cancer. *Dig Dis* 2011; **29**: 459-464 [PMID: 22095010 DOI: 10.1159/000332213]
19 **Tefera S**, Hatlebakk JG, Berstad A. The effect of Helicobacter pylori eradication on gastro-oesophageal reflux. *Aliment Pharmacol Ther* 1999; **13**: 915-920 [PMID: 10383526 DOI: 10.1046/j.1365-2036.1999.00544.x]
20 **Wu JC**, Chan FK, Wong SK, Lee YT, Leung WK, Sung JJ. Effect of Helicobacter pylori eradication on oesophageal acid exposure in patients with reflux oesophagitis. *Aliment Pharmacol Ther* 2002; **16**: 545-552 [PMID: 11876709 DOI: 10.1046/j.1365-2036.2002.01189.x]
21 **Shirota T**, Kusano M, Kawamura O, Horikoshi T, Mori M, Sekiguchi T. Helicobacter pylori infection correlates with severity of reflux esophagitis: with manometry findings. *J Gastroenterol* 1999; **34**: 553-559 [PMID: 10535481 DOI: 10.1007/s005350050372]
22 **Grande M**, Cadeddu F, Villa M, Attinà GM, Muzi MG, Nigro C, Rulli F, Farinon AM. Helicobacter pylori and gastroesophageal reflux disease. *World J Surg Oncol* 2008; **6**: 74 [PMID: 18601740 DOI: 10.1186/1477-7819-6-74]
23 **Wu JC**, Lai AC, Wong SK, Chan FK, Leung WK, Sung JJ. Dysfunction of oesophageal motility in Helicobacter pylori-infected patients with reflux oesophagitis. *Aliment Pharmacol Ther* 2001; **15**: 1913-1919 [PMID: 11736722 DOI: 10.1046/j.1365-2036.2001.01132.x]
24 **Saad AM**, Choudhary A, Bechtold ML. Effect of Helicobacter pylori treatment on gastroesophageal reflux disease (GERD): meta-analysis of randomized controlled trials. *Scand J Gastroenterol* 2012; **47**: 129-135 [PMID: 22229305]
25 **Ishiki K**, Mizuno M, Take S, Nagahara Y, Yoshida T, Yamamoto K, Okada H, Yokota K, Oguma K, Shiratori Y. Helicobacter pylori eradication improves pre-existing reflux esophagitis in patients with duodenal ulcer disease. *Clin Gastroenterol Hepatol* 2004; **2**: 474-479 [PMID: 15181615]
26 **Haruma K**, Mihara M, Okamoto E, Kusunoki H, Hananoki M, Tanaka S, Yoshihara M, Sumii K, Kajiyama G. Eradication of Helicobacter pylori increases gastric acidity in patients with atrophic gastritis of the corpus-evaluation of 24-h pH monitoring. *Aliment Pharmacol Ther* 1999; **13**: 155-162 [PMID: 10102944 DOI: 10.1046/j.1365-2036.1999.00459.x]

P- Reviewer Shimatani T **S- Editor** Gou SX **L- Editor** A **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i6.790

World J Gastroenterol 2013 February 14; 19(6): 790-796
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

FRONTIER

Current hurdles in the management of eosinophilic oesophagitis: The next steps

Stephen EA Attwood, Michael SJ Wilson

Stephen EA Attwood, Michael SJ Wilson, Department of General Surgery, North Tyneside Hospital, Rake Lane, North Shields, Tyne and Wear NE29 8NH, United Kingdom
Author contributions: Attwood SEA and Wilson MSJ made substantial contributions to the conception,design, drafting and final approval of the final version of the manuscript to be published.
Correspondence to: Stephen EA Attwood, Professor, Department of General Surgery, North Tyneside Hospital, Rake Lane, North Shields, Tyne and Wear NE29 8NH, United Kingdom. stephen.attwood@nhct.nhs.uk
Telephone: +44-191-2934079 Fax: +44-191-2934190
Received: August 23, 2012 Revised: October 26, 2012
Accepted: January 17, 2013
Published online: February 14, 2013

Abstract

Eosinophilic oesophagitis (EoE) is a chronic, antigen mediated disease of the disease of the oesophagus that may present in both adults and children. It is characterised by intermittent dysphagia, food bolus obstruction and weight loss. The pathogenesis is incompletely understood but is thought to culminate in poor compliance, or reduced distensibility. The condition is being reported and studied in the literature with increasing incidence, although equally it is highly likely that the diagnosis is being missed altogether with alarming frequency. Diagnosis of the condition requires at least one oesophageal biopsy with an eosinophil count greater than 15 per high power field. Endoscopic features include trachealisation, furrows, white exudate, narrowing and in the most severe cases stricture formation although none are pathognomonic of the condition. Therapy is often not required, but in the acute setting may take the form of dietary therapy or topical steroids. Long term maintenance therapy is usually only required in the most severe cases and the most effective treatment is the subject of ongoing research. There are a number of hurdles to be overcome in the management of patients with EoE. These include; improving our understanding of the aetiology of the condition, investigating the individual causes, assessing the true disease severity and planning the best long term maintenance therapy. Distinguishing EoE from EoE gastro-oesophageal reflux disease is also a hurdle because the two conditions, both being common, can co-exist. In order to overcome these hurdles, a multifaceted approach is required. The management of food bolus obstruction requires a management algorithm that is accepted and endorsed by a number of specialties. National and international disease registers should be established in order to facilitate future research but more importantly to address areas where further education or increased diagnostic capabilities may be required. Assessment of disease severity should become a key goal, and the development of specific biomarkers for EoE should also be a priority. Finally, randomised controlled trials of new agents are required to assess the best treatment in both the acute and long term setting.

© 2013 Baishideng. All rights reserved.

Key words: Eosinophil oesophagitis; Dysphagia; Food bolus obstruction; Therapy; Gastroscopy

Attwood SEA, Wilson MSJ. Current hurdles in the management of eosinophilic oesophagitis: The next steps. *World J Gastroenterol* 2013; 19(6): 790-796 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i6/790.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i6.790

INTRODUCTION

Eosinophilic oesophagitis (EoE) is an increasingly common cause of dysphagia or food bolus obstruction. EoE is a chronic antigen mediated disease of the oesophagus. It is characterised symptomatically by oesophageal dysfunction and histologically by eosinophil predominant inflammation. The condition was first described almost 30

years ago in a cohort of patients with > 20 intraepithelial eosinophils (IEE) per high power field seen on oesophageal biopsy, with 11 of the 12 demonstrating normal oesophageal acid exposure on 24 pH monitoring[1]. A further cohort of 10 patients with recurrent dysphagia and high concentrations of IEE at endoscopic biopsy was reported in 1994[2].

Until 2007 only 212 cases had been reported in the literature[3]. Since that time, the reporting of cases of EoE has increased dramatically. More than 750 articles incorporating several thousand patients have been published on the subject since being first described with seventy five per cent being published in the last five years.

EoE is sometimes described as "oesophageal asthma" on account of its association with atopy. Up to 50% of patients also have bronchial asthma or allergic rhinitis, and 20% have atopic dermatitis[4]. Males are affected three times more often than females, and patients typically present either in childhood or during the third or fourth decades of life.

The typical presenting features of this condition include intermittent dysphagia, food bolus obstruction and weight loss. In the paediatric population, patients may also present with nausea, vomiting, weight loss and failure to thrive.

Recent consensus guidelines state that histological evidence of at least 15 intraepithelial eosinophils per high power field (eos/hpf) in at least one oesophageal mucosal biopsy stained with haematoxylin and eosin is required for the diagnosis. However, in the correct clinical setting, patients may be considered to have EoE with < 15 eos/hpf. An example would be an atopic male on a proton pump inhibitor (PPI) at the time of diagnostic endoscopy with typical endoscopic findings. Gastro-oesophageal reflux disease (GORD) should be excluded by demonstrating a lack of response to high dose proton pump inhibitor therapy or normal oesophageal pH monitoring[5,6].

Not only is oesophageal biopsy essential in all patients with food bolus obstruction or dysphagia, regardless of the endoscopic appearance, the number of biopsies taken is also important. A minimum of six biopsies should be taken; two each from the upper, mid and lower oesophagus. Using a benchmark of 15 eos/hpf, it has been shown that the diagnostic sensitivity for EoE is 84%, 97% and 100% when 2, 3 and 6 biopsies are taken respectively[7]. A further study demonstrated 100% sensitivity when 5 biopsies were taken[8].

Estimates of the prevalence of EoE in the general population vary in the literature. In Switzerland, the adult prevalence has been estimated to be 0.02%[9], 0.44% in the United States[10] and 1% in Sweden[11]. In more targeted populations, such as those undergoing oesophageal endoscopy the prevalence is significantly greater and in the region of 6%-15%[12,13].

The current hurdles in the management of patients with EoE include; improving our understanding of the aetiology of the condition, investigating the individual causes, assessing the true disease severity and planning the best long term maintenance therapy. Distinguishing EoE from GORD is also a hurdle because the two conditions, both being common, can co-exist.

AETIOLOGY AND PATHOPHYSIOLOGY

The pathophysiology of this condition is incompletely understood and is the subject of ongoing research and debate. Expert consensus has concluded that the condition arises as a result of an antigen mediated immunologic process resulting in oesophageal inflammation. The specific allergen(s) is yet to be identified but a number of theories have been postulated. Skin prick testing has been shown to have a poor predictive value for the identification of specific food allergens in patients with EoE[14]. Given that the condition is being reported with increasing frequency this raises the possibility of an environmental allergen being involved in the pathogenesis. The "hygiene hypothesis" or declining incidence of *Helicobacter pylori* have both been proposed to play a role given that they have coincided with increased incidence of EoE[15,16].

Acid reflux (GORD) is not usually present in patients with EoE. However, it is standard practice to perform diagnostic biopsies while patients are on PPI to exclude the contribution of reflux injury[5]. When symptoms are atypical, or if there is a mixture of reflux symptoms and dysphagic EoE symptoms then 24 h pH monitoring should be employed to guide therapy. There is a subgroup of symptomatically typical EoE patients, with normal pH profiles who seem to respond to PPI, and these PPI responsive patients will benefit from with maintenance therapy[17].

Oesophageal biopsy specimens from patients with EoE compared to normal controls or patients with GORD show increased levels of the T helper 2 (Th2) cytokines, principally interleukin-5 (IL-5) and IL-13[18-20], with increased levels of the eosinophil chemoattractants eotaxin-1 and eotaxin-3 also reported[20-23]. Other studies have also demonstrated increased levels of other cytokines such as tumour necrosis factor-β1 and fibroblast growth factor-9[24,25]. GORD can be distinguished from EoE by its high level of COX-2 activation, while both show high rates of proliferation (Ki-67)[26].

It is thought that long standing oesophageal inflammation results in remodelling of the oesophageal wall in a similar manner to the airway remodelling seen in patients with bronchial asthma. A potential sequela is a fragile mucosa that is likely to tear easily as demonstrated by reported cases of spontaneous or procedure induced oesophageal rupture.

ASSESSING THE TRUE DISEASE SEVERITY

EoE can present at any age, but is more prevalent in childhood or during the third and fourth decades of life[5]. Adult patients typically present with a long history of intermittent, often severe dysphagia. In the most se-

vere cases the dysphagia may be continuous and associated with odynophagia and chest pain. EoE is also prevalent in the paediatric population, and is often associated with other atopic conditions. The clinical presentation varies with age. Infants and toddlers may present with prolonged feeding time or frank denial of food, whereas older children may present with vomiting and odynophagia. Adolescents, like adults tend to present with dysphagia. Less frequently, the child may present with failure to thrive and weight loss[27,28].

EoE is associated with a number of abnormalities of the oesophagus at endoscopy. These include; oesophageal rings (or trachealisation), furrows, white exudate, narrowing and in the most severe cases stricture formation. The pathogenesis of dysphagia in EoE is believed to be poor compliance, or reduced distensibility. If this affects the esophagus diffusely then a narrow bore oesophagus that fails to distend will result. If there is focal fibrosis, a stricture will be evident. The distinction is important when considering interventions such as dilation[29]. However, normal endoscopic findings do not exclude the diagnosis and neither are the above endoscopic findings pathognomonic of the condition. A number of studies have demonstrated histopathological evidence of EoE in patients with normal appearance of the oesophagus at endoscopy. This highlights the importance of oesophageal biopsy for all patients presenting with food bolus obstruction or dysphagia, even in the presence of a normal looking oesophagus[12,30].

Recent research has also shown that up to half of patients who present acutely with food bolus obstruction have underlying EoE. In a prospective series of 43 patients presenting with food bolus obstruction, of whom 29 had biopsies taken from the proximal and distal oesophagus, 14 (50%) fulfilled the histological criteria for EoE[31]. A further study reported that 17 of 31 (55%) patients presenting with food bolus obstruction fulfilled the histological criteria for EoE[32]. It is for these reasons that the most recent consensus guidelines stress the need for oesophageal biopsy of all patients presenting with dysphagia. In patients presenting with acute food bolus obstruction, oesophageal biopsies should also be taken at the time of disimpaction with arrangements made for appropriate clinical follow up[33]. In countries where the management of acute food bolus obstruction is principally managed by non EoE specialists, efforts should be made to raise awareness of the condition in order to avoid missed diagnoses[34].

A disease specific EoE health related quality of life questionnaire has also been developed[35]. This may be useful in identifying those who may benefit from treatment and in the assessment of response to therapy both in the clinical and research setting. There is a need to identify overall disease severity and to establish if symptoms, endoscopy or pathology can predict therapeutic outcomes.

Attention has also turned towards identifying potential biomarkers of the disease. Serum eosinophil derived neurotoxin has recently been highlighted as a potential diagnostic biomarker for EoE that may also be valid in assessing response to therapy and relapse of symptoms[36].

THERAPY

The acute management of EoE has a fairly well established pattern of treatment algorithms. In the paediatric setting, diet and topical steroids are the mainstay of treatment in the acute phase.In adults non obstructive disease is usually treated with topical steroids as the first line therapy, along with avoidance of known food precipitants. For obstructive EoE dilatation is worthwhile. Long term maintenance therapy is believed to be valuable but there is no evidence in controlled studies with long term follow up. Disease severity is both difficult to score and not predictive of long term natural history. The lack of long term therapy does not necessarily lead to the development of long term complications[37].

Dietary therapy in the acute setting can take on a number of forms. The introduction of an elemental diet has been used effectively to induce remission of symptoms and oesophageal inflammation particularly in the paediatric setting[38], with some recent success reported in adult[39]. In adults, the role of dietary therapy is being assessed but is not well established. The major drawback of this approach is the unpalatable nature of the diet. Some diets are intolerable in the long term, and recurrence of symptoms after cessation of the elemental diet is common. An alternative approach has been attempted, again with some success. This involved the introduction of a six-food elimination diet (wheat, milk, eggs, soya, nuts and rice) over a 6-12 wk period and improved patients' symptoms[40,41]. Further work by the same group highlighted the major drawback of dietary therapy in terms of compliance with the avoidance of particular food groups that are so common in western societies[42]. In the paediatric setting there has been some success with single food introduction following the six food elimination diet, with milk being identified as the most common causative food antigen[43].

Topical steroids in the acute setting are usually very effective with 60%-80% symptom resolution[44]. For long term maintenance topical corticosteroids have demonstrated some efficacy[44,45] although there is a risk of candida infection with long term use. Traditionally, topical steroid therapy is in widespread use for the management of bronchial asthma. In EoE the topical steroid is swallowed as opposed to inhaled. The most frequently employed topical steroid is fluticasone propionate at a dose of 300 to 500 micrograms twice daily, but doses of up to 880 micrograms twice daily have also been reported. The aim is to maximise exposure to the oesophagus and therefore spraying the back of the throat and swallowing the agent coats the oesophagus. This should be undertaken twice daily (morning and night time) to maximise the exposure of topical steroid to the oesophagus over a 24-h period. More recently, oral viscous budesonide has

also been shown to be effective[46,47] and in one case report a superior alternative to swallowed fluticasone[48]. Studies of clinical response to topical steroids do show variation in clinical outcome and also poor correlation of histological response with clinical improvement[49]. It is thought that topical corticosteroid therapy acts by reversing the oesophageal remodelling that occurs in EoE[45]. In a paediatric population fluticasone has been associated with improvements in the endoscopic, histologic and immunological parameters of EoE. However, the improvement was less marked in patients with a history of allergy[50].

In some patients reflux type symptoms and occasionally dysphagia is improved by PPI therapy. It is useful to measure the degree of acid reflux in these patients with 24 h pH monitoring to help gauge the need for combinations of PPI and topical steroid therapy.

Monteleukast, a leukotriene receptor antagonist has been proposed as a long term treatment. In an observational study of twelve patients, monteleukast was associated with a good symptomatic response (dysphagia scores and frequency of bolus obstruction) with an inconsistent reduction in the associated concentration of eosinophils[51]. A similar response has been demonstrated in a small cohort of paediatric patients[52]. The use of monteleukast has been criticised in some quarters on account of the lack of a placebo controlled group in the original paper reporting its use, and a further study has shown that monteleukast is inefficient in maintaining steroid reduced remission in EoE[53].

Oesophageal dilatation has been shown to be a safe and effective therapy in EoE, particularly in the presence of strictures[54]. However, mucosal tears are a worrisome occurrence but may be acceptable if an effective dilatation is achieved. Oesophageal perforation is a recognised complication of this procedure, but rare[55]. As a result, this intervention gained an adverse reputation somewhat prematurely. A recent systematic review has estimated a 0.1% risk of oesophageal perforation as a direct result of oesophageal dilatation[56,57], and this is no different to the risk of perforation when dilating a peptic stricture[58].

EoE may cause a stiffening of the oesophageal wall and produce poor compliance. Subsequent attempts to regurgitate or dislodge a food bolus may then cause a tear (usually partial) in the oesophagus[59-61]. The frequency of perforation seems to be greater from the food bolus itself than with dilatation. Management needs careful assessment because if the perforation is only a dissection, with a contained leak and no free fluid in the chest cavity, then non surgical management may be sufficient. The majority of such perforations heal with such a conservative approach[5].

More recently, attention has turned towards potential monoclonal antibody therapies against specific cytokines. Mepolizumab, a monoclonal antibody against IL-5 has been shown in a small randomised controlled trial to reduce the peak oesophageal eosinophil count, but with no improvement in clinical symptoms[62]. Similarly reslizumab (anti IL-5)[63], infliximab (anti-tumor necrosis factor-α)[64] and omalizumab (anti-IgE)[65] have been shown to reduce peak oesophageal eosinophil counts with no improvement in clinical symptoms. Results from larger randomised trials are awaited.

With thoughts towards the future, potential targets for therapy are being evaluated. One candidate currently in phase Ⅱ clinical trials is the chemoattractant receptor expressed on Th2 cells (CRTH2) antagonist[66]. CRTH2 is a receptor expressed on Th2 cells that is known to bind to prostaglandin in asthmatics. By developing a drug with the ability to block this receptor it is hoped that a key component of the inflammatory response in patients with EoE will be disabled with a subsequent clinical improvement for the patient.

FUTURE CHALLENGES

In order to facilitate future clinical research into this condition then the largest centres should collaborate closely. Both national and international disease registers should be established in order to better understand the epidemiology of this novel condition. National and international registers will enable the identification of the largest centres with the highest incidence and prevalence, but more importantly identify those centres with low or zero prevalence. This will enable targeted education to be focused upon these centres as undoubtedly this represents missed or misinterpreted diagnoses.

With recent research highlighting that up to half of patients presenting with acute food bolus obstruction fulfil the diagnostic criteria for EoE, formal protocols should be implemented to ensure that patients undergo oesophageal biopsy at the time of disimpaction to exclude EoE. In a number of countries where the management of acute food bolus obstruction is managed by specialties with little or no knowledge of the existence of this condition this will require collaborative links to be forged between gastroenterology, ENT and surgical colleagues as appropriate. Ensuring these patients undergo oesophageal biopsy is likely to result in a significant increase in the incidence of EoE and sufficient resources should be in place in order to cope. This will require dissemination of the existence of this condition into primary care.

REFERENCES

1 **Attwood SE**, Smyrk TC, Demeester TR, Jones JB. Esophageal eosinophilia with dysphagia. A distinct clinicopathologic syndrome. *Dig Dis Sci* 1993; **38**: 109-116 [PMID: 8420741]

2 **Straumann A**, Spichtin HP, Bernoulli R, Loosli J, Vögtlin J. [Idiopathic eosinophilic esophagitis: a frequently overlooked disease with typical clinical aspects and discrete endoscopic findings]. *Schweiz Med Wochenschr* 1994; **124**: 1419-1429 [PMID: 7939509]

3 **Pasha SF**, DiBaise JK, Kim HJ, De Petris G, Crowell MD, Fleischer DE, Sharma VK. Patient characteristics, clinical, endoscopic, and histologic findings in adult eosinophilic esophagitis: a case series and systematic review of the medical literature. *Dis Esophagus* 2007; **20**: 311-319 [PMID: 17617880 DOI: 10.1111/j.1442-2050.2007.00721.x]

4 **Attwood SE**, Lamb CA. Eosinophilic oesophagitis and other non-reflux inflammatory conditions of the oesophagus: diagnostic imaging and management. *Best Pract Res Clin Gastroenterol* 2008; **22**: 639-660 [PMID: 18656822 DOI: 10.1016/j.bpg.2007.12.003]

5 **Liacouras CA**, Furuta GT, Hirano I, Atkins D, Attwood SE, Bonis PA, Burks AW, Chehade M, Collins MH, Dellon ES, Dohil R, Falk GW, Gonsalves N, Gupta SK, Katzka DA, Lucendo AJ, Markowitz JE, Noel RJ, Odze RD, Putnam PE, Richter JE, Romero Y, Ruchelli E, Sampson HA, Schoepfer A, Shaheen NJ, Sicherer SH, Spechler S, Spergel JM, Straumann A, Wershil BK, Rothenberg ME, Aceves SS. Eosinophilic esophagitis: updated consensus recommendations for children and adults. *J Allergy Clin Immunol* 2011; **128**: 3-20.e6; quiz 21-22 [PMID: 21477849 DOI: 10.1016/j.jaci.2011.02.040]

6 **Furuta GT**, Liacouras CA, Collins MH, Gupta SK, Justinich C, Putnam PE, Bonis P, Hassall E, Straumann A, Rothenberg ME. Eosinophilic esophagitis in children and adults: a systematic review and consensus recommendations for diagnosis and treatment. *Gastroenterology* 2007; **133**: 1342-1363 [PMID: 17919504 DOI: 10.1053/j.gastro.2007.08.017]

7 **Shah A**, Kagalwalla AF, Gonsalves N, Melin-Aldana H, Li BU, Hirano I. Histopathologic variability in children with eosinophilic esophagitis. *Am J Gastroenterol* 2009; **104**: 716-721 [PMID: 19209168 DOI: 10.1038/ajg.2008.117]

8 **Gonsalves N**, Policarpio-Nicolas M, Zhang Q, Rao MS, Hirano I. Histopathologic variability and endoscopic correlates in adults with eosinophilic esophagitis. *Gastrointest Endosc* 2006; **64**: 313-319 [PMID: 16923475 DOI: 10.1016/j.gie.2006.04.037]

9 **Straumann A**, Simon HU. Eosinophilic esophagitis: escalating epidemiology? *J Allergy Clin Immunol* 2005; **115**: 418-419 [PMID: 15696105 DOI: 10.1016/j.jaci.2004.11.006]

10 **Kapel RC**, Miller JK, Torres C, Aksoy S, Lash R, Katzka DA. Eosinophilic esophagitis: a prevalent disease in the United States that affects all age groups. *Gastroenterology* 2008; **134**: 1316-1321 [PMID: 18471509 DOI: 10.1053/j.gastro.2008.02.016]

11 **Ronkainen J**, Talley NJ, Aro P, Storskrubb T, Johansson SE, Lind T, Bolling-Sternevald E, Vieth M, Stolte M, Walker MM, Agréus L. Prevalence of oesophageal eosinophils and eosinophilic oesophagitis in adults: the population-based Kalixanda study. *Gut* 2007; **56**: 615-620 [PMID: 17135307 DOI: 10.1136/gut.2006.107714]

12 **Prasad GA**, Talley NJ, Romero Y, Arora AS, Kryzer LA, Smyrk TC, Alexander JA. Prevalence and predictive factors of eosinophilic esophagitis in patients presenting with dysphagia: a prospective study. *Am J Gastroenterol* 2007; **102**: 2627-2632 [PMID: 17764492 DOI: 10.1111/j.1572-0241.2007.01512.x]

13 **Veerappan GR**, Perry JL, Duncan TJ, Baker TP, Maydonovitch C, Lake JM, Wong RK, Osgard EM. Prevalence of eosinophilic esophagitis in an adult population undergoing upper endoscopy: a prospective study. *Clin Gastroenterol Hepatol* 2009; **7**: 420-426, 426.e1-2 [PMID: 19162236 DOI: 10.1016/j.cgh.2008.10.009]

14 **Kamdar TA**, Ditto AM, Bryce PJ. Skin prick testing does not reflect the presence of IgE against food allergens in adult eosinophilic esophagitis patients: a case study. *Clin Mol Allergy* 2010; **8**: 16 [PMID: 21083924 DOI: 10.1186/1476-7961-8-16]

15 **Bonis PA**. Putting the puzzle together: epidemiological and clinical clues in the etiology of eosinophilic esophagitis. *Immunol Allergy Clin North Am* 2009; **29**: 41-52, viii [PMID: 19141340 DOI: 10.1016/j.iac.2008.09.005]

16 **Dellon ES**, Peery AF, Shaheen NJ, Morgan DR, Hurrell JM, Lash RH, Genta RM. Inverse association of esophageal eosinophilia with Helicobacter pylori based on analysis of a US pathology database. *Gastroenterology* 2011; **141**: 1586-1592 [PMID: 21762663 DOI: 10.1053/j.gastro.2011.06.081]

17 **Molina-Infante J**, Ferrando-Lamana L, Ripoll C, Hernandez-Alonso M, Mateos JM, Fernandez-Bermejo M, Dueñas C, Fernandez-Gonzalez N, Quintana EM, Gonzalez-Nuñez MA. Esophageal eosinophilic infiltration responds to proton pump inhibition in most adults. *Clin Gastroenterol Hepatol* 2011; **9**: 110-117 [PMID: 20920599 DOI: 10.1016/j.cgh.2010.09.019]

18 **Straumann A**, Bauer M, Fischer B, Blaser K, Simon HU. Idiopathic eosinophilic esophagitis is associated with a T(H)2-type allergic inflammatory response. *J Allergy Clin Immunol* 2001; **108**: 954-961 [PMID: 11742273 DOI: 10.1067/mai.2001.119917]

19 **Mishra A**, Hogan SP, Brandt EB, Rothenberg ME. IL-5 promotes eosinophil trafficking to the esophagus. *J Immunol* 2002; **168**: 2464-2469 [PMID: 11859139]

20 **Lucendo AJ**, De Rezende L, Comas C, Caballero T, Bellón T. Treatment with topical steroids downregulates IL-5, eotaxin-1/CCL11, and eotaxin-3/CCL26 gene expression in eosinophilic esophagitis. *Am J Gastroenterol* 2008; **103**: 2184-2193 [PMID: 18844613 DOI: 10.1111/j.1572-0241.2008.01937.x]

21 **Mishra A**, Rothenberg ME. Intratracheal IL-13 induces eosinophilic esophagitis by an IL-5, eotaxin-1, and STAT6-dependent mechanism. *Gastroenterology* 2003; **125**: 1419-1427 [PMID: 14598258]

22 **Konikoff MR**, Blanchard C, Kirby C, Buckmeier BK, Cohen MB, Heubi JE, Putnam PE, Rothenberg ME. Potential of blood eosinophils, eosinophil-derived neurotoxin, and eotaxin-3 as biomarkers of eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2006; **4**: 1328-1336 [PMID: 17059896 DOI: 10.1016/j.cgh.2006.08.013]

23 **Bhattacharya B**, Carlsten J, Sabo E, Kethu S, Meitner P, Tavares R, Jakate S, Mangray S, Aswad B, Resnick MB. Increased expression of eotaxin-3 distinguishes between eosinophilic esophagitis and gastroesophageal reflux disease. *Hum Pathol* 2007; **38**: 1744-1753 [PMID: 17900656 DOI: 10.1016/j.humpath.2007.05.008]

24 **Mulder DJ**, Pacheco I, Hurlbut DJ, Mak N, Furuta GT, MacLeod RJ, Justinich CJ. FGF9-induced proliferative response to eosinophilic inflammation in oesophagitis. *Gut* 2009; **58**: 166-173 [PMID: 18978176 DOI: 10.1136/gut.2008.157628]

25 **Aceves SS**, Newbury RO, Dohil R, Bastian JF, Broide DH. Esophageal remodeling in pediatric eosinophilic esophagitis. *J Allergy Clin Immunol* 2007; **119**: 206-212 [PMID: 17208603 DOI: 10.1016/j.jaci.2006.10.016]

26 **Lewis CJ**, Lamb CA, Kanakala V, Pritchard S, Armstrong GR, Attwood SE. Is the etiology of eosinophilic esophagitis in adults a response to allergy or reflux injury? Study of cellular proliferation markers. *Dis Esophagus* 2009; **22**: 249-255 [PMID: 19207551 DOI: 10.1111/j.1442-2050.2008.00896.x]

27 **Aceves SS**, Newbury RO, Dohil MA, Bastian JF, Dohil R. A symptom scoring tool for identifying pediatric patients with eosinophilic esophagitis and correlating symptoms with inflammation. *Ann Allergy Asthma Immunol* 2009; **103**: 401-406 [PMID: 19927538 DOI: 10.1016/S1081-1206[10)60359-6]

28 **Mukkada VA**, Haas A, Maune NC, Capocelli KE, Henry M, Gilman N, Petersburg S, Moore W, Lovell MA, Fleischer DM, Furuta GT, Atkins D. Feeding dysfunction in children with eosinophilic gastrointestinal diseases. *Pediatrics* 2010; **126**: e672-e677 [PMID: 20696733 DOI: 10.1542/peds.2009-2227]

29 **Kwiatek MA**, Hirano I, Kahrilas PJ, Rothe J, Luger D, Pandolfino JE. Mechanical properties of the esophagus in eosinophilic esophagitis. *Gastroenterology* 2011; **140**: 82-90 [PMID: 20858491 DOI: 10.1053/j.gastro.2010.09.037]

30 **Mackenzie SH**, Go M, Chadwick B, Thomas K, Fang J, Kuwada S, Lamphier S, Hilden K, Peterson K. Eosinophilic oesophagitis in patients presenting with dysphagia--a prospective analysis. *Aliment Pharmacol Ther* 2008; **28**: 1140-1146 [PMID: 18624788 DOI: 10.1111/j.1365-2036.2008.03795.x]

31 **Kerlin P**, Jones D, Remedios M, Campbell C. Prevalence of eosinophilic esophagitis in adults with food bolus obstruction of the esophagus. *J Clin Gastroenterol* 2007; **41**: 356-361 [PMID: 17413601 DOI: 10.1097/01.mcg.0000225590.08825.77]

32 **Desai TK**, Stecevic V, Chang CH, Goldstein NS, Badizadegan K, Furuta GT. Association of eosinophilic inflammation with esophageal food impaction in adults. *Gastrointest Endosc* 2005; **61**: 795-801 [PMID: 15933677]

33 **Hurtado CW**, Furuta GT, Kramer RE. Etiology of esophageal food impactions in children. *J Pediatr Gastroenterol Nutr* 2011; **52**: 43-46 [PMID: 20975581 DOI: 10.1097/MPG.0b013e3181e67072]

34 **Bergquist H**, Bove M. Eosinophilic esophagitis in adults: An ear, nose, and throat perspective. *Laryngoscope* 2009; **119**: 1467-1471 [PMID: 19504557 DOI: 10.1002/lary.20255]

35 **Taft TH**, Kern E, Kwiatek MA, Hirano I, Gonsalves N, Keefer L. The adult eosinophilic oesophagitis quality of life questionnaire: a new measure of health-related quality of life. *Aliment Pharmacol Ther* 2011; **34**: 790-798 [PMID: 21806649 DOI: 10.1111/j.1365-2036.2011.04791.x]

36 **Subbarao G**, Rosenman MB, Ohnuki L, Georgelas A, Davis M, Fitzgerald JF, Molleston JP, Croffie JM, Pfefferkorn MD, Corkins MR, Lim JR, Steiner SJ, Schaefer E, Gleich GJ, Gupta SK. Exploring potential noninvasive biomarkers in eosinophilic esophagitis in children. *J Pediatr Gastroenterol Nutr* 2011; **53**: 651-658 [PMID: 21694637 DOI: 10.1097/MPG.0b013e318228cee6]

37 **Kanakala V**, Lamb CA, Haigh C, Stirling RW, Attwood SE. The diagnosis of primary eosinophilic oesophagitis in adults: missed or misinterpreted? *Eur J Gastroenterol Hepatol* 2010; **22**: 848-855 [PMID: 20453656 DOI: 10.1097/MEG.0b013e32832c7709]

38 **Spergel JM**, Andrews T, Brown-Whitehorn TF, Beausoleil JL, Liacouras CA. Treatment of eosinophilic esophagitis with specific food elimination diet directed by a combination of skin prick and patch tests. *Ann Allergy Asthma Immunol* 2005; **95**: 336-343 [PMID: 16279563 DOI: 10.1016/S1081-1206[10)61151-9]

39 **Peterson K**, Clayton F, Vinson L, Fang J, Boynton K, Gleich G, Byrne K. Utility of an elemental diet in adult eosinophilic esophagitis. *Gastroenterology* 2011; **140**: 180

40 **Gonsalves N**, Yang G-Y, Doerfler B. A prospective clinical trial of six food elimination diet and reintroduction of causative agents in adults with eosinophilic oesophagitis. *Gastroenterology* 2009; **136**: 280

41 **Gonsalves N**, Yang GY, Doerfler B, Ritz S, Ditto AM, Hirano I. Elimination diet effectively treats eosinophilic esophagitis in adults; food reintroduction identifies causative factors. *Gastroenterology* 2012; **142**: 1451-1459.e1; quiz e14-15 [PMID: 22391333 DOI: 10.1053/j.gastro.2012.03.001]

42 **Gonsalves N**, Hirano I. Quality of Life Assessment in Adults with EoE At Baseline and After Treatment with Dietary Therapy: Is the Treatment Worse Than the Disease? *Gastroenterology* 2009; **136**: 137

43 **Kagalwalla AF**, Shah A, Li BU, Sentongo TA, Ritz S, Manuel-Rubio M, Jacques K, Wang D, Melin-Aldana H, Nelson SP. Identification of specific foods responsible for inflammation in children with eosinophilic esophagitis successfully treated with empiric elimination diet. *J Pediatr Gastroenterol Nutr* 2011; **53**: 145-149 [PMID: 21788754 DOI: 10.1097/MPG.0b013e31821cf503]

44 **Arora AS**, Perrault J, Smyrk TC. Topical corticosteroid treatment of dysphagia due to eosinophilic esophagitis in adults. *Mayo Clin Proc* 2003; **78**: 830-835 [PMID: 12839078]

45 **Aceves SS**, Newbury RO, Chen D, Mueller J, Dohil R, Hoffman H, Bastian JF, Broide DH. Resolution of remodeling in eosinophilic esophagitis correlates with epithelial response to topical corticosteroids. *Allergy* 2010; **65**: 109-116 [PMID: 19796194 DOI: 10.1111/j.1398-9995.2009.02142.x]

46 **Dohil R**, Newbury R, Fox L, Bastian J, Aceves S. Oral viscous budesonide is effective in children with eosinophilic esophagitis in a randomized, placebo-controlled trial. *Gastroenterology* 2010; **139**: 418-429 [PMID: 20457157 DOI: 10.1053/j.gastro.2010.05.001]

47 **Straumann A**, Conus S, Degen L, Felder S, Kummer M, Engel H, Bussmann C, Beglinger C, Schoepfer A, Simon HU. Budesonide is effective in adolescent and adult patients with active eosinophilic esophagitis. *Gastroenterology* 2010; **139**: 1526-1537, 1537.e1 [PMID: 20682320 DOI: 10.1053/j.gastro.2010.07.048]

48 **Krishna SG**, Kakati BR, Olden KW, Brown DK. Treatment of eosinophilic esophagitis: is oral viscous budesonide superior to swallowed fluticasone spray? *Gastroenterol Hepatol* (NY) 2011; **7**: 55-59 [PMID: 21346855]

49 **Alexander JA**, Jung KW, Arora AS, Enders F, Katzka DA, Kephardt GM, Kita H, Kryzer LA, Romero Y, Smyrk TC, Talley NJ. Swallowed fluticasone improves histologic but not symptomatic response of adults with eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2012; **10**: 742-749.e1 [PMID: 22475741 DOI: 10.1016/j.cgh.2012.03.018]

50 **Noel RJ**, Putnam PE, Collins MH, Assa'ad AH, Guajardo JR, Jameson SC, Rothenberg ME. Clinical and immunopathologic effects of swallowed fluticasone for eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2004; **2**: 568-575 [PMID: 15224281]

51 **Attwood SE**, Lewis CJ, Bronder CS, Morris CD, Armstrong GR, Whittam J. Eosinophilic oesophagitis: a novel treatment using Montelukast. *Gut* 2003; **52**: 181-185 [PMID: 12524397]

52 **Stumphy J**, Al-Zubeidi D, Guerin L, Mitros F, Rahhal R. Observations on use of montelukast in pediatric eosinophilic esophagitis: insights for the future. *Dis Esophagus* 2011; **24**: 229-234 [PMID: 21073625 DOI: 10.1111/j.1442-2050.2010.01134.x]

53 **Lucendo AJ**, De Rezende LC, Jiménez-Contreras S, Yagüe-Compadre JL, González-Cervera J, Mota-Huertas T, Guagnozzi D, Angueira T, González-Castillo S, Arias A. Montelukast was inefficient in maintaining steroid-induced remission in adult eosinophilic esophagitis. *Dig Dis Sci* 2011; **56**: 3551-3558 [PMID: 21674173 DOI: 10.1007/s10620-011-1775-y]

54 **Schoepfer AM**, Gschossmann J, Scheurer U, Seibold F, Straumann A. Esophageal strictures in adult eosinophilic esophagitis: dilation is an effective and safe alternative after failure of topical corticosteroids. *Endoscopy* 2008; **40**: 161-164 [PMID: 18253909 DOI: 10.1055/s-2007-995345]

55 **Straumann A**, Bussmann C, Zuber M, Vannini S, Simon HU, Schoepfer A. Eosinophilic esophagitis: analysis of food impaction and perforation in 251 adolescent and adult patients. *Clin Gastroenterol Hepatol* 2008; **6**: 598-600 [PMID: 18407800 DOI: 10.1016/j.cgh.2008.02.003]

56 **Cohen MS**, Kaufman AB, Palazzo JP, Nevin D, Dimarino AJ, Cohen S. An audit of endoscopic complications in adult eosinophilic esophagitis. *Clin Gastroenterol Hepatol* 2007; **5**: 1149-1153 [PMID: 17683993 DOI: 10.1016/j.cgh.2007.05.017]

57 **Jacobs JW**, Spechler SJ. A systematic review of the risk of perforation during esophageal dilation for patients with eosinophilic esophagitis. *Dig Dis Sci* 2010; **55**: 1512-1515 [PMID: 20238250 DOI: 10.1007/s10620-010-1165-x]

58 **Riley SA**, Attwood SE. Guidelines on the use of oesophageal dilatation in clinical practice. *Gut* 2004; **53** Suppl 1: i1-i6 [PMID: 14724139]

59 **Liguori G**, Cortale M, Cimino F, Sozzi M. Circumferential mucosal dissection and esophageal perforation in a patient with eosinophilic esophagitis. *World J Gastroenterol* 2008; **14**: 803-804 [PMID: 18205276]

60 **Quiroga J**, Prim JM, Moldes M, Ledo R. Spontaneous circumferential esophageal dissection in a young man with eosinophilic esophagitis. *Interact Cardiovasc Thorac Surg* 2009; **9**: 1040-1042 [PMID: 19752151 DOI: 10.1510/icvts.2009.208975]

61 **Shim LS**, Grehan M. Education and Imaging. Gastrointestinal: oesophageal perforation during endoscopy for food impaction in eosinophilic oesophagitis. *J Gastroenterol Hepatol* 2010; **25**: 428 [PMID: 20136991 DOI: 10.1111/j.1440-1746.2009.06181.x]

62 **Straumann A**, Conus S, Grzonka P, Kita H, Kephart G, Bussmann C, Beglinger C, Smith DA, Patel J, Byrne M, Simon HU. Anti-interleukin-5 antibody treatment (mepolizumab) in active eosinophilic oesophagitis: a randomised, placebo-controlled, double-blind trial. *Gut* 2010; **59**: 21-30 [PMID: 19828470 DOI: 10.1136/gut.2009.178558]

63 **Spergel JM**, Rothenberg ME, Collins MH, Furuta GT, Markowitz JE, Fuchs G, O'Gorman MA, Abonia JP, Young J, Henkel T, Wilkins HJ, Liacouras CA. Reslizumab in children and adolescents with eosinophilic esophagitis: results of a double-blind, randomized, placebo-controlled trial. *J Allergy Clin Immunol* 2012; **129**: 456-463, 463.e1-3 [PMID: 22206777 DOI: 10.1016/j.jaci.2011.11.044]

64 **Straumann A**, Bussmann C, Conus S, Beglinger C, Simon HU. Anti-TNF-alpha (infliximab) therapy for severe adult eosinophilic esophagitis. *J Allergy Clin Immunol* 2008; **122**: 425-427 [PMID: 18678345 DOI: 10.1016/j.jaci.2008.06.012]

65 **Fang J**, Hilden K, Gleich G, Emerson L, Ogorman M, Lowichik A, Peterson KA. A Pilot Study of the Treatment of Eosinophilic Esophagitis WithOmalizumab. *Gastroenterology* 2011; **140**: 235

66 **Pettipher R**, Hansel TT, Armer R. Antagonism of the prostaglandin D2 receptors DP1 and CRTH2 as an approach to treat allergic diseases. *Nat Rev Drug Discov* 2007; **6**: 313-325 [PMID: 17396136 DOI: 10.1038/nrd2266]

P- Reviewers Bove M, Moawad FJ **S- Editor** Zhai HH
L- Editor A **E- Editor** Li JY



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i6.797

World J Gastroenterol 2013 February 14; 19(6): 797-801
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

FIELD OF VISION

Hepatocellular carcinoma after ablation: The imaging follow-up scheme

Lin-Na Liu, Hui-Xiong Xu, Yi-Feng Zhang, Jun-Mei Xu

Lin-Na Liu, Hui-Xiong Xu, Yi-Feng Zhang, Jun-Mei Xu, Department of Medical Ultrasound, Shanghai Tenth People's Hospital, Tenth People's Hospital of Tongji University, Shanghai 200072, China

Author contributions: Liu LN collected the materials and wrote the manuscript; Zhang YF and Xu JM discussed the topic; and Xu HX supervised the publication of this commentary.

Supported by National Scientific Foundation Committee of China, No. 30970837; Key Project from Shanghai Health Bureau, No. 20114003; Shanghai Talent Development Project from Shanghai Human Resource and Social Security Bureau, No. 2012045

Correspondence to: Hui-Xiong Xu, MD, PhD, Professor, Department of Medical Ultrasound, Shanghai Tenth People's Hospital, Tenth People's Hospital of Tongji University, No. 301 Yanchangzhong Road, Shanghai 200072, China. xuhuixiong@hotmail.com
Telephone: +86-21-66301031 Fax: +86-21-66301031

Received: November 20, 2012 Revised: January 9, 2013
Accepted: January 18, 2013
Published online: February 14, 2013

Abstract

Percutaneous ablation using thermal or chemical methods has been widely used in the treatment of hepatocellular carcinoma (HCC). Nowadays, contrast-enhanced imaging modalities such as computed tomography (CT), magnetic resonance imaging (MRI), and contrast-enhanced ultrasound (CEUS) are widely used to evaluate local treatment response after ablation therapies. CEUS is gaining increasing attention due to its characteristics including real-time scanning, easy performance, lack of radiation, wide availability, and lack of allergy reactions. Several studies have documented that CEUS is comparable to CT or MRI in evaluating local treatment efficacy within 1 mo of treatment. However, little information is available regarding the role of CEUS in the follow-up assessment after first successful ablation treatment. Zheng *et al* found that in comparison with contrast-enhanced computed tomography (CECT), the sensitivity, specificity, positive predictive value, negative predictive value and overall accuracy of CEUS in detecting local tumor progression (LTP) were 67.5%, 97.4%, 81.8%, 94.4% and 92.3%, respectively, and were 77.7%, 92.0%, 92.4%, 76.7% and 84.0%, respectively for the detection of new intrahepatic recurrence. They concluded that the sensitivity of CEUS in detecting LTP and new intrahepatic recurrence after ablation is relatively low in comparison with CECT, and CEUS cannot replace CECT in the follow-up assessment after percutaneous ablation for HCC. These results are meaningful and instructive, and indicated that in the follow-up period, the use of CEUS alone is not sufficient. In this commentary, we discuss the discordance between CT and CEUS, as well as the underlying mechanisms involved. We propose the combined use of CT and CEUS which will reduce false positive and negative results in both modalities. We also discuss future issues, such as an evidence-based ideal imaging follow-up scheme, and a cost-effectiveness analysis of this imaging follow-up scheme.

© 2013 Baishideng. All rights reserved.

Key words: Hepatocellular carcinoma; Radiofrequency ablation; Ethanol ablation; Contrast-enhanced ultrasound; Follow-up; Treatment response; Computed tomography

Liu LN, Xu HX, Zhang YF, Xu JM. Hepatocellular carcinoma after ablation: The imaging follow-up scheme. *World J Gastroenterol* 2013; 19(6): 797-801 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i6/797.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i6.797

COMMENTARY ON HOT TOPICS

We read with great interest the recent article by Zheng *et al*[1] evaluating the usefulness of contrast-enhanced ultrasound (CEUS) in the follow-up of patients with he-

patocellular carcinoma (HCC) who had undergone local ablation therapies.

As a minimal invasive and safe treatment method, percutaneous ablation using thermal or chemical methods has been widely used in the treatment of early HCC, recurrent HCC, and even advanced HCC[2-11]. Percutaneous ablation such as radiofrequency ablation (RFA), microwave ablation and ethanol ablation (EA), is regarded as the curative treatment method for early HCC[12,13]. In contrast to surgical resection where the tumor is removed, the tumor is not eradicated from the body but is deactivated by ablation therapy, therefore, it is of paramount importance to evaluate the efficacy of this treatment to determine follow-up treatment and strategy. Currently, the use of contrast-enhanced imaging to detect residual viable tumor or recurrent HCC is widely accepted[14-19]. The underlying mechanism of this imaging method relates to the fact that viable tumor tissue shows arterial hypervascularity (*i.e.*, hyper-enhancement), whereas destroyed tumor shows absence of vascularity (*i.e.*, non-enhancement), thus the distinction between them is achievable. However, this method is not ideal as imaging studies may fail to detect tiny viable tumor tissue, especially when neoangiogenesis is not obvious. Percutaneous biopsy may be another option, however, this technique has significant sample error and it is not ethical and practical to sample all the lesion after ablation. Consequently, contrast-enhanced imaging modalities such as computed tomography (CT), magnetic resonance imaging (MRI), and CEUS are currently widely used to evaluate local treatment response after ablation therapies. Although CT and MRI are accepted as the reference standards, the newly introduced CEUS is also gaining increasing attention due to its characteristics including real-time scanning, easy performance, lack of radiation, wide availability, and lack of allergic reactions[20-38]. Several studies have documented that CEUS is comparable to CT or MRI in evaluating local treatment efficacy within one mo of treatment (Table 1)[18,39-41]. Kim *et al*[42] also reported that they were in favor of CEUS as it had the advantage of being able to detect lesions < 2 cm; therefore, CEUS has been used effectively in diagnostic algorithms for small 1-2 cm newly detected nodules in HCC patients. However, little information is available regarding the role of CEUS in the follow-up assessment after first successful ablation treatment. In the follow-up period, the patient may develop local tumor progression (LTP) or new intrahepatic recurrence, and imaging modalities are used to successfully detect these lesions.

In the study by Zheng *et al*[1], 141 patients with HCCs who underwent percutaneous ablation therapy were assessed by paired follow-up CEUS and contrast-enhanced computed tomography (CECT). Using CECT as the reference standard, the ability of CEUS to detect LTP or new intrahepatic recurrence during follow-up was evaluated. They found that 33 LTP and 131 new intrahepatic recurrent foci were detected on CEUS, whereas 40 and 183 were detected on CECT, respectively (both $P < 0.05$). Compared with CECT, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and overall accuracy of CEUS in detecting LTP were 67.5%, 97.4%, 81.8%, 94.4% and 92.3%, respectively, and were 77.7%, 92.0%, 92.4%, 76.7% and 84.0%, respectively for the detection of new intrahepatic recurrence. They concluded that the sensitivity of CEUS in detecting LTP and new intrahepatic recurrence after ablation was relatively low compared with CECT, and that CEUS can not replace CECT in the follow-up assessment after percutaneous ablation for HCC. Their results are interesting and meaningful, and we agree with the authors regarding the role of CEUS in the follow-up of HCC patients after ablation therapy.

The discordance in imaging features between CT and CEUS is well recognized, and is largely due to the difference in pharmacokinetics between CT contrast medium and ultrasound contrast agent[35,43]. The CT contrast medium diffuses into the interstitial space, while the ultrasound contrast agent is a pure blood pool tracer. The CEUS characteristic of real-time scanning is helpful in detecting subtle lesions with transient arterial hyper-enhancement which is hard to visualize on CT. Some lesions may show arterial iso-enhancement on CT and hyper-enhancement on CEUS due to the limited time window in CT scanning[44-49]. On the other hand, CEUS also has its shortcomings as shown by Zheng *et al*[1] who found that image quality was affected by lesions located near the liver dome, and obscuration due to gas from the lung or intestine. The development of new foci may be located in different lobes of the liver, and the arterial hyper-enhancement on CEUS only lasts for several seconds, thus it is difficult to detect all hypervascular lesions in one scanning procedure. Most importantly, in comparison with CT/MRI, there is universal bias in readers' minds with regards to ultrasound images. The quality of the procedure and subsequent results are largely operator-dependent, thus less uniformity is encountered in clinical practice (Table 2).

The data in the above-mentioned article are detailed and reasonable results have been obtained. However, there is still controversy over the role of CEUS *vs* CECT in the diagnosis of HCC after ablation, which is largely dependent on individual opinion and familiarity with the techniques. Frieser *et al*[39] concluded that CEUS is equal to CECT in evaluating treatment response. Gallotti *et al*[46] found that CEUS was excellent in evaluating treatment response after RFA, whereas it was inadequate for evaluating treatment response after EA. It should be noted that despite the vast number of published studies on the subject, a unanimous consensus has not been achieved.

There are a number of unsolved issues regarding the use of CEUS and CECT including the following: (1) The authors should recommend the combined use of CT and CEUS in clinical practice when CEUS is available, which will reduce the number of false positive and negative findings in both modalities[50]. In a study of liver lesion characterization, although no follow-up assessment after

Table 1 Diagnostic values of contrast-enhanced imaging in evaluating treatment response after ablation for liver cancer

Ref.	*n*	Imaging	Accuracy	Sen	Spe	PPV	NPV
[1]Lu *et al*[18]	151 patients	CEUS	96.6%	-	98.20%	-	-
Frieser *et al*[39]	76 patients 118 nodules	CEUS	93.8%	-	-	-	-
		CECT	86.2%	-	-	-	-
		CEUS	100%	-	-	-	-
		CEMRI	88.4%	-	-	-	-
[2]Ricci *et al*[40]	100 patients 100 nodules	CEUS	-	92.3%	100%	100%	97.4%
[2]Salvaggio *et al*[41]	148 nodules	CEUS	97%	83.3%	100%	-	-

[1]In comparison with contrast-enhanced computed tomography (CECT) or contrast-enhanced magnetic resonance imaging (CEMRI); [2]In comparison with CECT. -: Not applicable. Sen: Sensitivity; Spe: Specificity; PPV: Positive predictive value; NPV: Negative predictive value; CEUS: Contrast-enhanced ultrasound.

Table 2 Comparison between contrast-enhanced ultrasound and contrast-enhanced computed tomography in the follow-up scheme after liver cancer ablation

	CEUS	CECT
Pharmacokinetics of the contrast	Early vascular phase; followed by diffusion into the interstitial space	Pure blood pool tracer; without diffusion into the interstitial space
Strong points	Real-time scanning, easy to perform, no radiation, wide availability, and no allergic reactions	High image quality; operator-independent; panoramic imaging; easy to interpret
Weak points	Image quality is apt to be affected by lesions located near the liver dome, and obscuration by gas from the lung or intestine; Inability to imaging multiple lesions in one procedure; operator-dependent	Inferior temporal resolution; allergic reaction to the contrast-medium; unsuitable for patients with kidney function impairment; radiation; inferior availability

CEUS: Contrast-enhanced ultrasound; CECT: Contrast-enhanced computed tomography.

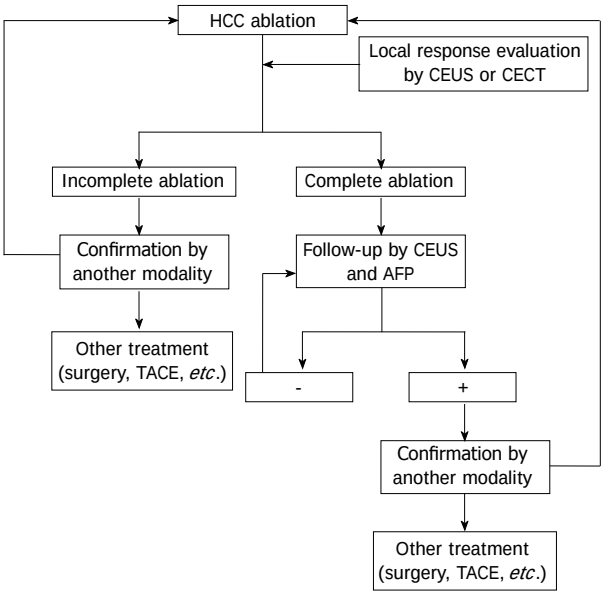


Figure 1 The proposed algorithm combining the strengths of the radiologic techniques of contrast-enhanced ultrasound and contrast-enhanced computed tomography. HCC: Hepatocellular carcinoma; CEUS: Contrast-enhanced ultrasound; CECT: Contrast-enhanced computed tomography; AFP: α fetoprotein; TACE: Transarterial chemoembolization; -: Negative finding; +: Positive finding.

ablation was carried out, the authors found that combined assessment using CEUS/CT provided higher sensitivity (97%, both readers) than separate assessment using CEUS (88% reader 1; 87% reader 2) and CT (74% reader 1; 71% reader 2; $P < 0.05$), while no change in specificity was observed using the combined analysis. The combined assessment of hepatocellular nodule vascularity using CT and CEUS improved diagnostic sensitivity of malignancy in patients with liver cirrhosis[50]. However, the study by Zheng *et al*[1] was retrospective, thus it is difficult to assess the value of the combined diagnostic procedures in clinical practice, which has prompted a future prospective study to evaluate this issue. Fusion imaging may be another solution which combines the virtues of both modalities[51]; (2) The ideal imaging follow-up scheme is not yet available. Future evidence-based studies are necessary to establish this scheme. Every practicing clinician must make a decision on the most accurate, cost-effective radiologic test to be used in the follow-up of liver lesions after local treatment. A reasonable algorithm is proposed and is shown in Figure 1, however, further study is mandatory to evaluate its efficacy; (3) A cost-effectiveness analysis should be performed to select the best imaging follow-up scheme; and (4) Long-term follow-up studies are needed to help guide our approach and therapy.

REFERENCES

1 **Zheng SG**, Xu HX, Lu MD, Xie XY, Xu ZF, Liu GJ, Liu LN. Role of contrast-enhanced ultrasound in follow-up assessment after ablation for hepatocellular carcinoma. *World J Gastroenterol* 2013; **6**: 855-865 [DOI: 10.3748/wjg.v19.i6.855]
2 **Xu HX**, Wang Y, Lu MD, Liu LN. Percutaneous ultrasound-guided thermal ablation for intrahepatic cholangiocarcinoma. *Br J Radiol* 2012; **85**: 1078-1084 [PMID: 22374282 DOI: 10.1259/bjr/24563774]
3 **Ohmoto K**, Yamamoto S. Comparison between radiofrequency ablation and percutaneous microwave coagulation

therapy for small hepatocellular carcinomas. *Clin Radiol* 2006; **61**: 800-801; author reply 801-802 [PMID: 16905390]
4 **Xu HX**, Lu MD, Xie XY, Yin XY, Kuang M, Chen JW, Xu ZF, Liu GJ. Prognostic factors for long-term outcome after percutaneous thermal ablation for hepatocellular carcinoma: a survival analysis of 137 consecutive patients. *Clin Radiol* 2005; **60**: 1018-1025 [PMID: 16124984 DOI: 10.1016/j.crad.2005.04.009]
5 **Xu HX**, Xie XY, Lu MD, Chen JW, Yin XY, Xu ZF, Liu GJ. Ultrasound-guided percutaneous thermal ablation of hepatocellular carcinoma using microwave and radiofrequency ablation. *Clin Radiol* 2004; **59**: 53-61 [PMID: 14697375 DOI: 10.1016/j.crad.2003.09.006]
6 **Yin XY**, Xie XY, Lu MD, Kuang M, Liu GJ, Xu ZF, Xu HX, Wang Z. Percutaneous ablative therapies of recurrent hepatocellular carcinoma after hepatectomy: proposal of a prognostic model. *Ann Surg Oncol* 2012; **19**: 4300-4306 [PMID: 22766980 DOI: 10.1245/s10434-012-2433-0]
7 **Kuang M**, Lu MD, Xie XY, Xu HX, Xu ZF, Liu GJ, Yin XY, Huang JF, Lencioni R. Ethanol ablation of hepatocellular carcinoma Up to 5.0 cm by using a multipronged injection needle with high-dose strategy. *Radiology* 2009; **253**: 552-561 [PMID: 19709992 DOI: 10.1148/radiol.2532082021]
8 **Yin XY**, Xie XY, Lu MD, Xu HX, Xu ZF, Kuang M, Liu GJ, Liang JY, Lau WY. Percutaneous thermal ablation of medium and large hepatocellular carcinoma: long-term outcome and prognostic factors. *Cancer* 2009; **115**: 1914-1923 [PMID: 19241423 DOI: 10.1002/cncr.24196]
9 **Lu MD**, Yin XY, Xie XY, Xu HX, Xu ZF, Liu GJ, Kuang M, Zheng YL. Percutaneous thermal ablation for recurrent hepatocellular carcinoma after hepatectomy. *Br J Surg* 2005; **92**: 1393-1398 [PMID: 16044409 DOI: 10.1002/bjs.5102]
10 **Lu MD**, Xu HX, Xie XY, Yin XY, Chen JW, Kuang M, Xu ZF, Liu GJ, Zheng YL. Percutaneous microwave and radiofrequency ablation for hepatocellular carcinoma: a retrospective comparative study. *J Gastroenterol* 2005; **40**: 1054-1060 [PMID: 16322950 DOI: 10.1007/s00535-005-1671-3]
11 **Liu LN**, Xu HX, Lu MD, Xie XY. Percutaneous ultrasound-guided thermal ablation for liver tumor with artificial pleural effusion or ascites. *Chin J Cancer* 2010; **29**: 830-835 [PMID: 20800026]
12 **Poon D**, Anderson BO, Chen LT, Tanaka K, Lau WY, Van Cutsem E, Singh H, Chow WC, Ooi LL, Chow P, Khin MW, Koo WH. Management of hepatocellular carcinoma in Asia: consensus statement from the Asian Oncology Summit 2009. *Lancet Oncol* 2009; **10**: 1111-1118 [PMID: 19880065 DOI: 10.1016/S1470-2045(09)70241-4]
13 **Bruix J**, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]
14 **Xu HX**, Lu MD, Xie XH, Xie XY, Kuang M, Xu ZF, Liu GJ, Wang Z, Chen LD, Lin MX. Treatment response evaluation with three-dimensional contrast-enhanced ultrasound for liver cancer after local therapies. *Eur J Radiol* 2010; **76**: 81-88 [PMID: 19500929 DOI: 10.1016/j.ejrad.2009.05.006]
15 **Xu HX**, Lu MD, Xie XH, Xie XY, Xu ZF, Chen LD, Liu GJ, Liang JY, Lin MX, Wang Z, Huang B. Three-dimensional contrast-enhanced ultrasound of the liver: experience of 92 cases. *Ultrasonics* 2009; **49**: 377-385 [PMID: 19041996 DOI: 10.1016/j.ultras.2008.10.009]
16 **Xu HX**. Era of diagnostic and interventional ultrasound. *World J Radiol* 2011; **3**: 141-146 [PMID: 21666820 DOI: 10.4329/wjr.v3.i5.141]
17 **Xu HX**. Contrast-enhanced ultrasound: The evolving applications. *World J Radiol* 2009; **1**: 15-24 [PMID: 21160717 DOI: 10.4329/wjr.v1.i1.15]
18 **Lu MD**, Yu XL, Li AH, Jiang TA, Chen MH, Zhao BZ, Zhou XD, Wang JR. Comparison of contrast enhanced ultrasound and contrast enhanced CT or MRI in monitoring percutaneous thermal ablation procedure in patients with hepatocellular carcinoma: a multi-center study in China. *Ultrasound Med Biol* 2007; **33**: 1736-1749 [PMID: 17629608 DOI: 10.1016/j.ultrasmedbio.2007.05.004]
19 **Ippolito D**, Bonaffini PA, Capraro C, Leni D, Corso R, Sironi S. Viable residual tumor tissue after radiofrequency ablation treatment in hepatocellular carcinoma: evaluation with CT perfusion. *Abdom Imaging* 2012; Epub ahead of print [PMID: 22743839 DOI: 10.1007/s00261-012-9924-0]
20 **Xu HX**, Lu MD, Liu LN, Zhang YF, Guo LH, Liu C, Wang S. Imaging Features of Intrahepatic Biliary Cystadenoma and Cystadenocarcinoma on B-Mode and Contrast-Enhanced Ultrasound. *Ultraschall Med* 2012; **33**: E241-E249 [PMID: 23154870 DOI: 10.1055/s-0031-1299276]
21 **Liu LN**, Xu HX, Lu MD, Xie XY, Wang WP, Hu B, Yan K, Ding H, Tang SS, Qian LX, Luo BM, Wen YL. Contrast-enhanced ultrasound in the diagnosis of gallbladder diseases: a multi-center experience. *PLoS One* 2012; **7**: e48371 [PMID: 23118996 DOI: 10.1371/journal.pone.0048371]
22 **Xu HX**, Lu MD, Liu LN, Zhang YF, Guo LH, Xu JM, Liu C. Discrimination between neoplastic and non-neoplastic lesions in cirrhotic liver using contrast-enhanced ultrasound. *Br J Radiol* 2012; **85**: 1376-1384 [PMID: 22553290 DOI: 10.1259/bjr/19932596]
23 **Xu HX**, Lu MD. The current status of contrast-enhanced ultrasound in China. *J Med Ultrason* 2010; **37**: 97-106 [DOI: 10.1007/s10396-010-0264-9]
24 **Xu ZF**, Xu HX, Xie XY, Liu GJ, Zheng YL, Lu MD. Renal cell carcinoma and renal angiomyolipoma: differential diagnosis with real-time contrast-enhanced ultrasonography. *J Ultrasound Med* 2010; **29**: 709-717 [PMID: 20427782]
25 **Xu ZF**, Xu HX, Xie XY, Liu GJ, Zheng YL, Liang JY, Lu MD. Renal cell carcinoma: real-time contrast-enhanced ultrasound findings. *Abdom Imaging* 2010; **35**: 750-756 [PMID: 19844755 DOI: .1007/s00261-009-9583-y]
26 **Wang Z**, Xu HX, Xie XY, Xie XH, Kuang M, Xu ZF, Liu GJ, Chen LD, Lin MX, Lu MD. Imaging features of hepatic angiomyolipomas on real-time contrast-enhanced ultrasound. *Br J Radiol* 2010; **83**: 411-418 [PMID: 19723766 DOI: 10.1259/bjr/81174247]
27 **Chen LD**, Xu HX, Xie XY, Xie XH, Xu ZF, Liu GJ, Wang Z, Lin MX, Lu MD. Intrahepatic cholangiocarcinoma and hepatocellular carcinoma: differential diagnosis with contrast-enhanced ultrasound. *Eur Radiol* 2010; **20**: 743-753 [PMID: 19760416 DOI: 10.1007/s00330-009-1599-8]
28 **Xie XH**, Xu HX, Xie XY, Lu MD, Kuang M, Xu ZF, Liu GJ, Wang Z, Liang JY, Chen LD, Lin MX. Differential diagnosis between benign and malignant gallbladder diseases with real-time contrast-enhanced ultrasound. *Eur Radiol* 2010; **20**: 239-248 [PMID: 19657645 DOI: 10.1007/s00330-009-1538-8]
29 **Lin MX**, Xu HX, Lu MD, Xie XY, Chen LD, Xu ZF, Liu GJ, Xie XH, Liang JY, Wang Z. Diagnostic performance of contrast-enhanced ultrasound for complex cystic focal liver lesions: blinded reader study. *Eur Radiol* 2009; **19**: 358-369 [PMID: 18795298 DOI: 10.1007/s00330-008-1166-8]
30 **Xu HX**, Xie XY, Lu MD, Liu GJ, Xu ZF, Zheng YL, Liang JY, Chen LD. Contrast-enhanced sonography in the diagnosis of small hepatocellular carcinoma < or = 2 cm. *J Clin Ultrasound* 2008; **36**: 257-266 [PMID: 18088056 DOI: 10.1002/jcu.20433]
31 **Xu HX**, Xie XY, Lu MD, Liu GJ, Xu ZF, Liang JY, Chen LD. Unusual benign focal liver lesions: findings on real-time contrast-enhanced sonography. *J Ultrasound Med* 2008; **27**: 243-254 [PMID: 18204015]
32 **Xu HX**, Liu GJ, Lu MD, Xie XY, Xu ZF, Zheng YL, Liang JY. Characterization of small focal liver lesions using real-time contrast-enhanced sonography: diagnostic performance analysis in 200 patients. *J Ultrasound Med* 2006; **25**: 349-361 [PMID: 16495496]
33 **Xu HX**, Liu GJ, Lu MD, Xie XY, Xu ZF, Zheng YL, Liang JY. Characterization of focal liver lesions using contrast-enhanced sonography with a low mechanical index mode

and a sulfur hexafluoride-filled microbubble contrast agent. *J Clin Ultrasound* 2006; **34**: 261-272 [PMID: 16788957 DOI: 10.1002/jcu.20234]

34 **Xu HX**, Lu MD, Liu GJ, Xie XY, Xu ZF, Zheng YL, Liang JY. Imaging of peripheral cholangiocarcinoma with low-mechanical index contrast-enhanced sonography and SonoVue: initial experience. *J Ultrasound Med* 2006; **25**: 23-33 [PMID: 16371552]

35 **Claudon M**, Dietrich CF, Choi BI, Cosgrove DO, Kudo M, Nolsøe CP, Piscaglia F, Wilson SR, Barr RG, Chammas MC, Chaubal NG, Chen MH, Clevert DA, Correas JM, Ding H, Forsberg F, Fowlkes JB, Gibson RN, Goldberg BB, Lassau N, Leen EL, Mattrey RF, Moriyasu F, Solbiati L, Weskott HP, Xu HX. Guidelines and Good Clinical Practice Recommendations for Contrast Enhanced Ultrasound (CEUS) in the Liver - Update 2012. *Ultraschall Med* 2013; **34**: 11-29 [PMID: 23129518 DOI: 10.1016/j.ultrasmedbio.2012.09.002]

36 **Wong GL**, Xu HX, Xie XY. Detection of focal liver lesions in cirrhotic liver using contrast-enhanced ultrasound. *World J Radiol* 2009; **1**: 25-36 [PMID: 21160718 DOI: 10.4329/wjr.v1.i1.25]

37 **Xu HX**. Contrast-enhanced ultrasound in the biliary system: Potential uses and indications. *World J Radiol* 2009; **1**: 37-44 [PMID: 21160719 DOI: 10.4329/wjr.v1.i1.37]

38 **Xu HX**, Chen LD, Liu LN, Zhang YF, Guo LH, Liu C. Contrast-enhanced ultrasound of intrahepatic cholangiocarcinoma: correlation with pathological examination. *Br J Radiol* 2012; **85**: 1029-1037 [PMID: 22374276 DOI: 10.1259/bjr/21653786]

39 **Frieser M**, Kiesel J, Lindner A, Bernatik T, Haensler JM, Janka R, Hahn EG, Strobel D. Efficacy of contrast-enhanced US versus CT or MRI for the therapeutic control of percutaneous radiofrequency ablation in the case of hepatic malignancies. *Ultraschall Med* 2011; **32**: 148-153 [PMID: 21225567 DOI: 10.1055/s-0029-1245934]

40 **Ricci P**, Cantisani V, Drudi F, Pagliara E, Bezzi M, Meloni F, Calliada F, Erturk SM, D'Andrea V, D'Ambrosio U, Passariello R. Is contrast-enhanced US alternative to spiral CT in the assessment of treatment outcome of radiofrequency ablation in hepatocellular carcinoma? *Ultraschall Med* 2009; **30**: 252-258 [PMID: 19280552 DOI: 10.1055/s-2008-1027727]

41 **Salvaggio G**, Campisi A, Lo Greco V, Cannella I, Meloni MF, Caruso G. Evaluation of posttreatment response of hepatocellular carcinoma: comparison of ultrasonography with second-generation ultrasound contrast agent and multidetector CT. *Abdom Imaging* 2010; **35**: 447-453 [PMID: 19562414 DOI: 10.1007/s00261-009-9551-6]

42 **Kim TK**, Lee KH, Khalili K, Jang HJ. Hepatocellular nodules in liver cirrhosis: contrast-enhanced ultrasound. *Abdom Imaging* 2011; **36**: 244-263 [PMID: 21253723 DOI: 10.1007/s00261-011-9686-0]

43 **Chen LD**, Xu HX, Xie XY, Lu MD, Xu ZF, Liu GJ, Liang JY, Lin MX. Enhancement patterns of intrahepatic cholangiocarcinoma: comparison between contrast-enhanced ultrasound and contrast-enhanced CT. *Br J Radiol* 2008; **81**: 881-889 [PMID: 18941048 DOI: 10.1259/bjr/22318475]

44 **Wiggermann P**, Zuber-Jerger I, Zausig Y, Loss M, Scherer MN, Schreyer AG, Stroszczynski C, Jung EM. Contrast-enhanced ultrasound improves real-time imaging of ablation region during radiofrequency ablation: preliminary results. *Clin Hemorheol Microcirc* 2011; **49**: 43-54 [PMID: 22214677 DOI: 10.3233/CH-2011-1456]

45 **Liu F**, Yu X, Liang P, Cheng Z, Han Z, Dong B. Contrast-enhanced ultrasound-guided microwave ablation for hepatocellular carcinoma inconspicuous on conventional ultrasound. *Int J Hyperthermia* 2011; **27**: 555-562 [PMID: 21797695 DOI: 10.3109/02656736.2011.564262]

46 **Gallotti A**, D'Onofrio M, Ruzzenente A, Martone E, De Robertis R, Guglielmi A, Pozzi Mucelli R. Contrast-enhanced ultrasonography (CEUS) immediately after percutaneous ablation of hepatocellular carcinoma. *Radiol Med* 2009; **114**: 1094-1105 [PMID: 19756947 DOI: 10.1007/s11547-009-0436-0]

47 **Chen MH**, Wu W, Yang W, Dai Y, Gao W, Yin SS, Yan K. The use of contrast-enhanced ultrasonography in the selection of patients with hepatocellular carcinoma for radio frequency ablation therapy. *J Ultrasound Med* 2007; **26**: 1055-1063 [PMID: 17646367]

48 **Chen MH**, Yang W, Yan K, Dai Y, Wu W, Fan ZH, Callstrom MR, Charboneau JW. The role of contrast-enhanced ultrasound in planning treatment protocols for hepatocellular carcinoma before radiofrequency ablation. *Clin Radiol* 2007; **62**: 752-760 [PMID: 17604763 DOI: 10.1016/j.crad.2006.12.013]

49 **Solbiati L**, Ierace T, Tonolini M, Cova L. Guidance and monitoring of radiofrequency liver tumor ablation with contrast-enhanced ultrasound. *Eur J Radiol* 2004; **51** Suppl: S19-S23 [PMID: 15311434]

50 **Quaia E**, Alaimo V, Baratella E, Medeot A, Midiri M, Cova MA. The added diagnostic value of 64-row multidetector CT combined with contrast-enhanced US in the evaluation of hepatocellular nodule vascularity: implications in the diagnosis of malignancy in patients with liver cirrhosis. *Eur Radiol* 2009; **19**: 651-663 [PMID: 18815790 DOI: 10.1007/s00330-008-1179-3]

51 **Xu HX**, Lu MD, Liu LN, Guo LH. Magnetic navigation in ultrasound-guided interventional radiology procedures. *Clin Radiol* 2012; **67**: 447-454 [PMID: 22153232 DOI: 10.1016/j.crad.2011.10.015]

P- Reviewers Koch TR, Adinolfi LE, Tischendorf J, Marin JJG
S- Editor Wen LL **L- Editor** Webster JR **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i6.802

World J Gastroenterol 2013 February 14; 19(6): 802-812
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

REVIEW

What is the role of adiponectin in obesity related non-alcoholic fatty liver disease?

Carmine Finelli, Giovanni Tarantino

Carmine Finelli, Giovanni Tarantino, Center of Obesity and Eating Disorder, Stella Maris Mediterraneum Foundation, 80035 Potenza, Italy
Carmine Finelli, Giovanni Tarantino, Department of Clinical and Experimental Medicine, Federico Ⅱ University Medical School of Naples, 80131 Naples, Italy
Author contributions: Both authors contributed equally to the design and draft of manuscript.
Correspondence to: Giovanni Tarantino, Professor, Department of Clinical and Experimental Medicine, Federico Ⅱ University Medical School of Naples, 80131 Naples, Italy. tarantin@unina.it
Telephone: +39-81-7462024 Fax: +39-81-5466152
Received: June 26, 2012 Revised: December 3, 2012
Accepted: December 15, 2012
Published online: February 14, 2013

Abstract

Non-alcoholic fatty liver disease (NAFLD) is recognized as the most common type of chronic liver disease in Western countries. Insulin resistance is a key factor in the pathogenesis of NAFLD, the latter being considered as the hepatic component of insulin resistance or obesity. Adiponectin is the most abundant adipose-specific adipokine. There is evidence that adiponectin decreases hepatic and systematic insulin resistance, and attenuates liver inflammation and fibrosis. Adiponectin generally predicts steatosis grade and the severity of NAFLD; however, to what extent this is a direct effect or related to the presence of more severe insulin resistance or obesity remains to be addressed. Although there is no proven pharmacotherapy for the treatment of NAFLD, recent therapeutic strategies have focused on the indirect upregulation of adiponectin through the administration of various therapeutic agents and/or lifestyle modifications. In this adiponectin-focused review, the pathogenetic role and the potential therapeutic benefits of adiponectin in NAFLD are analyzed systematically.

© 2013 Baishideng. All rights reserved.

Key words: Non-alcoholic fatty liver disease; Obesity; Adiponectin; Visceral fat; Adipokines

Finelli C, Tarantino G. What is the role of adiponectin in obesity related non-alcoholic fatty liver disease? *World J Gastroenterol* 2013; 19(6): 802-812 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i6/802.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i6.802

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is the most common type of chronic liver injury in many countries[1,2]. NAFLD includes a spectrum of syndromes, ranging from simple steatosis, non-alcoholic steatohepatitis (NASH) to fibrosis, cirrhosis and hepatocellular carcinoma[3]. The overall prevalence of NAFLD is 15%-40% in Western countries and 9%-40% in the Asian population[4]. NAFLD has dramatically increased over the past 15 years, mainly as a consequence of its close association with two major worldwide epidemics, obesity and type 2 diabetes mellitus (T2DM)[5]. Mortality in patients with NAFLD is significantly higher than in the age and gender-matched general population[6]. Disease progression to NASH and cirrhosis appears to be very slow, and only a few patients develop life-threatening advanced liver disease.

In many cases of NAFLD, the risks of developing metabolic and cardiovascular morbidities are much higher than the risks of developing hepatic diseases[7,8]. In fact, NAFLD is considered as the hepatic manifestation of the metabolic syndrome, which refers to a cluster of cardiovascular risk factors associated with insulin resistance, including central obesity, hypertension, dyslipidemia and T2DM[9]. The association between NAFLD and metabolic syndrome has been established in many cross-sectional and prospective studies[8]. NAFLD significantly increases the risk of diabetes and is a better predictor of the development of metabolic disorders than obesity

itself[10]. Some studies have reported an association of NAFLD with multiple classical and non-classical risk factors for cardiovascular diseases[7]. NAFLD predicts future cardiovascular events independently of other prognostic factors, including the component of metabolic syndrome. In summary, NAFLD is associated with a future high incidence of cardiovascular and metabolic complications and should be considered beyond a liver disease confined to classical boundaries. Understanding the disease and its management is a vital issue in current clinical practice.

PATHOGENESIS OF NAFLD

Although the pathogenesis of NAFLD remains largely unknown, insulin resistance, oxidative stress and inflammation play important roles in the development and progression of NAFLD[11,12]. Fatty liver itself is a status of insulin resistance. Hepatic fat accumulation can lead to hepatic insulin resistance, which may occur before the alterations in peripheral insulin actions and may induce peripheral insulin resistance[13,14]. Insulin regulates the uptake, oxidation and storage of fuel within insulin-sensitive tissues including the liver, skeletal muscle and fat. Peripheral insulin resistance impairs glucose uptake from blood into skeletal muscle and adipose tissue; serum non-esterified fatty acid (NEFA) levels may also be elevated because of the failure of insulin to suppress lipolysis[15,16]. In the liver, insulin resistance is associated with increased cellular contents of fatty acids and their metabolites (fatty acyl-CoAs, diacylglycerides and ceramides)[17-19]. Hyperinsulinemia caused by insulin resistance, in the presence of increased circulating levels of NEFA, enhances the hepatic uptake of fatty acid and promotes lipogenesis[1,20]. In addition, defects in mitochondrial β-oxidation, enhanced fatty acid synthesis and impaired secretion of triacylglyceride-rich very low density lipoproteins also contribute to hepatic steatosis[21-23]. A growing body of evidence from animal models suggests a "two-hit" hypothesis as being responsible for the development of NAFLD[24-26]. According to this theory, the first hit is the occurrence of fatty liver (steatosis), followed by a second event leading to the development of NASH. The potential secondary hits include endotoxin exposure, alcohol consumption and virus infections, which expand hepatic lipid stores, cause hepatocellular injury, and promote oxidative stress and inflammation in the liver. Lipotoxicity, and the release of cytokines and other pro-inflammatory mediators, play important roles during this process. Moreover, inflammation in the development of NASH can further impede insulin signaling[27]. Histologically, NASH is manifested by hepatocyte nuclear ballooning, hepatocyte apoptosis, Mallory's hyaline and inflammation foci[28]. NAFLD patients have a high circulating free fatty acids (FFAs) level that correlates with the severity of liver disease. Overloaded FFAs may exhibit lipotoxicity by inducing the expression of proinflammatory cytokines, such as tumor necrosis factor alpha (TNF-α)[29].

VISCERAL OBESITY, ADIPOKINES AND NAFLD

Obesity, especially visceral obesity, is frequently associated with NAFLD and their coexistence in the same individual increases the likelihood of having more advanced forms of liver disease[30,31]. NAFLD occurs in 60%-95% of people with obesity[32]. Visceral fat is a key mediator of NASH and is strongly associated with alanine aminotransferase (ALT) levels in the nondiabetic obese population[31,33,34]. The importance of visceral fat in the pathogenesis of NAFLD has also been shown in many animal models, including *fa/fa* obese rats. In these animals, surgical resection of intra abdominal fat depots reverses hepatic insulin resistance and steatosis[35].

Recent evidence suggests that visceral adipose tissue is a metabolic and inflammatory organ that signals and modulates the action and metabolism of the brain, liver, muscle and cardiovascular system[36,37]. The imbalanced production of pro- and anti-inflammatory adipokines secreted from fat contributes to the pathogenesis of NAFLD[38]. Modulation of endocrine/immune/inflammatory interactions of adipose tissue may provide novel therapeutic (pharmacological) targets for the treatment of NAFLD. For example, in patients with severe lipodystrophy, injection with leptin reverses nonalcoholic fatty liver diseases[39,40]. However, in cases of NAFLD associated with obesity, serum levels of leptin are increased, and the liver becomes refractory to the "anti-steatotic" effects of leptin[41-43]. Leptin infusion is therefore unlikely to be of therapeutic value for patients with NAFLD. TNF-α, a pro-inflammatory adipokine that interferes with insulin signaling and favors steatosis, may play a casual role in the pathogenesis of NASH[38]. Circulating levels of TNF-α and hepatic expression of its type 1 receptor are increased in NASH, but could not discriminate steatohepatitis from steatosis[44-46]. Neutralization of TNF-α activity improves fatty liver disease in animals[47]. Conversely, nutritional steatohepatitis can still be produced experimentally in both TNF-α and TNF-α type 1 receptor knockout mice, suggesting that this adipokine might not be an essential mediator of NAFLD[48,49]. In contrast to leptin and TNF-α, adiponectin is more closely implicated in the pathogenesis of NAFLD/NASH. Unlike other adipokines, serum levels of adiponectin are decreased in obesity and its associated medical complications[50]. A negative association between serum levels of adiponectin and liver enzyme levels has been shown in healthy subjects[51]. Numerous epidemiological investigations in diverse ethnic groups have identified lower adiponectin level as an independent risk factor for NAFLDs and liver dysfunctions[37]. Compared with healthy controls, adiponectin levels are lower by more than 50% in NASH patients[52]. Adiponectin expression is decreased by 20%-40% during the development of NAFLD, from simple steatosis to NASH[52,53]. Moreover, NASH patients with lower levels of adiponectin show higher grades of inflammation, suggesting that adiponectin deficiency is an important risk factor

for the development of fatty liver, steatohepatitis and other forms of liver injuries[52-55]. In patients with T2DM, plasma adiponectin concentrations are inversely related to hepatic fat content[56]. There is a direct relationship between hypoadiponectinemia and NASH, independent of insulin resistance[52]. Animal-based studies have demonstrated that adiponectin possesses potent protective activities against various forms of liver injury, including those induced by carbon tetrachloride, lipopolysaccharide (LPS)/*D*-galactosamine, pharmacological compounds, bile duct ligations and methionine-deficient diet[57-61]. In animal models of both alcoholic and nonalcoholic steatohepatitis, exogenous adiponectin reduces hepatomegaly, depletes lipid accumulation, quenches hepatic inflammation and decreases hepatic expression and plasma concentrations of TNF-α[62]. Adiponectin knockout mice exhibit an enhanced pattern of hepatic fibrosis induced by carbon tetrachloride[58]. The lack of adiponectin expression could accelerate hepatic tumor formation in a NASH model in mice[63]. Among the known adipokines, adiponectin stands out for its insulin-sensitizing and anti-inflammatory roles, and may be used as a promising drug candidate for the treatment of liver diseases.

HEPATOPROTECTIVE FUNCTIONS OF ADIPONECTIN: STRUCTURAL BASIS AND SIGNALLING MECHANISMS

Four independent groups originally identified adiponectin, also termed Acrp30, AdipoQ, apM1 or GBP28, in both mice and humans[64-67]. This adipokine has attracted much attention because of its multiple beneficial effects on a cluster of obesity-related metabolic and cardiovascular dysfunctions. Hypoadiponectinemia is a key etiological factor contributing to almost all the major pathological conditions associated with obesity[68]. The physiological functions and clinical relevance of adiponectin in obesity-related medical complications have been extensively reviewed elsewhere[50,69-72]. In the following sections, we will discuss recent advances on the structural regulations of adiponectin as well as the molecular evidence supporting the role of adiponectin as a major protective agent against obesity-related NAFLD.

Polymorphism of the multimeric structures of adiponectin

A unique feature of the structure of adiponectin is its ability to assemble into several characteristic oligomeric isoforms, including trimers [low molecular weight (LMW)], hexamers [middle molecular weight (MMW)] and the oligomeric complexes comprising 18 protomers or above [high molecular weight (HMW)][73]. Adiponectin presents predominantly in the circulation as these three oligomeric complexes[74-79]. Trimeric adiponectin is the basic building block of adiponectin. The subunits in the trimer are associated *via* hydrophobic interactions. Two LMW adiponectin molecules linked by disulfide bonds form hexameric adiponectin. The structural properties of the HMW adiponectin remain poorly characterized because of the heterogeneous nature of this isoform. Analysis of adiponectin oligomers by non-denaturing and non-heating gel electrophoresis shows that the human HMW adiponectin composes of a mixture of 18-30 mers, or even larger molecular weight species[73,78,80,81]. Dynamic light scattering and transmission electron microscopy shows that the bovine HMW adiponectin forms a bouquet-like architecture resembling that of complement C1q[82]. Six globular objects can be seen atop a thin stalk, which presumably correspond to the six LMW adiponectins. The stalks bunch together in a manner that is consistent with the requirement for NH2-terminal disulfide bonding. The side views of HMW adiponectin suggest a conical structure of the oligomer with the COOH-terminal portion forming the base. Interestingly, these globular domains are arranged in a tight ring. This circular arrangement might enable polyvalent interactions of the globular domains with a single receptor. Recently, the HMW oligomeric structures formed by multiples of adiponectin trimers have been determined by single-particle analysis of electron micrographs[83]. Pleiomorphic ensembles of collagen-like stretches of the trimers lead to a highly dynamic structure of HMW adiponectin, which can be classified into two major classes: the fan-shaped (Class Ⅰ) and bouquet-shaped (Class Ⅱ). In both of these conformations, the globular domains assume a variety of arrangements, covering an area of up to 4.9 × 105 Å^2 and up to 320 Å apart. The conformational flexibility of the HMW oligomer can allow it to access and cluster disparate target ligands or receptors, which may be necessary to activate cellular signaling leading to the remarkable functional diversity of adiponectin.

HMW adiponectin as a major bioactive form in liver

Obese individuals have different distribution of adiponectin oligomers compared with lean controls. Relatively lower content of HMW adiponectin is closely associated with obesity-related metabolic complications[81]. The increases in the ratio of HMW *vs* total adiponectin, but not total adiponectin level *per se*, correlate well with improved insulin sensitivity during treatment with the insulin-sensitizing drug thiazolidinediones, in both diabetic mice and patients with T2DM. On the other hand, weight reduction by either calorie restriction or gastric bypass surgery results in a selective elevation of the HMW adiponectin, but not the trimeric and hexameric complexes[84-86]. An independent inverse association exists between ALT and HMW adiponectin[87]. Taken together, these epidemiological and genetic data suggest that the beneficial effects of adiponectin in humans might be mediated primarily by its HMW isoform, and the deficiency of this oligomer is an important etiological factor that links obesity with its medical complications.

Evidence from both *in vitro* and animal-based studies also supports the role of the HMW oligomer as the major active form in mediating the multiple actions of adiponectin in liver tissue. Recombinant adiponectin pro-

duced from mammalian cells, which can form the HMW oligomers, potently decreases hyperglycemia in diabetic mice through inhibition of hepatic glucose production[88]. However, bacterially generated full-length adiponectin, which lacks the capacity to form the HMW adiponectin, is almost inactive. Intravenous injection of the HMW adiponectin, but not the hexameric adiponectin, leads to a dose-dependent decrease in serum glucose levels[81]. The formation of the HMW oligomers is obligatory to mediate the insulin sensitizing effects of adiponectin on suppression of hepatic gluconeogenesis in primary rat hepatocytes[80]. Acute injection of recombinant adiponectin enriched with the HMW oligomers results in a marked activation of AMP-activated kinase (AMPK) in the liver, while chronic infusion with this protein leads to prolonged alleviation of hyperglycemia and insulin resistance in *db/db* diabetic mice[89]. This animal-based evidence is consistent with the clinical observations showing that the ratio of HMW/total adiponectin correlates closely with hepatic insulin sensitivity[81]. The role of the HMW oligomer as a predominant active form of adiponectin mediating its hepatic actions is also supported by two recent independent reports demonstrating that the insulin-sensitizing effects of the peroxisome proliferator-activated receptor gamma (PPAR-γ) agonists thiazolidinediones were diminished in *ob/ob* obese mice with the targeted mutation of the adiponectin gene[90,91]. Notably, treatment with thiazolidinediones causes a selective elevation of the HMW oligomeric adiponectin[79,81]. In addition to the hepatic insulin-sensitizing activity, the HMW adiponectin has also been suggested to be the most potent isoform for alleviation of fatty liver disease in high fat diet-induced obese mice[92], and inhibition of apolipoprotein B and E release from human hepatocytes[93]. HMW adiponectin dose-dependently suppressed growth factor-induced hepatic stellate cell proliferation[94]. Taken together, these data suggest that the HMW form predominantly mediates the beneficial effects of adiponectin in hepatic tissue.

Receptors and postreceptor signaling pathways mediating the hepato-protective functions of adiponectin

Two adiponectin receptors (adipoR1 and adipoR2) have been identified and found to be expressed in various tissues[95]. AdipoR1 is abundantly expressed in skeletal muscles, whereas adipoR2 is present predominantly in the liver, suggesting a role of adipoR2 in hepatic adiponectin signaling[68,96]. Recently, several laboratories have investigated the physiological roles of adipoR1 and adipoR2 in *adipoR1/2* knockout mice. Both *adipoR1* and *adipoR2* knockout mice exhibit mild insulin resistance[97]. In *adipoR1/R2* double knockout mice, the binding and actions of adiponectin are abolished, resulting in increased tissue triglyceride content, inflammation and oxidative stress[97]. *AdipoR2* knockout mice reported by Liu *et al*[98] displayed reduced diet-induced insulin resistance, but promoted T2DM. These data support the physiological roles of adipoR1 and adipoR2 as the predominant receptors for adiponectin in the regulation of glucose and lipid metabolism. Despite this information, the detailed roles and expression of adipoRs in NAFLD are not conclusive[38,99-102].

Adiponectin stimulates AMPK in almost all its major target tissues, including skeletal muscle, liver, heart, endothelium, adipocytes and brain[75,89,103-106]. Notably, most biological effects of adiponectin in these target tissues are abrogated by expression of a dominant negative version of AMPK, supporting its obligatory role in mediating adiponectin's multiple actions. The precise mechanisms whereby adiponectin activates AMPK through its receptors remain to be determined. APPL1, an adaptor protein containing a pleckstrin homology domain, a phosphotyrosine binding domain and a leucine zipper motif, appears to be a key signaling molecule that couples adiponectin receptors and its downstream AMPK activation[103,107]. Adiponectin enhances the binding of APPL1 to both adipoR1 and adipoR2, and these interactions are essential for subsequent phosphorylation and activation of AMPK. Studies also indicate the important role of APPL1 in the metabolic syndrome[108,109]. AMPK activation in turn phosphorylates acetyl Coenzyme A carboxylase (ACC) and attenuates ACC activity. Inhibition of ACC reduces lipid synthesis and enhances fatty acid oxidation by blocking the production of malonyl-CoA, an allosteric inhibitor of carnitine palmitoyl transferase 1, the rate-limiting enzyme in fatty acid oxidation. In addition, activation of AMPK downregulates the expression of sterol regulatory element-binding protein 1c (SREBP1c), a transcription factor that regulates cholesterol and lipid synthesis. Reduction of SREBP1c results in downregulation of genes involved in lipogenesis, including ACC, fatty acid synthase, and glycerol-3-phosphate acyltransferase[104,110,111].

PPARα is a transcription factor controlling the transcription of a panel of genes encoding fatty acid oxidation enzymes, such as FATP, acyl-CoA oxidase and long chain acyl-CoA synthetase. Adiponectin stimulates PPARα activity possibly through PPARγ coactivator-1α[112]. These adiponectin-mediated signaling pathways lead to enhanced fat oxidation, reduced lipid synthesis and prevention of hepatic steatosis (Figure 1).

Cellular mechanisms contributing to the anti-inflammatory activities of adiponectin in NAFLD

Inflammatory cytokines are key mediators of hepatic inflammation, cell death, and fibrosis, as well as regeneration after massive or focal liver injury[38,113]. Adiponectin levels are negatively associated with mediators of inflammation, including interleukin-6 (IL-6) and C-reactive protein; but positively related to anti-inflammatory cytokine IL-10[114,115]. It suppresses TNF-α functions by inhibiting its expression and antagonizing its activities[61,62,116,117]. In the liver, cytokines such as IL-6 and TNF-α, are mainly produced from Kupffer cells and hepatic stellate cells (HSC), and partly from inflamed hepatocytes[52,118,119]. Adiponectin ameliorates NASH and liver fibrosis by suppressing the activation of Kupffer

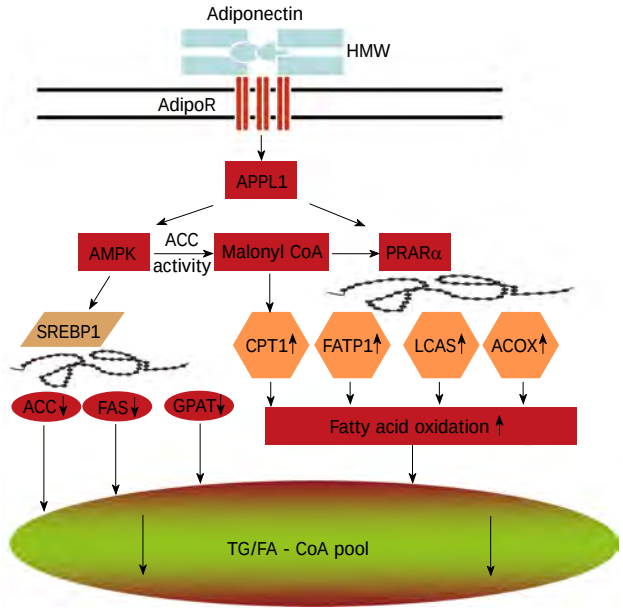


Figure 1 Summary of multiple signaling pathways that mediate the anti-steatotic effects of adiponectin. HMW: High molecular weight; AdipoR: Adiponectin receptor; APPL1: Adaptor protein, phosphotyrosine interaction, PH domain and leucine zipper containing 1; AMPK: AMP-activated kinase; ACC: Acetyl Coenzyme A carboxylase; CPT1: Carnitine palmitoyl transferase 1; SREBP1: Sterol regulatory element-binding protein 1; FAS: Fatty acid synthase; GPAT: Glycerol-3-phosphate acyltransferase; ACOX: Acyl-CoA oxidase; LCAS: Long chain acyl-CoA synthetase; FATP: Fatty acid transport protein; PPARα: Peroxisome proliferator-activated receptor α; TG: Triacylglyceride; FA: Fatty acid.

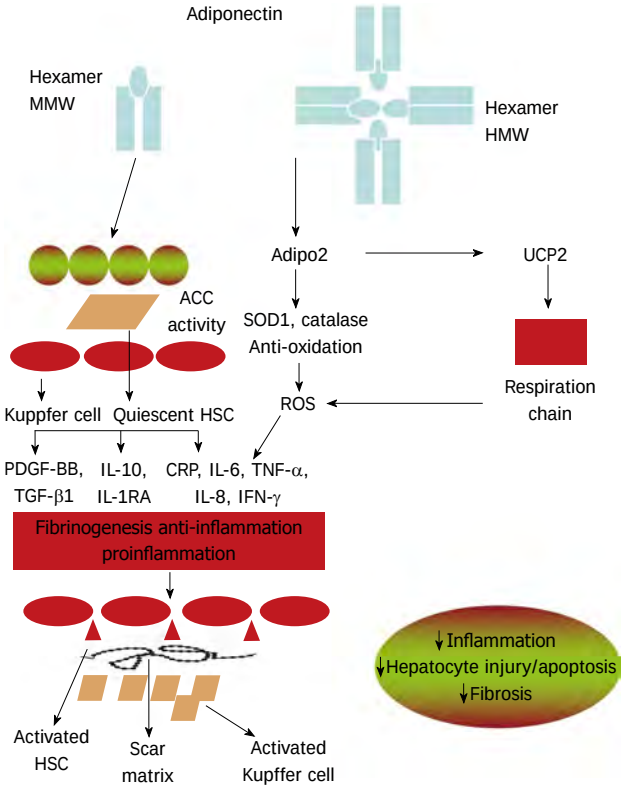


Figure 2 Summary of multiple pathways underlying the protective effects of adiponectin against liver injury. MMW: Middle molecular weight; HMW: High molecular weight; AdipoR: Adiponectin receptor; UCP: Uncoupling protein; SOD1: Superoxide dismutase 1; ROS: Reactive oxygen species; PDGF-BB: Platelet-derived growth factor BB; TGF-β1: Transforming growth factor-β1; CRP: C-reactive protein; IL: Interleukin; IL-1RA: Interleukin-1-receptor antagonist; TNF-α: Tumor necrosis factor-α; HSC: Hepatic stellate cells; IFN-γ: Interferon-γ.

cells and HSC (Figure 2). In porcine blood-derived macrophages, adiponectin suppresses both TNF-α and IL-6 production stimulated by LPS and induces IL10 expression. The attenuation of proinflammatory cytokine production by adiponectin is mediated in part by attenuating the translocation of nuclear factor kappa B (NF-κB) to the nucleus[120]. Adiponectin can also induce the expression of the anti-inflammation cytokine interleukin-1-receptor antagonist[121,122]. The anti-inflammatory effects of adiponectin in macrophages may involve the toll-like receptor-4 (TLR-4) signaling pathway. However, the mechanisms by which adiponectin suppresses TLR-4 mediated responses are not well understood[123].

The transformation of HSC into myofibroblasts is the key step that initiates the fibrotic process during liver injury[124,125]. The activated hepatic stellate cells increase the accumulation of extracellular matrix. Both adiponectin receptors, adipoR1 and adipoR2, are expressed in HSC. Adiponectin treatment maintains HSC quiescence, inhibits platelet-derived growth factor-stimulated proliferation and migration of human HSCs, and reduces the secretion and of monocyte chemoattractant protein-1 through AMPK-dependent mechanisms[94,125,126]. Additionally, adiponectin also regulates hepatic expression of TGFβ1, a pro-fibrotic factor involved in HSC activation[58,127] that plays an important role in neofibrogenesis of NAFLD[128].

Inhibition of adipoR2 expression by short hairpin RNAi-expressing adenovirus can induce TGFβ1 expression, and overexpression of adipoR2 diminishes TGFβ1 mRNA level.

Regulatory role of adiponectin on mitochondria activities

Mitochondrial dysfunction represents a central mechanism linking obesity with associated metabolic complications[129]. In patients with NASH, the hepatic mitochondria exhibit ultrastructural lesions and decreased activity of the respiratory chain complexes[130,131]. In this condition, the decreased activity of the respiratory chain results in accumulation of reactive oxygen species (ROS) that oxidize fat deposits to form lipid peroxidation products, which in turn, cause steatohepatitis, necrosis, inflammation and fibrosis. The increased mitochondrial ROS formation in steatohepatitis could directly damage mitochondria DNA and respiratory chain polypeptides, induce NF-κB activation and the hepatic synthesis of TNFα[132]. Oxidative phosphorylation reactions mediated by mitochondria respiratory chain (MRC) complexes are directly involved in regulating intracellular ROS activities and preventing accumulation of lipids and lipid peroxidation products in the liver.

Mice without adiponectin show an increased lipid

accumulation even under normal chow feeding[117]. This pre-existing hepatic steatotic condition might be the direct consequence of dysregulated mitochondria functions[117]. Adiponectin treatment restores the MRC activities, decreases the levels of mitochondrial lipid peroxidation products through regulating hepatic mitochondrial functions, which might represent a common mechanism underlying the multiple beneficial activities of this hormone in various obesity-related pathologies. Moreover, we have provided evidence supporting an essential role of uncoupling protein 2 (UCP2), a mitochondria inner membrane transporter, in mediating the beneficial effects of adiponectin on MRC activities. The protein and mRNA levels of UCP2 are decreased in the liver tissues of adiponectin knockout mice and can be significantly upregulated by adiponectin treatment. Overexpression of adipoR2 upregulates mRNA levels of UCP2, catalase, and superoxide dismutase 1 in the liver[97]. Furthermore, the effects of adiponectin on MRC activities are dramatically attenuated in *Ucp2*-deficient mice, suggesting that the increased UCP2 expression might be obligatory for adiponectin to elicit its activities on mitochondria functions (Figure 2). UCP2 possesses anti-oxidant activities through inhibition of ROS production from mitochondria[133]. It can also inhibit the production of pro-inflammatory cytokines in both macrophage and Kupffer cells[134]. A growing body of evidence suggests that UCP2 may play a beneficial role in various stages of fatty liver diseases[134,135]. These results suggest the existence of a reciprocal relationship between uncoupling proteins and adiponectin. However, the detailed signaling mechanisms underlying adiponectin-induced UCP2 expression are not clear and warrant further investigation.

ELEVATION OF ADIPONECTIN PRODUCTION AS A THERAPEUTIC STRATEGY FOR TREATMENT OF NAFLD

To date, there have been very few effective drug treatments for NAFLD and NASH. Early diagnosis and management of the underlying condition remains the mainstay of treatment. The present "gold standard" for treatment of NAFLD is weight reduction or a reduction of central obesity[4]. These "life-style adjustment" or anti-obesity measures (including bariatric surgery) impressively reduce liver cell injury, inflammation and hepatic fibrosis, as well as steatosis[136,137]. The potential for correcting steatosis by dietary or pharmacological approaches should provide a sound therapeutic approach for the treatment of steatosis and steatohepatitis. Strategies to block oxidative stress are of great interest, with some evidence that ALT normalization or histological improvement occurs with vitamin E (alone or with vitamin C or pioglitazone) and betaine[138].

Adiponectin and its agonists might represent emerging therapeutic agents for the treatment and/or prevention of liver dysfunctions[139-141]. Adiponectin replacement therapy is not yet available as a treatment option. Pharmacological intervention aimed at elevating adiponectin production might hold promise for the treatment and/or prevention of NAFLD.

CONCLUDING REMARKS

Based on our data, polymorphic UCP1 (AG + GG) obese patients with low adiponectin levels appear to be high-risk subjects for worsening of liver steatosis, an NAFLD, possibly requiring a second-step evaluation by liver biopsy[142].

The role of adiponectin in systemic inflammation and critical illness is not well defined. Early data suggest that plasma levels of adiponectin are decreased in critical illness[143]. Whether this is a result of the disease process itself or whether patients with lower levels of this hormone are more susceptible to developing a critical illness is not known. This observation of lower adiponectin levels then raises the possibility of therapeutic options to increase circulating adiponectin levels[143]. The various options for modulation of serum adiponectin (recombinant adiponectin, thiazolidinediones) are discussed.

Nevertheless, adiponectin-based therapeutics for NAFLD represent a promising area for further investigation.

CONCLUSION

Adiponectin is an abundant adipocyte-derived hormone with well established anti-inflammatory and insulin sensitizing properties. The significance of adiponectin in protecting obesity-related NAFLD has been increasingly recognized. Despite the advances made in recent years, the detailed molecular and cellular mechanisms underlying its hepato-protective functions remain largely uncharacterized.

REFERENCES

1 **Tarantino G**, Saldalamacchia G, Conca P, Arena A. Non-alcoholic fatty liver disease: further expression of the metabolic syndrome. *J Gastroenterol Hepatol* 2007; **22**: 293-303 [PMID: 17295757 DOI: 10.1111/j.1440-1746.2007.04824.x]

2 **Fan JG**, Saibara T, Chitturi S, Kim BI, Sung JJ, Chutaputti A. What are the risk factors and settings for non-alcoholic fatty liver disease in Asia-Pacific? *J Gastroenterol Hepatol* 2007; **22**: 794-800 [PMID: 17498218 DOI: 10.1111/j.1440-1746.2007.04952.x]

3 **Tajiri K**, Shimizu Y. Role of NKT Cells in the Pathogenesis of NAFLD. *Int J Hepatol* 2012; **2012**: 850836 [PMID: 22577564]

4 **Farrell GC**, Larter CZ. Nonalcoholic fatty liver disease: from steatosis to cirrhosis. *Hepatology* 2006; **43**: S99-S112 [PMID: 16447287 DOI: 10.1002/hep.20973]

5 **Hamaguchi M**, Takeda N, Kojima T, Ohbora A, Kato T, Sarui H, Fukui M, Nagata C, Takeda J. Identification of individuals with non-alcoholic fatty liver disease by the diagnostic criteria for the metabolic syndrome. *World J Gastroenterol* 2012; **18**: 1508-1516 [PMID: 22509083 DOI: 10.3748/wjg.v18.i13.1508]

6 **Lazo M**, Hernaez R, Bonekamp S, Kamel IR, Brancati FL, Guallar E, Clark JM. Non-alcoholic fatty liver disease and mortality among US adults: prospective cohort study. *BMJ* 2011; **343**: d6891 [PMID: 22102439 DOI: 10.1136/bmj.d6891]

7 **Edens MA**, Kuipers F, Stolk RP. Non-alcoholic fatty liver disease is associated with cardiovascular disease risk markers. *Obes Rev* 2009; **10**: 412-419 [PMID: 19413701 DOI: 10.1111/j.1467-789X.2009.00594.x]

8 **Watanabe S**, Yaginuma R, Ikejima K, Miyazaki A. Liver diseases and metabolic syndrome. *J Gastroenterol* 2008; **43**: 509-518 [PMID: 18648737 DOI: 10.1007/s00535-008-2193-6]

9 **Tsochatzis EA**, Papatheodoridis GV. Is there any progress in the treatment of non-alcoholic fatty liver disease? *World J Gastrointest Pharmacol Ther* 2011; **2**: 1-5 [PMID: 21577310 DOI: 10.4292/wjgpt.v2.i1.1]

10 **Lee J**, Chung DS, Kang JH, Yu BY. Comparison of visceral fat and liver fat as risk factors of metabolic syndrome. *J Korean Med Sci* 2012; **27**: 184-189 [PMID: 22323866 DOI: 10.3346/jkms.2012.27.2.184]

11 **Obika M**, Noguchi H. Diagnosis and evaluation of nonalcoholic fatty liver disease. *Exp Diabetes Res* 2012; **2012**: 145754 [PMID: 22110476]

12 **Tarantino G**, Colao A, Capone D, Conca P, Tarantino M, Grimaldi E, Chianese D, Finelli C, Contaldo F, Scopacasa F, Savastano S. Circulating levels of cytochrome C, gamma-glutamyl transferase, triglycerides and unconjugated bilirubin in overweight/obese patients with non-alcoholic fatty liver disease. *J Biol Regul Homeost Agents* 2011; **25**: 47-56 [PMID: 21382273]

13 **Tarantino G**, Finelli C, Colao A, Capone D, Tarantino M, Grimaldi E, Chianese D, Gioia S, Pasanisi F, Contaldo F, Scopacasa F, Savastano S. Are hepatic steatosis and carotid intima media thickness associated in obese patients with normal or slightly elevated gamma-glutamyl-transferase? *J Transl Med* 2012; **10**: 50 [PMID: 22424154 DOI: 10.1186/1479-5876-10-50]

14 **Thorn SR**, Rozance PJ, Brown LD, Hay WW. The intrauterine growth restriction phenotype: fetal adaptations and potential implications for later life insulin resistance and diabetes. *Semin Reprod Med* 2011; **29**: 225-236 [PMID: 21710398 DOI: 10.1055/s-0031-1275516]

15 **Houmard JA**. Intramuscular lipid oxidation and obesity. *Am J Physiol Regul Integr Comp Physiol* 2008; **294**: R1111-R1116 [PMID: 18256136 DOI: 10.1152/ajpregu.00396.2007]

16 **Joseph AM**, Joanisse DR, Baillot RG, Hood DA. Mitochondrial dysregulation in the pathogenesis of diabetes: potential for mitochondrial biogenesis-mediated interventions. *Exp Diabetes Res* 2012; **2012**: 642038 [PMID: 22203837]

17 **Rocha PM**, Barata JT, Minderico CS, Silva AM, Teixeira PJ, Sardinha LB. Visceral abdominal and subfascial femoral adipose tissue have opposite associations with liver fat in overweight and obese premenopausal caucasian women. *J Lipids* 2011; **2011**: 154672 [PMID: 21961071]

18 **Longato L**, Ripp K, Setshedi M, Dostalek M, Akhlaghi F, Branda M, Wands JR, de la Monte SM. Insulin resistance, ceramide accumulation, and endoplasmic reticulum stress in human chronic alcohol-related liver disease. *Oxid Med Cell Longev* 2012; **2012**: 479348 [PMID: 22577490]

19 **Yoshimura E**, Kumahara H, Tobina T, Matono S, Kiyonaga A, Kimura M, Tsukikawa H, Kono S, Etou T, Irie S, Anzai K, Tanaka H. Relationships between fat deposition in the liver and skeletal muscle and insulin sensitivity in Japanese individuals: a pilot study. *Diabetes Metab Syndr Obes* 2011; **4**: 35-43 [PMID: 21448320 DOI: 10.2147/DMSO.S16175]

20 **Gathercole LL**, Morgan SA, Bujalska IJ, Hauton D, Stewart PM, Tomlinson JW. Regulation of lipogenesis by glucocorticoids and insulin in human adipose tissue. *PLoS One* 2011; **6**: e26223 [PMID: 22022575 DOI: 10.1371/journal.pone.0026223]

21 **Choi SH**, Ginsberg HN. Increased very low density lipoprotein (VLDL) secretion, hepatic steatosis, and insulin resistance. *Trends Endocrinol Metab* 2011; **22**: 353-363 [PMID: 21616678 DOI: 10.1016/j.tem.2011.04.007]

22 **Capeau J**. Insulin resistance and steatosis in humans. *Diabetes Metab* 2008; **34**: 649-657 [PMID: 19195626 DOI: 10.1016/S1262-3636(08)74600-7]

23 **Nagarajan P**, Mahesh Kumar MJ, Venkatesan R, Majundar SS, Juyal RC. Genetically modified mouse models for the study of nonalcoholic fatty liver disease. *World J Gastroenterol* 2012; **18**: 1141-1153 [PMID: 22468076 DOI: 10.3748/wjg.v18.i11.1141]

24 **Nagata K**, Suzuki H, Sakaguchi S. Common pathogenic mechanism in development progression of liver injury caused by non-alcoholic or alcoholic steatohepatitis. *J Toxicol Sci* 2007; **32**: 453-468 [PMID: 18198478 DOI: 10.2131/jts.32.453]

25 **Mirza MS**. Obesity, Visceral Fat, and NAFLD: Querying the Role of Adipokines in the Progression of Nonalcoholic Fatty Liver Disease. *ISRN Gastroenterol* 2011; **2011**: 592404 [PMID: 21991518]

26 **Charlton M**. Noninvasive indices of fibrosis in NAFLD: starting to think about a three-hit (at least) phenomenon. *Am J Gastroenterol* 2007; **102**: 409-411 [PMID: 17311653 DOI: 10.1111/j.1572-0241.2006.01039.x]

27 **de Luca C**, Olefsky JM. Inflammation and insulin resistance. *FEBS Lett* 2008; **582**: 97-105 [PMID: 18053812 DOI: 10.1016/j.febslet.2007.11.057]

28 **Liu Q**, Bengmark S, Qu S. The role of hepatic fat accumulation in pathogenesis of non-alcoholic fatty liver disease (NAFLD). *Lipids Health Dis* 2010; **9**: 42 [PMID: 20426802 DOI: 10.1186/1476-511X-9-42]

29 **Polyzos SA**, Kountouras J, Zavos C. Nonalcoholic fatty liver disease: the pathogenetic roles of insulin resistance and adipocytokines. *Curr Mol Med* 2009; **9**: 299-314 [PMID: 19355912 DOI: 10.2174/156652409787847191]

30 **van der Poorten D**, Milner KL, Hui J, Hodge A, Trenell MI, Kench JG, London R, Peduto T, Chisholm DJ, George J. Visceral fat: a key mediator of steatohepatitis in metabolic liver disease. *Hepatology* 2008; **48**: 449-457 [PMID: 18627003 DOI: 10.1002/hep.22350]

31 **El-Koofy NM**, Anwar GM, El-Raziky MS, El-Hennawy AM, El-Mougy FM, El-Karaksy HM, Hassanin FM, Helmy HM. The association of metabolic syndrome, insulin resistance and non-alcoholic fatty liver disease in overweight/obese children. *Saudi J Gastroenterol* 2012; **18**: 44-49 [PMID: 22249092 DOI: 10.4103/1319-3767.91738]

32 **Jang S**, Lee CH, Choi KM, Lee J, Choi JW, Kim KA, Park CM. Correlation of fatty liver and abdominal fat distribution using a simple fat computed tomography protocol. *World J Gastroenterol* 2011; **17**: 3335-3341 [PMID: 21876622 DOI: 10.3748/wjg.v17.i28.3335]

33 **Verrijken A**, Francque S, Mertens I, Talloen M, Peiffer F, Van Gaal L. Visceral adipose tissue and inflammation correlate with elevated liver tests in a cohort of overweight and obese patients. *Int J Obes* (Lond) 2010; **34**: 899-907 [PMID: 20142825 DOI: 10.1038/ijo.2010.4]

34 **Koda M**, Kawakami M, Murawaki Y, Senda M. The impact of visceral fat in nonalcoholic fatty liver disease: cross-sectional and longitudinal studies. *J Gastroenterol* 2007; **42**: 897-903 [PMID: 18008034 DOI: 10.1007/s00535-007-2107-z]

35 **Foster MT**, Shi H, Seeley RJ, Woods SC. Transplantation or removal of intra-abdominal adipose tissue prevents age-induced glucose insensitivity. *Physiol Behav* 2010; **101**: 282-288 [PMID: 20570685 DOI: 10.1016/j.physbeh.2010.05.014]

36 **Antuna-Puente B**, Feve B, Fellahi S, Bastard JP. Adipokines: the missing link between insulin resistance and obesity. *Diabetes Metab* 2008; **34**: 2-11 [PMID: 18093861 DOI: 10.1016/j.diabet.2007.09.004]

37 **Matsuzawa Y**. Adiponectin: a key player in obesity related disorders. *Curr Pharm Des* 2010; **16**: 1896-1901 [PMID: 20370675 DOI: 10.2174/138161210791208893]

38 **Tarantino G**, Savastano S, Colao A. Hepatic steatosis, low-grade chronic inflammation and hormone/growth factor/adipokine imbalance. *World J Gastroenterol* 2010; **16**: 4773-4783 [PMID: 20939105 DOI: 10.3748/wjg.v16.i38.4773]

39 **Fiorenza CG**, Chou SH, Mantzoros CS. Lipodystrophy: pathophysiology and advances in treatment. *Nat Rev Endocrinol* 2011; **7**: 137-150 [PMID: 21079616 DOI: 10.1038/nrendo.2010.199]

40 **Chong AY**, Lupsa BC, Cochran EK, Gorden P. Efficacy of leptin therapy in the different forms of human lipodystrophy. *Diabetologia* 2010; **53**: 27-35 [PMID: 19727665 DOI: 10.1007/s00125-009-1502-9]

41 **Huang XD**, Fan Y, Zhang H, Wang P, Yuan JP, Li MJ, Zhan XY. Serum leptin and soluble leptin receptor in non-alcoholic fatty liver disease. *World J Gastroenterol* 2008; **14**: 2888-2893 [PMID: 18473416 DOI: 10.3748/wjg.14.2888]

42 **Kim IK**, Kim J, Kang JH, Song J. Serum leptin as a predictor of fatty liver in 7-year-old Korean children. *Ann Nutr Metab* 2008; **53**: 109-116 [PMID: 18946208 DOI: 10.1159/000165360]

43 **Kukla M**, Mazur W, Bułdak RJ, Zwirska-Korczala K. Potential role of leptin, adiponectin and three novel adipokines--visfatin, chemerin and vaspin--in chronic hepatitis. *Mol Med* 2011; **17**: 1397-1410 [PMID: 21738955 DOI: 10.2119/molmed.2010.00105]

44 **Braunersreuther V**, Viviani GL, Mach F, Montecucco F. Role of cytokines and chemokines in non-alcoholic fatty liver disease. *World J Gastroenterol* 2012; **18**: 727-735 [PMID: 22371632 DOI: 10.3748/wjg.v18.i8.727]

45 **Tarantino G**, Scopacasa F, Colao A, Capone D, Tarantino M, Grimaldi E, Savastano S. Serum Bcl-2 concentrations in overweight-obese subjects with nonalcoholic fatty liver disease. *World J Gastroenterol* 2011; **17**: 5280-5288 [PMID: 22219597 DOI: 10.3748/wjg.v17.i48.5280]

46 **Chakraborty JB**, Oakley F, Walsh MJ. Mechanisms and biomarkers of apoptosis in liver disease and fibrosis. *Int J Hepatol* 2012; **2012**: 648915 [PMID: 22567408]

47 **Farrell GC**, van Rooyen D, Gan L, Chitturi S. NASH is an Inflammatory Disorder: Pathogenic, Prognostic and Therapeutic Implications. *Gut Liver* 2012; **6**: 149-171 [PMID: 22570745 DOI: 10.5009/gnl.2012.6.2.149]

48 **Maher JJ**, Leon P, Ryan JC. Beyond insulin resistance: Innate immunity in nonalcoholic steatohepatitis. *Hepatology* 2008; **48**: 670-678 [PMID: 18666225 DOI: 10.1002/hep.22399]

49 **Di Minno MN**, Iervolino S, Peluso R, Russolillo A, Lupoli R, Scarpa R, Di Minno G, Tarantino G. Hepatic steatosis and disease activity in subjects with psoriatic arthritis receiving tumor necrosis factor-α blockers. *J Rheumatol* 2012; **39**: 1042-1046 [PMID: 22422493 DOI: 10.3899/jrheum.111391]

50 **Chu SH**, Lee MK, Ahn KY, Im JA, Park MS, Lee DC, Jeon JY, Lee JW. Chemerin and adiponectin contribute reciprocally to metabolic syndrome. *PLoS One* 2012; **7**: e34710 [PMID: 22509348 DOI: 10.1371/journal.pone.0034710]

51 **Buechler C**, Wanninger J, Neumeier M. Adiponectin, a key adipokine in obesity related liver diseases. *World J Gastroenterol* 2011; **17**: 2801-2811 [PMID: 21734787]

52 **Lemoine M**, Ratziu V, Kim M, Maachi M, Wendum D, Paye F, Bastard JP, Poupon R, Housset C, Capeau J, Serfaty L. Serum adipokine levels predictive of liver injury in non-alcoholic fatty liver disease. *Liver Int* 2009; **29**: 1431-1438 [PMID: 19422483 DOI: 10.1111/j.1478-3231.2009.02022.x]

53 **Dowman JK**, Tomlinson JW, Newsome PN. Systematic review: the diagnosis and staging of non-alcoholic fatty liver disease and non-alcoholic steatohepatitis. *Aliment Pharmacol Ther* 2011; **33**: 525-540 [PMID: 21198708 DOI: 10.1111/j.1365-2036.2010.04556.x]

54 **Musso G**, Gambino R, Biroli G, Carello M, Fagà E, Pacini G, De Michieli F, Cassader M, Durazzo M, Rizzetto M, Pagano G. Hypoadiponectinemia predicts the severity of hepatic fibrosis and pancreatic Beta-cell dysfunction in nondiabetic nonobese patients with nonalcoholic steatohepatitis. *Am J Gastroenterol* 2005; **100**: 2438-2446 [PMID: 16279898 DOI: 10.1111/j.1572-0241.2005.00297.x]

55 **Polyzos SA**, Toulis KA, Goulis DG, Zavos C, Kountouras J. Serum total adiponectin in nonalcoholic fatty liver disease: a systematic review and meta-analysis. *Metabolism* 2011; **60**: 313-326 [PMID: 21040935 DOI: 10.1016/j.metabol.2010.09.003]

56 **Van Wagner LB**, Rinella ME. The role of insulin-sensitizing agents in the treatment of nonalcoholic steatohepatitis. *Therap Adv Gastroenterol* 2011; **4**: 249-263 [PMID: 21765869 DOI: 10.1177/1756283X11403809]

57 **Lee UE**, Friedman SL. Mechanisms of hepatic fibrogenesis. *Best Pract Res Clin Gastroenterol* 2011; **25**: 195-206 [PMID: 21497738 DOI: 10.1016/j.bpg.2011.02.005]

58 **Yang Z**, Wang X, Wen J, Ye Z, Li Q, He M, Lu B, Ling C, Wu S, Hu R. Prevalence of non-alcoholic fatty liver disease and its relation to hypoadiponectinaemia in the middle-aged and elderly Chinese population. *Arch Med Sci* 2011; **7**: 665-672 [PMID: 22291803 DOI: 10.5114/aoms.2011.24137]

59 **Takahashi Y**, Soejima Y, Fukusato T. Animal models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *World J Gastroenterol* 2012; **18**: 2300-2308 [PMID: 22654421]

60 **Matsumoto H**, Tamura S, Kamada Y, Kiso S, Fukushima J, Wada A, Maeda N, Kihara S, Funahashi T, Matsuzawa Y, Shimomura I, Hayashi N. Adiponectin deficiency exacerbates lipopolysaccharide/D-galactosamine-induced liver injury in mice. *World J Gastroenterol* 2006; **12**: 3352-3358 [PMID: 16733851]

61 **Mandal P**, Park PH, McMullen MR, Pratt BT, Nagy LE. The anti-inflammatory effects of adiponectin are mediated via a heme oxygenase-1-dependent pathway in rat Kupffer cells. *Hepatology* 2010; **51**: 1420-1429 [PMID: 20052772 DOI: 10.1002/hep.23427]

62 **Rogers CQ**, Ajmo JM, You M. Adiponectin and alcoholic fatty liver disease. *IUBMB Life* 2008; **60**: 790-797 [PMID: 18709650 DOI: 10.1002/iub.124]

63 **Fukushima J**, Kamada Y, Matsumoto H, Yoshida Y, Ezaki H, Takemura T, Saji Y, Igura T, Tsutsui S, Kihara S, Funahashi T, Shimomura I, Tamura S, Kiso S, Hayashi N. Adiponectin prevents progression of steatohepatitis in mice by regulating oxidative stress and Kupffer cell phenotype polarization. *Hepatol Res* 2009; **39**: 724-738 [PMID: 19473437 DOI: 10.1111/j.1872-034X.2009.00509.x]

64 **Díez JJ**, Iglesias P. The role of the novel adipocyte-derived hormone adiponectin in human disease. *Eur J Endocrinol* 2003; **148**: 293-300 [PMID: 12611609 DOI: 10.1530/eje.0.1480293]

65 **Fukuhara Y**, Suda T, Kobayashi M, Tamura Y, Igarashi M, Waguri N, Kawai H, Aoyagi Y. Identification of cellular genes showing differential expression associated with hepatitis B virus infection. *World J Hepatol* 2012; **4**: 139-148 [PMID: 22567186 DOI: 10.4254/wjh.v4.i4.139]

66 **Daniele A**, Cammarata R, Pasanisi F, Finelli C, Salvatori G, Calcagno G, Bracale R, Labruna G, Nardelli C, Buono P, Sacchetti L, Contaldo F, Oriani G. Molecular analysis of the adiponectin gene in severely obese patients from southern Italy. *Ann Nutr Metab* 2008; **53**: 155-161 [PMID: 19011278 DOI: 10.1159/000172976]

67 **Bracale R**, Labruna G, Finelli C, Daniele A, Sacchetti L, Oriani G, Contaldo F, Pasanisi F. The absence of polymorphisms in ADRB3, UCP1, PPARγ, and ADIPOQ genes protects morbid obese patients toward insulin resistance. *J Endocrinol Invest* 2012; **35**: 2-4 [PMID: 22391136]

68 **Woo YC**, Tso AW, Xu A, Law LS, Fong CH, Lam TH, Lo SV, Wat NM, Cheung BM, Lam KS. Combined use of serum adiponectin and tumor necrosis factor-alpha receptor 2 levels was comparable to 2-hour post-load glucose in diabetes prediction. *PLoS One* 2012; **7**: e36868 [PMID: 22615828 DOI: 10.1371/journal.pone.0036868]

69 **Kawano J**, Arora R. The role of adiponectin in obesity, diabetes, and cardiovascular disease. *J Cardiometab Syndr* 2009; **4**: 44-49 [PMID: 19245516 DOI: 10.1111/j.1559-4572.2008.00030.x]

70 **Deng Y**, Scherer PE. Adipokines as novel biomarkers and regulators of the metabolic syndrome. *Ann N Y Acad*

Sci 2010; **1212**: E1-E19 [PMID: 21276002 DOI: 10.1111/j.1749-6632.2010.05875.x]
71 **Li R**, Lau WB, Ma XL. Adiponectin resistance and vascular dysfunction in the hyperlipidemic state. *Acta Pharmacol Sin* 2010; **31**: 1258-1266 [PMID: 20802503 DOI: 10.1038/aps.2010.95]
72 **Oh DK**, Ciaraldi T, Henry RR. Adiponectin in health and disease. *Diabetes Obes Metab* 2007; **9**: 282-289 [PMID: 17391153 DOI: 10.1111/j.1463-1326.2006.00610.x]
73 **Simpson F**, Whitehead JP. Adiponectin--it's all about the modifications. *Int J Biochem Cell Biol* 2010; **42**: 785-788 [PMID: 20044026 DOI: 10.1016/j.biocel.2009.12.021]
74 **Briggs DB**, Giron RM, Malinowski PR, Nuñez M, Tsao TS. Role of redox environment on the oligomerization of higher molecular weight adiponectin. *BMC Biochem* 2011; **12**: 24 [PMID: 21600065 DOI: 10.1186/1471-2091-12-24]
75 **Jungtrakoon P**, Plengvidhya N, Tangjittipokin W, Chimnaronk S, Salaemae W, Chongjaroen N, Chanprasert K, Sujjitjoon J, Srisawat C, Yenchitsomanus PT. Novel adiponectin variants identified in type 2 diabetic patients reveal multimerization and secretion defects. *PLoS One* 2011; **6**: e26792 [PMID: 22046359 DOI: 10.1371/journal.pone.0026792]
76 **Misu H**, Ishikura K, Kurita S, Takeshita Y, Ota T, Saito Y, Takahashi K, Kaneko S, Takamura T. Inverse correlation between serum levels of selenoprotein P and adiponectin in patients with type 2 diabetes. *PLoS One* 2012; **7**: e34952 [PMID: 22496878 DOI: 10.1371/journal.pone.0034952]
77 **Mohammadzadeh G**, Zarghami N. Hypoadiponectinemia in obese subjects with type II diabetes: A close association with central obesity indices. *J Res Med Sci* 2011; **16**: 713-723 [PMID: 22091299]
78 **Richards AA**, Stephens T, Charlton HK, Jones A, Macdonald GA, Prins JB, Whitehead JP. Adiponectin multimerization is dependent on conserved lysines in the collagenous domain: evidence for regulation of multimerization by alterations in posttranslational modifications. *Mol Endocrinol* 2006; **20**: 1673-1687 [PMID: 16497731 DOI: 10.1210/me.2005-0390]
79 **Rothan HA**, Teh SH, Haron K, Mohamed Z. A Comparative Study on the Expression, Purification and Functional Characterization of Human Adiponectin in Pichia pastoris and Escherichia coli. *Int J Mol Sci* 2012; **13**: 3549-3562 [PMID: 22489167 DOI: 10.3390/ijms13033549]
80 **Dadson K**, Liu Y, Sweeney G. Adiponectin action: a combination of endocrine and autocrine/paracrine effects. *Front Endocrinol* (Lausanne) 2011; **2**: 62 [PMID: 22649379]
81 **Vrachnis N**, Belitsos P, Sifakis S, Dafopoulos K, Siristatidis C, Pappa KI, Iliodromiti Z. Role of adipokines and other inflammatory mediators in gestational diabetes mellitus and previous gestational diabetes mellitus. *Int J Endocrinol* 2012; **2012**: 549748 [PMID: 22550485]
82 **Suzuki S**, Wilson-Kubalek EM, Wert D, Tsao TS, Lee DH. The oligomeric structure of high molecular weight adiponectin. *FEBS Lett* 2007; **581**: 809-814 [PMID: 17292892 DOI: 10.1016/j.febslet.2007.01.046]
83 **Yamauchi T**, Kadowaki T. Physiological and pathophysiological roles of adiponectin and adiponectin receptors in the integrated regulation of metabolic and cardiovascular diseases. *Int J Obes (Lond)* 2008; **32** Suppl 7: S13-S18 [PMID: 19136982 DOI: 10.1038/ijo.2008.233]
84 **Polak J**, Kovacova Z, Holst C, Verdich C, Astrup A, Blaak E, Patel K, Oppert JM, Langin D, Martinez JA, Sørensen TI, Stich V. Total adiponectin and adiponectin multimeric complexes in relation to weight loss-induced improvements in insulin sensitivity in obese women: the NUGENOB study. *Eur J Endocrinol* 2008; **158**: 533-541 [PMID: 18362301 DOI: 10.1530/EJE-07-0512]
85 **Salani B**, Briatore L, Andraghetti G, Adami GF, Maggi D, Cordera R. High-molecular weight adiponectin isoforms increase after biliopancreatic diversion in obese subjects. *Obesity* (Silver Spring) 2006; **14**: 1511-1514 [PMID: 17030961 DOI: 10.1038/oby.2006.174]
86 **Hage MP**, Safadi B, Salti I, Nasrallah M. Role of Gut-Related Peptides and Other Hormones in the Amelioration of Type 2 Diabetes after Roux-en-Y Gastric Bypass Surgery. *ISRN Endocrinol* 2012; **2012**: 504756 [PMID: 22619730]
87 **Liu Y**, Retnakaran R, Hanley A, Tungtrongchitr R, Shaw C, Sweeney G. Total and high molecular weight but not trimeric or hexameric forms of adiponectin correlate with markers of the metabolic syndrome and liver injury in Thai subjects. *J Clin Endocrinol Metab* 2007; **92**: 4313-4318 [PMID: 17698903 DOI: 10.1210/jc.2007-0890]
88 **Berg AH**, Combs TP, Du X, Brownlee M, Scherer PE. The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med* 2001; **7**: 947-953 [PMID: 11479628 DOI: 10.1038/90992]
89 **Xie L**, Boyle D, Sanford D, Scherer PE, Pessin JE, Mora S. Intracellular trafficking and secretion of adiponectin is dependent on GGA-coated vesicles. *J Biol Chem* 2006; **281**: 7253-7259 [PMID: 16407204]
90 **Gastaldelli A**, Harrison SA, Belfort-Aguilar R, Hardies LJ, Balas B, Schenker S, Cusi K. Importance of changes in adipose tissue insulin resistance to histological response during thiazolidinedione treatment of patients with nonalcoholic steatohepatitis. *Hepatology* 2009; **50**: 1087-1093 [PMID: 19670459 DOI: 10.1002/hep.23116]
91 **Cao J**, Puri N, Sodhi K, Bellner L, Abraham NG, Kappas A. Apo A1 Mimetic Rescues the Diabetic Phenotype of HO-2 Knockout Mice via an Increase in HO-1 Adiponectin and LKBI Signaling Pathway. *Int J Hypertens* 2012; **2012**: 628147 [PMID: 22577519]
92 **Hendricks GL**, Hadley JA, Krzysik-Walker SM, Prabhu KS, Vasilatos-Younken R, Ramachandran R. Unique profile of chicken adiponectin, a predominantly heavy molecular weight multimer, and relationship to visceral adiposity. *Endocrinology* 2009; **150**: 3092-3100 [PMID: 19299452 DOI: 10.1210/en.2008-1558]
93 **Neumeier M**, Sigruener A, Eggenhofer E, Weigert J, Weiss TS, Schaeffler A, Schlitt HJ, Aslanidis C, Piso P, Langmann T, Schmitz G, Schölmerich J, Buechler C. High molecular weight adiponectin reduces apolipoprotein B and E release in human hepatocytes. *Biochem Biophys Res Commun* 2007; **352**: 543-548 [PMID: 17129575 DOI: 10.1016/j.bbrc.2006.11.058]
94 **Adachi M**, Brenner DA. High molecular weight adiponectin inhibits proliferation of hepatic stellate cells via activation of adenosine monophosphate-activated protein kinase. *Hepatology* 2008; **47**: 677-685 [PMID: 18220291 DOI: 10.1002/hep.21991]
95 **Kadowaki T**, Yamauchi T. Adiponectin and adiponectin receptors. *Endocr Rev* 2005; **26**: 439-451 [PMID: 15897298 DOI: 10.1210/er.2005-0005]
96 **Kadowaki T**, Yamauchi T, Kubota N. The physiological and pathophysiological role of adiponectin and adiponectin receptors in the peripheral tissues and CNS. *FEBS Lett* 2008; **582**: 74-80 [PMID: 18054335 DOI: 10.1016/j.febslet.2007.11.070]
97 **Yamauchi T**, Nio Y, Maki T, Kobayashi M, Takazawa T, Iwabu M, Okada-Iwabu M, Kawamoto S, Kubota N, Kubota T, Ito Y, Kamon J, Tsuchida A, Kumagai K, Kozono H, Hada Y, Ogata H, Tokuyama K, Tsunoda M, Ide T, Murakami K, Awazawa M, Takamoto I, Froguel P, Hara K, Tobe K, Nagai R, Ueki K, Kadowaki T. Targeted disruption of AdipoR1 and AdipoR2 causes abrogation of adiponectin binding and metabolic actions. *Nat Med* 2007; **13**: 332-339 [PMID: 17268472 DOI: 10.1038/nm1557]
98 **Liu Y**, Michael MD, Kash S, Bensch WR, Monia BP, Murray SF, Otto KA, Syed SK, Bhanot S, Sloop KW, Sullivan JM, Reifel-Miller A. Deficiency of adiponectin receptor 2 reduces diet-induced insulin resistance but promotes type 2 diabetes. *Endocrinology* 2007; **148**: 683-692 [PMID: 17068142 DOI: 10.1210/en.2006-0708]

99 **Ma H**, Gomez V, Lu L, Yang X, Wu X, Xiao SY. Expression of adiponectin and its receptors in livers of morbidly obese patients with non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2009; **24**: 233-237 [PMID: 18713296 DOI: 10.1111/j.1440-1746.2008.05548.x]

100 **Nannipieri M**, Cecchetti F, Anselmino M, Mancini E, Marchetti G, Bonotti A, Baldi S, Solito B, Giannetti M, Pinchera A, Santini F, Ferrannini E. Pattern of expression of adiponectin receptors in human liver and its relation to nonalcoholic steatohepatitis. *Obes Surg* 2009; **19**: 467-474 [PMID: 18923878 DOI: 10.1007/s11695-008-9701-x]

101 **Tiniakos DG**. Nonalcoholic fatty liver disease/nonalcoholic steatohepatitis: histological diagnostic criteria and scoring systems. *Eur J Gastroenterol Hepatol* 2010; **22**: 643-650 [PMID: 19478676]

102 **Vuppalanchi R**, Marri S, Kolwankar D, Considine RV, Chalasani N. Is adiponectin involved in the pathogenesis of nonalcoholic steatohepatitis? A preliminary human study. *J Clin Gastroenterol* 2005; **39**: 237-242 [PMID: 15718867 DOI: 10.1097/01.mcg.0000152747.79773.2f]

103 **Deepa SS**, Dong LQ. APPL1: role in adiponectin signaling and beyond. *Am J Physiol Endocrinol Metab* 2009; **296**: E22-E36 [PMID: 18854421 DOI: 10.1152/ajpendo.90731.2008]

104 **Yamauchi T**, Kamon J, Minokoshi Y, Ito Y, Waki H, Uchida S, Yamashita S, Noda M, Kita S, Ueki K, Eto K, Akanuma Y, Froguel P, Foufelle F, Ferre P, Carling D, Kimura S, Nagai R, Kahn BB, Kadowaki T. Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. *Nat Med* 2002; **8**: 1288-1295 [PMID: 12368907 DOI: 10.1038/nm788]

105 **Shimano M**, Ouchi N, Shibata R, Ohashi K, Pimentel DR, Murohara T, Walsh K. Adiponectin deficiency exacerbates cardiac dysfunction following pressure overload through disruption of an AMPK-dependent angiogenic response. *J Mol Cell Cardiol* 2010; **49**: 210-220 [PMID: 20206634 DOI: 10.1016/j.yjmcc.2010.02.021]

106 **Kubota N**, Yano W, Kubota T, Yamauchi T, Itoh S, Kumagai H, Kozono H, Takamoto I, Okamoto S, Shiuchi T, Suzuki R, Satoh H, Tsuchida A, Moroi M, Sugi K, Noda T, Ebinuma H, Ueta Y, Kondo T, Araki E, Ezaki O, Nagai R, Tobe K, Terauchi Y, Ueki K, Minokoshi Y, Kadowaki T. Adiponectin stimulates AMP-activated protein kinase in the hypothalamus and increases food intake. *Cell Metab* 2007; **6**: 55-68 [PMID: 17618856 DOI: 10.1016/j.cmet.2007.06.003]

107 **Holmes RM**, Yi Z, De Filippis E, Berria R, Shahani S, Sathyanarayana P, Sherman V, Fujiwara K, Meyer C, Christ-Roberts C, Hwang H, Finlayson J, Dong LQ, Mandarino LJ, Bajaj M. Increased abundance of the adaptor protein containing pleckstrin homology domain, phosphotyrosine binding domain and leucine zipper motif (APPL1) in patients with obesity and type 2 diabetes: evidence for altered adiponectin signalling. *Diabetologia* 2011; **54**: 2122-2131 [PMID: 21562756 DOI: 10.1007/s00125-011-2173-x]

108 **Gu W**, Li Y. The therapeutic potential of the adiponectin pathway. *BioDrugs* 2012; **26**: 1-8 [PMID: 22050309 DOI: 10.2165/11594790-000000000-00000]

109 **Cleasby ME**, Lau Q, Polkinghorne E, Patel SA, Leslie SJ, Turner N, Cooney GJ, Xu A, Kraegen EW. The adaptor protein APPL1 increases glycogen accumulation in rat skeletal muscle through activation of the PI3-kinase signalling pathway. *J Endocrinol* 2011; **210**: 81-92 [PMID: 21543456 DOI: 10.1530/JOE-11-0039]

110 **Woods A**, Azzout-Marniche D, Foretz M, Stein SC, Lemarchand P, Ferré P, Foufelle F, Carling D. Characterization of the role of AMP-activated protein kinase in the regulation of glucose-activated gene expression using constitutively active and dominant negative forms of the kinase. *Mol Cell Biol* 2000; **20**: 6704-6711 [PMID: 10958668 DOI: 10.1128/MCB.20.18.6704-6711.2000]

111 **Polakof S**, Panserat S, Craig PM, Martyres DJ, Plagnes-Juan E, Savari S, Aris-Brosou S, Moon TW. The metabolic consequences of hepatic AMP-kinase phosphorylation in rainbow trout. *PLoS One* 2011; **6**: e20228 [PMID: 21625448 DOI: 10.1371/journal.pone.0020228]

112 **You M**, Rogers CQ. Adiponectin: a key adipokine in alcoholic fatty liver. *Exp Biol Med* (Maywood) 2009; **234**: 850-859 [PMID: 19491377 DOI: 10.3181/0902-MR-61]

113 **Carter-Kent C**, Zein NN, Feldstein AE. Cytokines in the pathogenesis of fatty liver and disease progression to steatohepatitis: implications for treatment. *Am J Gastroenterol* 2008; **103**: 1036-1042 [PMID: 18177455 DOI: 10.1111/j.1572-0241.2007.01709.x]

114 **Garg MK**, Dutta MK, Mahalle N. Adipokines (adiponectin and plasminogen activator inhhibitor-1) in metabolic syndrome. *Indian J Endocrinol Metab* 2012; **16**: 116-123 [PMID: 22276262 DOI: 10.4103/2230-8210.91206]

115 **Savastano S**, Valentino R, Di Somma C, Orio F, Pivonello C, Passaretti F, Brancato V, Formisano P, Colao A, Beguinot F, Tarantino G. Serum 25-Hydroxyvitamin D Levels, phosphoprotein enriched in diabetes gene product (PED/PEA-15) and leptin-to-adiponectin ratio in women with PCOS. *Nutr Metab* (Lond) 2011; **8**: 84 [PMID: 22112520 DOI: 10.1186/1743-7075-8-84]

116 **Huang H**, Park PH, McMullen MR, Nagy LE. Mechanisms for the anti-inflammatory effects of adiponectin in macrophages. *J Gastroenterol Hepatol* 2008; **23** Suppl 1: S50-S53 [PMID: 18336664 DOI: 10.1111/j.1440-1746.2007.05284.x]

117 **Begriche K**, Massart J, Robin MA, Borgne-Sanchez A, Fromenty B. Drug-induced toxicity on mitochondria and lipid metabolism: mechanistic diversity and deleterious consequences for the liver. *J Hepatol* 2011; **54**: 773-794 [PMID: 21145849 DOI: 10.1016/j.jhep.2010.11.006]

118 **Adler M**, Taylor S, Okebugwu K, Yee H, Fielding C, Fielding G, Poles M. Intrahepatic natural killer T cell populations are increased in human hepatic steatosis. *World J Gastroenterol* 2011; **17**: 1725-1731 [PMID: 21483633 DOI: 10.3748/wjg.v17.i13.1725]

119 **Jarrar MH**, Baranova A, Collantes R, Ranard B, Stepanova M, Bennett C, Fang Y, Elariny H, Goodman Z, Chandhoke V, Younossi ZM. Adipokines and cytokines in non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2008; **27**: 412-421 [PMID: 18081738 DOI: 10.1111/j.1365-2036.2007.03586.x]

120 **Wulster-Radcliffe MC**, Ajuwon KM, Wang J, Christian JA, Spurlock ME. Adiponectin differentially regulates cytokines in porcine macrophages. *Biochem Biophys Res Commun* 2004; **316**: 924-929 [PMID: 15033490 DOI: 10.1016/j.bbrc.2004.02.130]

121 **Kumada M**, Kihara S, Ouchi N, Kobayashi H, Okamoto Y, Ohashi K, Maeda K, Nagaretani H, Kishida K, Maeda N, Nagasawa A, Funahashi T, Matsuzawa Y. Adiponectin specifically increased tissue inhibitor of metalloproteinase-1 through interleukin-10 expression in human macrophages. *Circulation* 2004; **109**: 2046-2049 [PMID: 15096450 DOI: 10.1161/01.CIR.0000127953.98131.ED]

122 **Wolf AM**, Wolf D, Rumpold H, Enrich B, Tilg H. Adiponectin induces the anti-inflammatory cytokines IL-10 and IL-1RA in human leukocytes. *Biochem Biophys Res Commun* 2004; **323**: 630-635 [PMID: 15369797 DOI: 10.1016/j.bbrc.2004.08.145]

123 **Wang SN**, Wang ST, Lee KT. The potential interplay of adipokines with toll-like receptors in the development of hepatocellular carcinoma. *Gastroenterol Res Pract* 2011; **2011**: 215986 [PMID: 21960997]

124 **Bertolani C**, Marra F. Role of adipocytokines in hepatic fibrosis. *Curr Pharm Des* 2010; **16**: 1929-1940 [PMID: 20370673 DOI: 10.2174/138161210791208857]

125 **Handy JA**, Fu PP, Kumar P, Mells JE, Sharma S, Saxena NK, Anania FA. Adiponectin inhibits leptin signalling via multiple mechanisms to exert protective effects against hepatic fibrosis. *Biochem J* 2011; **440**: 385-395 [PMID: 21846328 DOI: 10.1042/BJ20102148]

126 **Caligiuri A**, Bertolani C, Guerra CT, Aleffi S, Galastri S, Trappoliere M, Vizzutti F, Gelmini S, Laffi G, Pinzani M, Marra F. Adenosine monophosphate-activated protein kinase modulates the activated phenotype of hepatic stellate cells. *Hepatology* 2008; **47**: 668-676 [PMID: 18098312 DOI: 10.1002/hep.21995]
127 **Tomita K**, Oike Y, Teratani T, Taguchi T, Noguchi M, Suzuki T, Mizutani A, Yokoyama H, Irie R, Sumimoto H, Takayanagi A, Miyashita K, Akao M, Tabata M, Tamiya G, Ohkura T, Hibi T. Hepatic AdipoR2 signaling plays a protective role against progression of nonalcoholic steatohepatitis in mice. *Hepatology* 2008; **48**: 458-473 [PMID: 18666257 DOI: 10.1002/hep.22365]
128 **Tarantino G**, Conca P, Riccio A, Tarantino M, Di Minno MN, Chianese D, Pasanisi F, Contaldo F, Scopacasa F, Capone D. Enhanced serum concentrations of transforming growth factor-beta1 in simple fatty liver: is it really benign? *J Transl Med* 2008; **6**: 72 [PMID: 19038040 DOI: 10.1186/1479-5876-6-72]
129 **Ruiz JR**, Lasa A, Simon E, Larrarte E, Labayen I. Lower plasma NAMPT/visfatin levels are associated with impaired hepatic mitochondrial function in non-diabetic obese women: a potential link between obesity and non-alcoholic fatty liver disease. *Nutr Metab Cardiovasc Dis* 2012; **22**: e1-e2 [PMID: 22227071 DOI: 10.1016/j.numecd.2011.03.003]
130 **Ren LP**, Chan SM, Zeng XY, Laybutt DR, Iseli TJ, Sun RQ, Kraegen EW, Cooney GJ, Turner N, Ye JM. Differing endoplasmic reticulum stress response to excess lipogenesis versus lipid oversupply in relation to hepatic steatosis and insulin resistance. *PLoS One* 2012; **7**: e30816 [PMID: 22355328 DOI: 10.1371/journal.pone.0030816]
131 **Hsieh PS**, Hsieh YJ. Impact of liver diseases on the development of type 2 diabetes mellitus. *World J Gastroenterol* 2011; **17**: 5240-5245 [PMID: 22219592 DOI: 10.3748/wjg.v17.i48.5240]
132 **Pessayre D**. Role of mitochondria in non-alcoholic fatty liver disease. *J Gastroenterol Hepatol* 2007; **22** Suppl 1: S20-S27 [PMID: 17567459 DOI: 10.1111/j.1440-1746.2006.04640.x]
133 **Deng S**, Yang Y, Han Y, Li X, Wang X, Li X, Zhang Z, Wang Y. UCP2 inhibits ROS-mediated apoptosis in A549 under hypoxic conditions. *PLoS One* 2012; **7**: e30714 [PMID: 22292025 DOI: 10.1371/journal.pone.0030714]
134 **Bai Y**, Onuma H, Bai X, Medvedev AV, Misukonis M, Weinberg JB, Cao W, Robidoux J, Floering LM, Daniel KW, Collins S. Persistent nuclear factor-kappa B activation in Ucp2-/- mice leads to enhanced nitric oxide and inflammatory cytokine production. *J Biol Chem* 2005; **280**: 19062-19069 [PMID: 15757894 DOI: 10.1074/jbc.M500566200]
135 **Baffy G**. Kupffer cells in non-alcoholic fatty liver disease: the emerging view. *J Hepatol* 2009; **51**: 212-223 [PMID: 19447517 DOI: 10.1016/j.jhep.2009.03.008]
136 **Petersen KF**, Dufour S, Morino K, Yoo PS, Cline GW, Shulman GI. Reversal of muscle insulin resistance by weight reduction in young, lean, insulin-resistant offspring of parents with type 2 diabetes. *Proc Natl Acad Sci USA* 2012; **109**: 8236-8240 [PMID: 22547801 DOI: 10.1073/pnas.1205675109]
137 **Mummadi RR**, Kasturi KS, Chennareddygari S, Sood GK. Effect of bariatric surgery on nonalcoholic fatty liver disease: systematic review and meta-analysis. *Clin Gastroenterol Hepatol* 2008; **6**: 1396-1402 [PMID: 18986848 DOI: 10.1016/j.cgh.2008.08.012]
138 **Ahmed MH**, Byrne CD. Current treatment of non-alcoholic fatty liver disease. *Diabetes Obes Metab* 2009; **11**: 188-195 [PMID: 18564173 DOI: 10.1111/j.1463-1326.2008.00926.x]
139 **Massip-Salcedo M**, Zaouali MA, Padrissa-Altés S, Casillas-Ramirez A, Rodés J, Roselló-Catafau J, Peralta C. Activation of peroxisome proliferator-activated receptor-alpha inhibits the injurious effects of adiponectin in rat steatotic liver undergoing ischemia-reperfusion. *Hepatology* 2008; **47**: 461-472 [PMID: 18098300 DOI: 10.1002/hep.21935]
140 **Walter R**, Wanninger J, Bauer S, Eisinger K, Neumeier M, Weiss TS, Amann T, Hellerbrand C, Schäffler A, Schölmerich J, Buechler C. Adiponectin reduces connective tissue growth factor in human hepatocytes which is already induced in non-fibrotic non-alcoholic steatohepatitis. *Exp Mol Pathol* 2011; **91**: 740-744 [PMID: 21946149 DOI: 10.1016/j.yexmp.2011.09.006]
141 **Elias-Miró M**, Jiménez-Castro MB, Mendes-Braz M, Casillas-Ramírez A, Peralta C. The Current Knowledge of the Role of PPAR in Hepatic Ischemia-Reperfusion Injury. *PPAR Res* 2012; **2012**: 802384 [PMID: 22675337]
142 **Labruna G**, Pasanisi F, Nardelli C, Tarantino G, Vitale DF, Bracale R, Finelli C, Genua MP, Contaldo F, Sacchetti L. UCP1 -3826 AG+GG genotypes, adiponectin, and leptin/adiponectin ratio in severe obesity. *J Endocrinol Invest* 2009; **32**: 525-529 [PMID: 19474520]
143 **Robinson K**, Prins J, Venkatesh B. Clinical review: adiponectin biology and its role in inflammation and critical illness. *Crit Care* 2011; **15**: 221 [PMID: 21586104 DOI: 10.1186/cc10021]

P- Reviewers Bala S, van der Velde A **S- Editor** Gou SX
L- Editor Stewart GJ **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i6.813

World J Gastroenterol 2013 February 14; 19(6): 813-828
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

REVIEW

Current evidence for histone deacetylase inhibitors in pancreatic cancer

Ioannis Koutsounas, Constantinos Giaginis, Efstratios Patsouris, Stamatios Theocharis

Ioannis Koutsounas, Constantinos Giaginis, Efstratios Patsouris, Stamatios Theocharis, First Department of Pathology, Medical School, National and Kapodistrian University of Athens, GR-11527 Athens, Greece
Constantinos Giaginis, Department of Food Science and Nutrition, University of the Aegean, GR-81400 Lemnos, Greece
Author contributions: Koutsounas I collected and analyzed literature data and wrote the paper; Giaginis C collected and analyzed literature data; Patsouris E collected funds for the study; and Theocharis S designed the study and analyzed literature data.
Correspondence to: Stamatios Theocharis, MD, PhD, Pathologist, Associate Professor of the Department of Pathology, Medical School, National and Kapodistrian University of Athens, GR-11527 Athens, Greece. theocharis@ath.forthnet.gr
Telephone: +30-21-7462413 Fax: +30-21-7716098
Received: June 9, 2011 Revised: October 18, 2011
Accepted: January 5, 2013
Published online: February 14, 2013

Abstract

Pancreatic cancer is one of the most aggressive human cancers, with more than 200 000 deaths worldwide every year. Despite recent efforts, conventional treatment approaches, such as surgery and classic chemotherapy, have only slightly improved patient outcomes. More effective and well-tolerated therapies are required to reverse the current poor prognosis of this type of neoplasm. Among new agents, histone deacetylase inhibitors (HDACIs) are now being tested. HDACIs have multiple biological effects related to acetylation of histones and many non-histone proteins that are involved in regulation of gene expression, apoptosis, cell cycle progression and angiogenesis. HDACIs induce cell cycle arrest and can activate the extrinsic and intrinsic pathways of apoptosis in different cancer cell lines. In the present review, the main mechanisms by which HDACIs act in pancreatic cancer cells *in vitro*, as well as their antiproliferative effects in animal models are presented. HDACIs constitute a promising treatment for pancreatic cancer with encouraging anti-tumor effects, at well-tolerated doses.

© 2013 Baishideng. All rights reserved.

Key words: Pancreatic cancer; Histone deacetylases; Histone deacetylase inhibitors; Experimental studies

Koutsounas I, Giaginis C, Patsouris E, Theocharis S. Current evidence for histone deacetylase inhibitors in pancreatic cancer. *World J Gastroenterol* 2013; 19(6): 813-828 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i6/813.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i6.813

INTRODUCTION

Pancreatic cancer is one of the most lethal human cancers and continues to be a major unsolved health problem at the beginning of the 21st century. Worldwide, over 200 000 people die annually of pancreatic cancer, with the highest incidence and mortality rates found in developed countries. Pancreatic cancer is the 4th and 6th leading cause of cancer death in United States and Europe, respectively. Pancreatic cancer incidence and mortality rates are almost equal because of the high fatality rate. A lack of illness indicators and screening tests mean that pancreatic cancer is usually diagnosed at the late stages of the natural history of the disease[1,2].

In Western communities, the 1- and 5-year survival rates for pancreatic cancer are less than 25% and 5%, respectively, and the mortality rates are essentially identical. Although survival rates are highest (16.6%) when the tumor is localized at diagnosis, less than 10% of tumors are detected at that time. On the other hand, the survival rates have been only slightly improved over the past decade because of a lack of significant medical advances in early detection and the poor outcome of treatment approaches. Pancreatic cancer is rare in the first three decades of life. The majority of pancreatic cancers occur

in the exocrine pancreas and the vast majority (> 90%) have ductal differentiation. Men consistently have higher incidence and mortality rates than women, worldwide[3].

Treatment of advanced pancreatic cancer

Traditionally, 5-fluorouracil (5-FU)-based chemotherapy and/or radiotherapy have been used in the treatment of locally advanced pancreatic cancer; however, the value of radiotherapy remains unclear[4]. Today, *gemcitabine*-based therapy is the acceptable treatment approach for both unresectable locally advanced and metastatic pancreatic cancer. Several phase Ⅲ trials were undertaken with gemcitabine in combination with a range of chemotherapy agents. However, combining gemcitabine with 5-FU[5], as well as *irinotecan*, *oxaliplatin*, *pemetrexed*, *exatecan* and *cisplatin*[6-10], all failed to show superiority over gemcitabine monotherapy. In a recent phase Ⅲ trial, the combination of *capecitabine* with gemcitabine significantly improved the objective response rate and progression-free survival, but did not show superiority in overall survival in patients with advanced pancreatic cancer[11]. Additionally, in a randomized phase Ⅱ trial of *folfirinox* (5-FU/leucovorin, irinotecan, and oxaliplatin) versus gemcitabine, the median overall survival was 11.1 mo in the folfirinox group compared with 6.8 mo in the gemcitabine group, indicating that folfirinox is an option for the treatment of patients with metastatic pancreatic cancer[12].

Targeted therapies have also been investigated for advanced pancreatic cancer. Erlotinib is a small-molecule tyrosine kinase inhibitor of the human epidermal growth factor receptor (EGFR). A multicenter, randomized, double-blind, placebo-controlled phase Ⅲ clinical trial of erlotinib in combination with gemcitabine, in patients with locally advanced or metastatic pancreatic adenocarcinoma met its primary endpoint, with the combination regimen being the first gemcitabine combination to demonstrate a statistically significant survival advantage over gemcitabine monotherapy and the regimen was consequently approved for metastatic disease[13].

Many molecular-targeted agents that interact with crucial pathways for cell survival in pancreatic cancer are currently being explored. These include agents that target *poly ADP*-ribose polymerase, histone deacetylase (*HDAC*), *Src/Abl kinases,* and mammalian targets of rapamycin[14].

Histone acetyltransferases and deacetylases

The principal structure of eukaryotic chromatin is the nucleosome. Each nucleosome consists of approximately 146 bp of DNA wrapped around a core of eight basic proteins called histones, two each of H2A, H2B, H3 and H4. Nucleosomal structure not only facilitates packing DNA into a relatively small nucleus, but also exerts important regulatory functions. The N- and C- terminal tails of the nucleosomal core undergo post-translational modifications, participating in chromatin assembly regulation and/or DNA accessibility. Nucleosomes containing highly charged hypoacetylated histones bind tightly to the phosphate backbone of DNA, inhibiting transcription, preventing transcription factors, regulatory complexes and RNA polymerase to access the DNA. Acetylation neutralizes the charge of the histones generating a more open DNA conformation. Transcription factors may then access the DNA, promoting the expression of the corresponding genes. Therefore, histone acetylation is generally associated with transcriptional activation. Histone acetylation is carried out by a group of proteins called histone acetyl transferases (HATs), and the acetyl groups can be removed by HDACs. These molecules play a pivotal role in cellular functions, such as chromosome remodelling, gene transcription and cell proliferation[15,16].

Eighteen different human HDAC isoforms have been described and are classified into four classes. Class Ⅰ HDACs (HDACs 1, 2, 3 and 8) are associated with RPD3 deacetylase, and are primarily located in the nucleus. Class Ⅱ HDACs are divided into two subclasses, class Ⅱa (HDACs 4, 5, 7 and 9) and class Ⅱb (HDACs 6 and 10) and are homologous to the yeast Hda1 deacetylase. Class Ⅲ HDACs consists of seven HDACs (SIRT1 to SIRT7) that share homologies with the yeast silent information regulator 2 (Sir2) family. The class Ⅳ family of HDACs has only one member, HDAC11. Classes Ⅰ, Ⅱ and Ⅳ require Zn^{2+} for activity, while class Ⅲ has a unique catalytic mechanism that requires the co-factor NAD^{+}. To achieve the acetylation of histones, an acetyl group of acetyl coenzyme A is linked to the ε-amino group of lysine by HATs, which can be removed by HDACs. When HDACs remove the acetyl group from a histone lysine, a positive charge on the lysine residue condensing the structure of nucleosomes is restored. The active site of HDACs consists of a cylindrical pocket in which the lysine residue fits when deacetylation takes place. Zn^{2+} is located near the bottom of the cylindrical pocket[17].

In addition to deacetylating histones, HDACs have also been reported to interact with non-histone proteins. Such protein targets of HDACs include transcription factors and regulators, signal transduction mediators, DNA repair enzymes, nuclear import regulators, chaperone proteins, structural proteins, inflammation mediators and viral proteins. These HDAC substrates are involved in numerous important cell pathways, including control of gene expression, regulation of cell proliferation, differentiation, migration and death. Altered expression of HDACs has been reported in several types of human neoplasms[18,19].

HDAC inhibitors

HDAC inhibitors (HDACIs) have three common structural characteristics: a Zn binding moiety, an opposite capping group, and a straight chain alkyl, vinyl or aryl linker connecting the two. The majority of HDACIs are designed to interfere with the catalytic domain of HDACs, blocking substrate recognition and inducing gene expression. Aberrant expression of different HDAC isoforms has been associated with different malignancies; thus, HDACIs represent a potent and specific strategy for cancer treatment. Most of the described HDACIs only

affect the Zn-dependent classes I and Ⅱ HDACs. The HDACIs described so far vary in structure and origin, being divided into different classes based on their chemical properties. The *hydroxamic acids* include trichostatin A (TSA), SAHA (vorinostat), LBH589 (panobinostat) and PXD101 (belinostat). The *short-chain fatty acids* comprise another class, including sodium butyrate (NaBu), 4-phenylbutyrate and valproic acid. A third class includes the *cyclic tetrapeptides*, such as FK228/depsipeptide (romidepsin). A fourth class of HDACIs is the *benzamides*, including MS-275 (entinostat), CI-994 and MGCD0103[20,21].

The mechanisms of action of HDACIs are complex and not completely understood. HDACIs have multiple biological effects related to acetylation of histones and many non-histone proteins, such as those involved in regulation of gene expression, apoptosis, cell cycle progression, DNA repair, cell migration and angiogenesis. HDACIs induce cell cycle growth arrest in both normal and transformed cells, and can activate the extrinsic and intrinsic pathways of apoptosis. Both *in vitro* and *in vivo* data and ongoing clinical trials have indicated that HDACIs could be used against different solid tumors and hematological malignancies; thus, comprising one of the most promising classes of new anticancer agents[22,23]. In the present review, the latest knowledge on the effect of HDACIs on pancreatic cancer is discussed.

EXPERIMENTAL *IN VITRO* STUDIES

The data available so far regarding the different classes of HDACIs used in pancreatic cancer cell lines are presented in the following section. Additionally, the targets modulated by different HDACI compounds are listed in Table 1.

Hydroxamic acids

Suberoylanilide hydroxamic acid (SAHA, *N*-hydroxy-*N*'-phenyl-octanediamide, vorinostat) is a synthetic hydroxamic acid that is structurally related to the natural product, trichostatin A {TSA, 7-[4-(dimethylamino)phenyl]-*N*-hydroxyl-4,6-dimethyl-7-oxo-(2*E*,4*E*,6*R*)-2,4-heptadienamide}, which is produced by selected strains of *Streptomyces platensis*, *Streptomyces hygroscopicus* Y-50 or *Streptomyces sioyaensis*. Hydroxamic acids have a high affinity to biometals, including Fe^{3+}, Ni^{2+} and Zn^{2+}. The synthesis of SAHA and its potency to induce differentiation of murine erythroleukemia (MEL) cells was first reported in 1996. SAHA and TSA contain a hydroxamic acid-based metal-binding domain that coordinates the catalytic Zn^{2+} in the HDAC active site, a 5 (TSA) or 6 (SAHA)-membered carbon-based linker that mimics the Cα functional group of lysine, and a hydrophobic motif that interacts with the periphery of the HDAC binding pocket[24].

TSA: TSA strongly inhibited the cellular growth of nine pancreatic adenocarcinoma cell lines (MiaPaCa-2, PANC1, PSN1, PT45P1, CFPAC1, HPAF-Ⅱ, T3M4, PaCa44 and PC), although a marked difference in sensitivity to the drug was noted. TSA-induced cell cycle arrest was associated with a block in the G2 phase and apoptotic death. TSA treatment in T3M4 and PaCa44 cell lines resulted in p21WAF1/CIP1 induction, an increase in caspase-3 activity and the downregulation of p27 and cyclin A2 mRNA expression[25].

Global gene expression profiles were also examined in several pancreatic cancer cell lines (CFPAC1, HPAF, MiaPaCa-2, Panc1, PC, PSN1, PT-45P1 and PaCa44) post-TSA treatment. Three point four percents of genes involved in a wide variety of cellular processes, such as cell proliferation, signaling, regulation of transcription, and apoptosis, were altered after TSA treatment. The cyclin-dependent kinase (cdk) inhibitors p21, p19 and p57 were all upregulated, while cyclin A and cdk10 were downregulated. Additionally, BIM, a proapoptotic BCL-2 family member, was significantly induced, while the expression of the antiapoptotic genes *BCL-XL* and *BCL-W* was repressed by TSA treatment[26].

Different pancreatic cancer cell lines co-express high-level TNF-related apoptosis-inducing ligand receptor (TRAIL-R), Fas and TNF-R1 but are strongly resistant to apoptosis triggered by the death receptors. The drug combinations geldanamycin/PS-341, TSA/PS-341 and TSA/geldanamycin with low-dose TRAIL were tested and all were found to be effective in initiating apoptosis in four pancreatic cancer cell lines (AsPC-1, BxPC-3, MiaPaCa-2 and Panc-1) compared with single drug-based treatments. This killing effect was enhanced when Bcl-XL was depleted. When Bcl-XL-depleted cells and control counterparts were exposed to TSA/PS-341, TRAIL induced cell death in Bcl-XL knockdown cells. However, under the same experimental conditions fewer control cells were killed, indicating that Bcl-XL depletion significantly increased TSA/PS-341 killing effects on pancreatic cancer cells in the presence of TRAIL[27].

TSA and SAHA induced apoptosis in pancreatic cancer cell lines IMIM-PC-1, IMIM-PC-2 and RWP-1, independently of their intrinsic resistance to conventional antineoplastic agents. Caspase-3 activity was slightly increased in IMIM-PC-1 and RWP-1 cells, but significantly increased in IMIM-PC-2 cells after TSA treatment. On the other hand, caspase-8 and -9 activities were not altered. In addition, PARP-1 was only partially cleaved after TSA treatment. An inhibitor of the human serine protease Omi/HtrA2, called ucf-101, was able to block the cell death induced by TSA in the three cell lines through a caspase-independent mechanism. In the same experimental setting, Bax protein levels were dramatically increased, but those of Bcl-2 and p21 were not significantly modified[28].

TSA and SK-7041, a novel hybrid synthetic HDACI, both induced apoptosis and G2-M cell cycle arrest in the pancreatic cancer cell lines Panc-1 and ASPC-1. They caused increased H4 histone acetylation, and also suppressed the expression of the antiapoptotic proteins Mcl-1 and Bcl-XL, but did not affect either Bcl-2 or the proapoptotic Bax and Bak proteins. TSA and SK-7041 also enhanced the expression of p21 and of cyclin D2

Table 1 Histone deacetylase inhibitors and targets modulated in different pancreatic cancer cell lines

HDACI compound		Targets modulated		Ref.
		Upregulated	Downregulated	
Hydroxamic acids	TSA	p21, p19, p57, Bim, Bax, caspase-3, -7, cyclin D2, TGF-β, OC18, MGMT, TFAR19, maspin, CDKN1C, MUC2, MUC5B, miR-107	p27, cyclin A2 , -B1, cdk6, cdk10, Bcl-XL, Mcl-1, PARP-1, NF-κB, K-Ras, MEK1/2, phosph. MEK, ERK1/2, NPM, TCTP	[25-30,37-52]
	SAHA	p21, p27, p57, Bax, RARa, E-cadherin, C/EBPa, caspase-3, -7, HHIP, RELN, DAB1	Bcl-2, cyclin D1, -B1, c-myc, Ptc-1, survivin, EGFR, NF-κB, RelA/p65	[53-60,62,63,66]
Cyclic peptides	FK228	p21, p16, caspase-3, DR5, GATA4	Survivin	[68-70]
Short chain fatty acids	VPA	NEP/CD10, RELN, DAB1		[62,73]
	4-PB	p21, p16, miR-127, caspase-8, Bid, JNK	Bcl-6, PARP	[78-80]
	NaBu	ALP, K23, NEP/CD10, caspase-3, -7, -9, GnT-Ⅳa, 5-hydroxytryptamine, CEA, DU-PAN-2, CA19-9, IRT, Lcu7, synaptophysin, p21, p27, Bax	Bcl-XL, TGF-α, PKC, b4 and b7 integrin, EGFR, ezrin, cyclin D1, mut-p53, Bcl-2, survivin	[73,82-99]
Benzamides	MS-275	p21, p27, gelsolin, caspase-3	pRb, Bcl-2, cyclin D1	[101,102]

HDACI: Histone deacetylase inhibitor; TSA: Trichostatin A; SAHA: Suberoylanilide hydroxamic acid; VPA: Valproic acid; NF-κB: Nuclear factor kappa B; TGF: Transforming growth factor; EGFR: Epidermal growth factor receptor; CEA: Carcinoembryonic antigen.

and reduced that of cyclin B1[29].

TSA and the selective 26S proteasome inhibitor PS-341, synergistically induced apoptosis in eight pancreatic adenocarcinoma cell lines (AsPC-1, BxPC-3, CFPAC-1, Capan-2, Mia PaCa-2, Panc-1, SU86, and SW1990). Combining TSA with PS-341 induced apoptosis by increasing caspase-3 and -7 activities and enhanced PARP cleavage. Their combination also effectively blocked nuclear factor kappa B (NF-κB) signaling pathway and downregulated the NF-κB dependent anti-apoptotic factor Bcl-XL. Moreover, they inactivated the Ras-MAP kinase pathway by depleting several key components of MAP kinase cascades, including K-Ras, MEK1/2, phosphorylated MEK and ERK1/2[30].

TSA strongly inhibited proliferation of pancreatic endocrine carcinoma cell lines (CM, metastatic insulinoma; BON, metastatic carcinoid; and QGP-1, somatostatinoma) by causing cell cycle G2/M arrest and apoptosis. TSA-induced apoptosis of CM cells was shown to be a retarded event with respect to that observed in BON and QGP-1 cells. Such effect was ascribed to modifications in the expression of proteins related to cell proliferation, gene expression, signal transduction, cytoskeleton organization, chromatin organization, as also RNA splicing and protein folding[31]. Another study examined the effect of TSA or 5-Aza-C, a DNA methyltransferase inhibitor, treatment on the proliferation of the pancreatic endocrine cancer cell lines QGP-1, CM and BON. TSA treatment resulted in cell cycle arrest at G1 (QGP-1) or G2 (BON and CM) phase, whereas 5-Aza-C blocked the cell cycle in G2 phase only in BON cells. The combined treatment did not significantly increase the cytostatic effect obtained with TSA alone, suggesting that the synergistic cell growth inhibition by the two drugs may be not caused by cell cycle arrest[32].

TSA and gemcitabine synergistically inhibited the proliferation of several human pancreatic adenocarcinoma cell lines (T3M4, PANC1, PC, CFPAC1, YAPC, DANG and Panc-89). In the cell lines tested, TSA enhanced apoptosis, but not the cell cycle arrest induced by gemcitabine[33,34]. TSA significantly inhibited the viability of BxPC-3 cells in a time- and dose-dependent manner by inhibition of cell proliferation and induction of apoptosis. Cell cycle analysis showed an increase of cells in the G0/G1 phase post-TSA treatment, indicating cell cycle arrest. Additionally, TSA induced the apoptosis of BxPC-3 cells and led to alterations in the expression levels of miRNAs. Although some variation at the gene transcription level was observed among Panc-1, BxPC-3, SOJ-6 and MiaPaCa-2 cell lines, the amount of HDAC proteins produced seemed to be comparable[35]. The effects of known inhibitors of class Ⅲ HDACs, such as Nicotinamide and Sirtinol, on the growth of pancreatic cancer cells, in addition to those of TSA, were also examined. Treatment of pancreatic cells with different drugs concentrations resulted in a dose-dependent inhibition of cell growth, with TSA being the most effective compound. Sirtinol induced G1 arrest in SOJ-6; however, TSA induced G1 arrest in BxPC-3 cells. Treatment of cells with HDACIs resulted in elevated cell numbers in the sub-G1 peak region, suggesting induction of cell DNA degradation by Sirtinol and TSA. Sirtinol and TSA treatment also involved the mitochondrial pathway of apoptosis induction[36].

Transforming growth factor beta (TGF-β) plays a significant role in the growth inhibition of most normal epithelial, and some cancer, cells. TGF-β mediates its biological affects through cell surface receptors known as type Ⅰ (RⅠ) and Ⅱ (RⅡ) receptors. TGF-β resistance caused by loss of receptors expression has been linked to tumor formation and progression. The TGF-β RⅡ promoter contains two consensus Sp1 sites. The Sp gene family consists of four members, whose protein products are referred to as Sp1-Sp4. Sp1, Sp2 and Sp4 are activators of gene transcription, whereas Sp3 can be an activator or a repressor. In this aspect, TSA treatment of MiaPaCa-2 cell line induced accumulation of acetylated histones in chromatin associated with the TGF-β RⅡ gene. MiaPaCa-2 pancreatic cancer cells acquired resistance to growth inhibition by TGF-β associated with

reduced transcription of TGF-β RⅡ. Accumulation of TGF-β RⅡ with highly acetylated histones H3 and H4 was noted in TSA-treated compared to untreated MiaPaCa-2 cells[37]. Furthermore, TSA activated TGF-β RⅡ promoter activity in a panel of five pancreatic cancer cell lines (BxPC-3, PANC-1, CFPAC-1, MiaPaCa-2 and UK Pan-1), by mechanisms involving induction of Sp1 acetylation and changes in a multiprotein complex containing p300, PCAF, Sp1 and NF-Y[38,39].

Treating the pancreatic carcinoma cell line MIA PaCa-2 with TSA, increased the O(6)-Methylguanine-DNA methyltransferase (MGMT) mRNA and protein levels by 2-3-fold, caused by increased histone acetylation in the endogenous MGMT promoter region, which was also associated with CBP/p300. MGMT is a suicide enzyme that repairs pre-mutagenic, pre-carcinogenic and pre-toxic DNA damage O(6)-methylguanine. MGMT also likely protects against therapy-related tumor formation caused by highly mutagenic drugs. The MGMT expression level provides important information on cancer susceptibility and the success of therapy[40]. TSA treatment also resulted in a marked (5-fold) induction of 2.3% of genes in AsPC1, 1.9% in Hs766T, 1.1% in MiaPaCa2, and 2.5% in Panc1. A large panel of novel targets for silencing by histone deacetylation were identified, including several known tumor suppressor or cell cycle-regulatory genes, such as *ING1*, *p57KIP2*, *CHES1*, *CHFR* and *GADD45B*. One of the novel findings of this study was that TSA alone induced the expression of four of the 11 genes whose CpG islands were identified as aberrantly methylated in pancreatic cancer[41]. Among the proteins with altered expression post-TSA treatment in Paca44 and T3M4 pancreatic cancer cell lines, of particular interest are the two downregulated proteins nucleophosmin and translationally controlled tumor protein, which are involved in oncogenesis and tumor reversion, respectively. Additionally, several other proteins were found to be upregulated, including programmed cell death protein 5 (TFAR19), which is involved in the regulation of cell apoptosis, and stathmin (OC18), which promotes microtubule depolymerization during interphase and late mitosis. TSA could inhibit cell proliferation of the pancreatic adenocarcinoma cell line Paca44 by cell growth arrest at the G2 phase and apoptosis[42,43].

Maspin is a unique member of the serpin family of protease inhibitors with tumor suppressive activity in different cancer types. Interestingly, the maspin gene is located on chromosome 18q21.3 and its promoter region contains the binding sites of several transcription factors that positively regulate its expression, including Ets, AP1, HER and p53. When PANC-1 cells were exposed to 5-Aza-C, maspin mRNA expression was restored in a dose dependent manner. TSA also led to re-expression of maspin[44]. Five pancreatic cancer cell lines (AsPC1, CFPAC1, Hs766T, MiaPaCa-2, and Panc1) were screened for genes that displayed expression patterns associated with hypomethylation. This analysis identified 1485 transcripts that were likely to be variably expressed in pancreatic cancer cell lines. Among the 1485 transcripts identified, 392 were found to be elevated by 3-fold or greater in any of the pancreatic cancer cell lines after combined treatment with 5-Aza-C and TSA. This list included several genes that have been reported to be overexpressed in pancreatic cancer, such as those encoding cysteine-rich protein 1 (CRIP1), decay accelerating factor for complement (CD55), maspin/SERPINB5, S100 calcium-binding protein P (S100P), and tissue-type plasminogen activator (PLAT). Treatment of MiaPaCa-2 cells with 5-Aza-C restored the expression of maspin mRNA, whereas treatment with TSA alone did not, but combined treatment with 5-Aza-C and TSA strongly induced the maspin expression in a synergistic manner[45,46]. CDKN1C is a potent inhibitor of several G1 cyclin complexes, and is a negative regulator of cell proliferation. Treatment of the pancreatic cancer cell lines AsPC1 and BxPC3, where the CDKN1C gene was silenced, with 5-Aza-C or TSA, or their combination, resulted in restoration of CDKN1C expression, more potently with TSA and the combined treatment[47].

MUC2, *MUC5AC*, *MUC5B* and *MUC6* mucin genes encode large secreted O-glycoproteins that participate in mucus formation and play an important role as a physiological barrier against various attacks on the underlying epithelia. Among the four 11p15 mucin genes, *MUC2* and *MUC5B* were highly susceptible to DNA methylation and histone modifications, whereas *MUC5AC* was rarely influenced by epigenetic regulation and *MUC6* was not. In this context, pancreatic cell lines CAPAN-1 and PANC-1 were treated with 5-Aza-C or TSA. In PANC-1 cells TSA treatment induced MUC2 expression, while in CAPAN-1 cells 5-Aza-C treatment induced an increase of *MUC5AC* mRNA and *MUC5B* expression. Histone deacetylation was also involved in *MUC5B* repression, as TSA treatment induced its expression[48]. Treatment of the human pancreatic cancer cell lines PANC1 (MUC2-negative) and BxPC3 (MUC2-positive) with both 5-Aza-C and TSA, resulted in a definite increase of the expression level of *MUC2* mRNA[49]. In pancreatic cancer, *MUC4* overexpression is associated with a bad prognosis and has become an important molecular target. In this aspect, the pancreatic cancer cell lines PANC-1, CAPAN-1 and CAPAN-2 were tested. In *MUC4*-nonexpressing pancreatic PANC-1 cells, treatment with 5-Aza-C and TSA induced *MUC4* expression at the mRNA level, and at protein level in a small number of cells. In *MUC4*-high expressing pancreatic CAPAN-1, 5-Aza-C treatment did not affect MUC4 mRNA, whereas TSA treatment induced a strong inhibition of *MUC4* mRNA levels, correlated to a strong decrease of the apomucin level in the cells[50].

Two pancreatic cancer cell lines, MiaPACA-2 and PANC-1, were treated with 5-Aza-C or TSA, and their combination. Fourteen miRNAs were upregulated by ⩾ 2-fold in each of the cell lines following exposure to both agents. Enforced expression of miR-107 in MiaPACA-2 and PANC-1 cells downregulated *in vitro* growth that was associated with repression of the putative miR-107 target,

cdk6, thereby providing a functional basis for the epigenetic inactivation of this miRNA in pancreatic cancer[51].

Evaluating the *in vitro* growth inhibition of several pancreatic cancer cell lines established from primary tumors, as well as that of others established from metastatic tumors, to gemcitabine and 5-FU, newer generation cytotoxic agents (oxaliplatin, irinotecan), targeted therapy (gefitinib) and TSA, demonstrated that the combination of TSA and irinotecan increased growth inhibition on the highest percentage of cell lines (80%). TSA proved to be the best partner for all drugs, with the exception of 5-FU. Notably, PSN1, the most sensitive cell line to single-drug treatments, became the most resistant cell line to all combined treatments. In addition, all pairwise combinations were less effective in PaCa3, which contains a functional p53 gene, supporting the hypothesis that the p53 gene status may by not relevant for cell sensitization by TSA[52].

SAHA: Both SAHA and its novel compounds 17a and 9, inhibited PANC-1 and PT-45 cells' proliferation in a dose dependent manner, with the novel compounds having a more potent antiproliferative activity. Although p21 gene expression of PANC-1 cells was significantly increased after treatment with SAHA and 17a, none of the HDACIs tested affected the expression of the p27 gene[53].

In another study, SAHA inhibited the growth of BxPC-3 and COLO-357 cell lines, by 42% and 50%, respectively, but not that of PANC-1 cells. SAHA induced a G1 cell cycle arrest and upregulation of p21 in BxPC-3 and COLO-357. On the other hand, PANC-1 cells remained unaffected. According to this study, p21 upregulation was necessary for SAHA-induced cell cycle arrest in COLO-357, but not in BxPC-3 cells. PANC-1 cells, which were resistant to gemcitabine alone, exhibited a marked increase in sensitivity when treated with both gemcitabine and SAHA[54].

Additive and time-dependent reduction of cell proliferation and induction of apoptosis by SAHA and the novel DNA methyltransferase inhibitor Zebularine was reported in pancreatic cancer cell lines YAP C, DAN G and Panc-89. In fact, the apoptosis induction was associated with downregulation of Bcl-2 and upregulation of Bax[55]. SAHA inhibited the cell growth in six pancreatic cancer cell lines in a dose-dependent manner. G2/M cell cycle arrest was also induced by SAHA in most cell lines. Remarkably, SAHA and 5-Aza-C treated cells, presented higher levels of acetylated-H3 than those noted in cells treated with SAHA alone. Treatment with SAHA markedly enhanced histone H3 acetylation in the promoter region of the p21 gene. In PANC-1 cells, p21 protein expression increased to the same levels after exposure to either 5-Aza-C, SAHA or both, while p27, Bax and Bcl-2 levels remained unaltered. Levels of p57, E-cadherin and RARa increased in the presence of SAHA, either alone or with 5-Aza-C. Additionally, SAHA decreased expression of cyclin D1, B1 and c-myc independently of the β-catenin pathway and increased C/EBPa[56].

Treatment of PANC1, MiaPaca2 and ASPC-1 cells with concentrations of SAHA and sorafenib that are sustainable in patient serum resulted in a greater than additive increase in tumor cell killing, as assessed by short-term death assays, and a synergistic increase in killing assessed by colony formation assays. Suppression of caspase 8 function, as well as expression of dominant negative caspase 9 or Bcl-XL also blunted sorafenib-SAHA lethality. Sorafenib-SAHA, but not treatment with the individual drugs, activated CD95 and caused formation of a death-inducing signal complex containing caspase 8, FADD, ATG5 and Grp78/BiP. Additionally, a clinically relevant and sustainable concentration of sodium valproate, another HDACI, enhanced sorafenib lethality in a synergistic fashion in pancreatic tumor cells derived from either humans or rodents. Finally, it was speculated that small-molecule antagonists of Bcl-2 family proteins (HA14-1, GX15-070) enhanced sorafenib-HDACI lethality *via* autophagy and partial activation of the intrinsic apoptosis pathway, independently of death receptor functionality[57-59].

A combination of SAHA and the Smoothened antagonist, SANT-1, was evaluated for their ability to suppress growth of the gemcitabine-resistant pancreatic adenocarcinoma cell lines Panc-1 and BxPC-3. The combination of SAHA and SANT-1 supra-additively suppressed cellular proliferation and colony formation *via* induction of apoptotic cell death, cell cycle arrest in G0/G1 phase and ductal epithelial differentiation. Cell death was associated with nuclear localization of survivin, increased Bax expression and activation of caspases-3 and -7. Consistent with the cell cycle arrest and cytodifferentiation, the CdkIs p21 and p27 were upregulated and cyclin D1 was downregulated. The potentiated anti-proliferative effect by the combination of SAHA and SANT-1 was attributed to cooperative suppression of the Hedgehog pathway activity, as shown by the upregulation of hedgehog interacting protein by SAHA, and enhanced repression of Ptc-1 mRNA expression[60].

SAHA induced apoptosis in IMIM-PC-1, IMIM-PC-2 and RWP-1 cell lines with a serine protease-dependent and caspase-independent mechanism. SAHA induced a decrease in the number of cells in S phase in all three cell lines, while an increase in the sub-G1 peak was noted, suggesting that the three cell lines underwent apoptosis[28].

SAHA and the proteasome inhibitor bortezomib (PS-341) were tested in a panel of pancreatic cancer cell lines. Both SAHA and TSA blocked bortezomib-induced aggresome formation in pancreatic cells, which is a cytoprotective mechanism, and dramatically sensitized aggresome-positive cells to bortezomib-induced apoptosis[61].

RELN, a key regulator of neuronal migration, is frequently silenced in pancreatic cancers. RELN is a secreted extracellular protein that plays an essential role in brain development and function. Underexpression of RELN and its components (ApoER2, VLDLR and DAB1) is related to cell motility, invasiveness, and colony-forming ability in cancer. Treatment of pancreatic adenocarcinoma cell lines Panc1 and AsPC1 with SAHA and valproic acid, restored

the expression of RELN and DAB1 in a dose-dependent manner, and also inhibited cell migration[62]. SAHA inhibited proliferation of MiaPaCa-2 and AsPC-1 PDAC cells in a dose-dependent manner and further enhanced the radiation-induced apoptosis (additive but not synergistic). Radiosensitization of these cells was ascribed to inhibition of DNA repair and suppression of radiation-induced EGFR and NF-κB prosurvival signaling pathways[63]. In another study, using 3-D agarose colonies of MiaPaCA cells, synergy occurred when SAHA was combined with carboplatin at short exposure times, providing evidence that SAHA may allow a reduction in the standard dose of carboplatin, with improvement in the overall therapeutic index[64]. Thirty genes were identified as constitutively silenced in PANC-1 cells, and were upregulated post-SAHA and/or 5-Aza-C treatment. Interestingly, among them, 10 genes were known cancer antigens, suggesting that many of these antigens may be silenced by acetylation and/or methylation in PANC-1 cells[65]. The Rel/NF-κB family consists of various members of transcription factors, such as RelA/p65, which are responsible for the regulation of cytokines, their receptors and cell adhesion molecules. Overexpression or dysregulation of certain regulatory proteins of the NF-κB pathway, have been associated with poor prognosis in different cancer types. Treatment of the pancreatic cancer cell line PANC-1 with SAHA resulted in a time dependent reduction of RelA/p65 activity of up to 50%, while valproic acid (VPA) treatment decreased RelA/p65 activity by approximately 25%, affecting also its subcellular localization. Neither SAHA nor VPA affected the protein levels of IκBα, but inhibited its phosphorylation. According to this study, strong antineoplastic effects of SAHA could be partly based on an alteration of the NF-κB signaling pathway[66].

Cyclic peptides

FK228 (FR901228, depsipeptide, romidepsin): Depsipeptide (1S,4S,7Z,10S,16E,21R)-7-ethylidene -4,21-bis (1-methyletheyl)-2-oxa-12,13-dithia-5,8,20,23-tetraazabicyclo [8.7.6] tricos-16-ene-3,6,9,19,22-pentone, is a bicyclic peptide isolated from *Chromobacterium Violaceum* and has demonstrated potent *in vitro* cytotoxic activity against human tumor cell lines and *in vivo* efficacy against human tumor xenografts. Upon entering cells, FK228 is reduced to an active compound, capable of preferentially interacting with the zinc in the active site of the HDAC class I enzymes; however, it is still generally classified as a broad-spectrum inhibitor as it also inhibits class Ⅱ enzymes. It was approved by the United States Food and Drug Administration for the treatment of cutaneous T-cell lymphoma[67].

FK228 markedly inhibited the proliferation of five pancreatic cancer cell lines, with the greatest effect on MIAPaCa-2 cells. FK228 treatment induced cell cycle arrest at the G1 or G2/M phase and subsequent apoptosis. The induced hyperacetylation of histone H3 was accompanied by p21 overexpression and caspase-3 activation, leading to cleavage of p21, and by dramatic downregulation of survivin[68]. Treatment with FK228, bortezomib or both, sensitized two out of three pancreatic cancer cell lines tested, to NK cells. Tumors sensitized to NK cell cytotoxicity showed a significant increase in surface expression of DR5, which induces the TRAIL pathway of apoptosis. Expressions of MHC class I , MIC-A/B, DR4 and Fas did not alter in different cell lines[69]. Interestingly, in another study, depsipeptide could induce demethylation of both the p16 and GATA4 promoters in PANC1 cells, among other cancer cell lines, assayed with bisulfite sequencing (region D of the p16 promoter and region B of the GATA4 promoter). Additionally, depsipeptide could induce a significant inhibition of cell proliferation in PANC1 cells. In this study, a novel mechanism of HDACI-mediated DNA demethylation *via* suppression of histone methyltransferases was suggested[70].

Short chain fatty acids

VPA: VPA is now an established antiepileptic drug through its effect on the function of the neurotransmitter GABA. The finding that VPA was an effective inhibitor of HDACs came from the observations that VPA was able to relieve transcriptional repression of a peroxisomal proliferation and activation of a glucocorticoid receptor (GR)-peroxisome-proliferation-associated receptor (PPAR)ε hybrid receptor and a RAR-dependent reporter gene expression system, suggesting that it acts on a common factor in gene regulation, such as corepressor-associated HDACs, rather than on individual transcription factors or receptors. Consistent with this finding, it was shown that VPA causes hyperacetylation of the N-terminal tails of histones H3 and H4 *in vitro* and *in vivo* and was found to inhibit HDAC enzymatic activity at a concentration of 0.5 mmol/L[71]. VPA has shown potent antitumor effects in a variety of *in vitro* and *in vivo* systems, by modulating multiple pathways, including cell cycle arrest, apoptosis, angiogenesis, metastasis, differentiation and senescence. Most of preclinical and clinical data on the anticancer effects of VPA has been generated for malignant hematological diseases[72].

Neutral endopeptidase (NEP/CD10) is a cell surface Zn metalloprotease that inactivates multiple physiologically active peptides. Loss of, or decrease in, NEP/CD10 expression has been reported in many types of malignancy. VPA treatment resulted in increase of NEP/CD10 protein expression accompanied with a significantly reduced growth in PATU-8988T and HUP-T4 cells. The VPA effect on the proliferation in HUP-T4, as well as in HUP-T3, cells was much lower than in the high NEP/CD10-expressing PATU-8988T cells[73]. VPA treatment of DanG cells resulted in a significant reduction of cell proliferation, an effect that was depended on the drug exposure time. VPA evoked a significant blockade of both DanG tumor cell adhesion to HUVECs, particularly when the compound was applied for 5 days. Additionally, VPA treatment modified the integrin surface profile on pancreatic cancer DanG cells[74]. MiaPaCa2 and Panc1 cell lines were also treated with the topoisomerase Ⅱ inhibitor etoposide and VPA. VPA-mediated depletion of HDAC2

was observed in MiaPaCa2 and Panc1 cells. The apoptotic fraction of VPA/etoposide co-treated MiaPaCa2 and Panc1 cells was significantly increased compared to etoposide-treated pancreatic cancer cells. Sensitization by VPA to etoposide (DNA-damage induced apoptosis) was noted in both cell lines. This effect was not observed when VPA was used in combination with other drugs, such as gemcitabine, 5-FU or oxaliplatin. Such data suggested that HDAC2 inhibition might result in specific sensitization towards DNA damage-induced apoptosis[75]. Treatment of Panc1 cells with VPA and SAHA, at different concentrations, induced the mRNA expression of RELN and DAB1 in a dose-dependent manner. VPA was also shown to prevent the epigenetic downregulation of RELN, leading to the inhibition of migration of Panc-1 cells[62].

4-phenylbutyrate: 4-phenylbutyrate (4-PB) is a short-chain fatty acid that reversibly inhibits class I and Ⅱ HDACs. It is considered as an HDAC inhibitor of the first generation, as the HDAC inhibitory effect is not specific. Working concentrations are rather high, in the millimolar range, and the effects are pleiotropic. 4-PB exerts multiple effects in the cell, including the modulation of protein isoprenylation, which regulates the ras proto-oncoprotein, and activation of the nuclear steroid PPAR[76]. 4-PB exerts a potent antitumor effect *in vitro* and causes growth inhibition and differentiation in various human cancer cell lines[77].

A set of normal fibroblast and cancer cell lines, among them the pancreatic cancer CFPAC-1, were treated with 5-Aza-C and 4-PB. Upregulation of the cell cycle inhibitors p16 and p21 was induced post-5-Aza-C and 4-PB treatment. Interestingly, both normal and cancer cells presented very similar induction levels after combination treatment[78]. Expression profiling of different cancer cell lines revealed upregulation of several miRNAs by simultaneous treatment with 5-Aza-C and 4-PB. One of these, miR-127, is embedded in a CpG island and is highly induced from its own promoter after treatment. miR-127 is usually expressed as part of an miRNA cluster in normal but not in cancer cells, suggesting that it is subject to epigenetic silencing. Additionally, the proto-oncogene *BCL6,* a potential target of miR-127, was translationally downregulated after treatment of several lines with the combination of drugs[79]. 4-PB inhibited HDAC activity by 60%-70% in the cancer cell lines T3M-4 and BxPc3. Treatment with 4-PB inhibited growth and induced apoptosis of Panc1, T3M-4, COLO 357 and BxPc3 pancreatic adenocarcinoma cell lines in a dose- and time-dependent manner. Concentration-dependent cell cycle arrest was noted in T3M-4 and COLO 357 cells, which was not verified in Panc 1 and BxPc3 cells. In addition, 4-PB increased gap junction communication between T3M-4 cells, allowing exchange of apoptotic signals between neighboring cells. Furthermore, 4-PB inhibited cellular export mechanisms. 4-PB increased gemcitabine-mediated apoptosis of two resistant cell lines T3M-4 and BxPc3. Activation of caspase-8 was enhanced, as well as that of Bid and PARP-cleavage. No differences in the expression levels of caspase -2 and -3 and IAPs were noted. Finally, no influence of MEK or p38 on gemcitabine-mediated cell death in these cells was found. In contrast, inhibition of JNK completely abolished the sensitizing effect of 4-PB[80].

Sodium butyrate (NaBu): Sodium butyrate has multiple effects on cultured mammalian cells, including inhibition of proliferation, induction of differentiation and induction or repression of gene expression. NaBu inhibits most HDACs, except class Ⅲ HDAC and class Ⅱ HDAC6 and -10. Promoters of butyrate-responsive genes have butyrate response elements, and the action of butyrate is often mediated through Sp1/Sp3 binding sites[81].

NaBu induced a dramatic decrease in cell proliferation and an increase in ALP activity in PANC-1 cells. NaBu also induced a number of morphologic alterations in these cells, including increase in the cytoplasmic secretory elements and enhancement of differentiation[82]. Both NaBu and the natural butyrate prodrug tributyrin, inhibited growth and induced apoptosis in MiaPaca-2 and Capan-l cells and stimulated differentiation in Capan-l cells, as indicated by alterations of ALP levels[83]. NaBu also induced differentiation and apoptosis in the human pancreatic cancer cell line AsPC-1, as well as increased K23 mRNA levels[84]. NaBu treatment resulted in a significant reduced cell growth of PATU-8988T cells and an increase of NEP/CD10 protein levels[73]. Additionally, NaBu treatment induced cell growth inhibition and apoptosis in four lines (ASPC-1, PANC-1, PT45 and PACA44) with different susceptibility. Bcl-xL expression was strongly downregulated by NaBu in a time-dependent manner, whereas Bax expression was not affected. NaBu enhanced the intrinsic pathway of apoptosis, including mitochondrial membrane depolarization, cytochrome c translocation to the cytosol, caspase-3 and -9 activation, although it had no effect on caspase-8. NaBu also enhanced the extrinsic pathway of apoptosis, sensitizing pancreatic cancer cell lines to Fas-mediated signals[85]. Moreover, NaBu inhibited the ability of several pancreatic cell lines to form colonies in soft agar. Cellular ALP levels were markedly increased post-treatment[86,87]. Treatment of pancreatic cancer cell line CAPAN-1 with 1 mmol/L NaBu reduced the rate of cellular growth and inhibited colony forming ability in soft agar, but did not suppress cell growth. These effects were completely reversible on removal of NaBu and were therefore not causing a terminal differentiation step or a loss of cell viability. Significant changes in proteins and glycoproteins of CAPAN-1 occurred with NaBu treatment[88]. Treatment with NaBu strongly inhibited growth of pancreatic carcinoid BON cell line. It was found that NaBu increased levels of 5-hydroxytryptamine in the cells, as a differential effect[89]. TGF-α but not TGF-β mRNA levels were decreased after NaBu treatment in CAPAN-1 cells, while the membrane-bound protein kinase C activity was also reduced[90].

Oligosaccharide antigens are commonly used as tumor markers. Such antigens control tumor cell adhesion,

motility and invasiveness, being synthesized by a series of glycosyltransferases. In MiaPaCa-2, PSN-1 and PK59 cell lines, the expression of GnT-IVa (N-acetylglucosaminyltransferase-IVa) was increased after NaBu treatment[91]. Carcinoembryonic antigen (CEA) expression of human pancreatic adenocarcinoma cells and differentiation features were studied and compared in the well differentiated and CEA-producing CAPAN-1 and the poorly differentiated PANC-1 cell line post-NaBu treatment. NaBu reduced colony formation in both cell lines by approximately 50%. Significant ultrastructural alterations were noted only in the PANC-1 cells, including increased intercellular desmosomes, tonofilaments and lipid droplets. NaBu increased CEA expression in CAPAN-1 cells, but had no effect on CEA expression in PANC-1 cells. Thus, CEA expression and state of differentiation were independently affected[92]. NaBu inhibited the growth of pancreatic cancer cell lines PC-1 and PC-1.0 (hamster) and HPAF, CD11, CD18 and PANC-1 (human), and induced cell enlargement, an increase in secretory material, microfilaments and pseudopodia. NaBu increased the expression of blood group A, DU-PAN-2 and CA 19-9 tumor associated antigens[93]. Treatment with NaBu, slightly increased immunoreactive trypsin 1 (IRT) levels in both human pancreatic adenocarcinoma cell lines CF-PAC-1 (established from a patient with cystic fibrosis) and CAPAN-1, while growth inhibition was significant. Consequently, IRT levels or differentiation state did not correlate with cellular growth[94].

NaBu treatment inhibited the cell growth of four pancreatic cancer lines (PT45, PaTu-Ⅱ, Panc-1 and A818-1) more potently than all-trans retinoic acid (ATRA). Additionally, neuroendocrine markers synaptophysin and Lcu7 in Panc-1 cells were highly expressed[95]. The expression of b4 and b7 integrin chains correlates with tumor invasiveness. In this aspect, it was documented that NaBu inhibited b4 integrin expression in AsPC-1 cells, inhibiting pancreatic tumor invasion. NaBu also reduced the expression of the b7 integrin chain, which was expressed only in the more aggressive pancreatic cancer cell lines[96]. The cellular morphological characteristics of the PANC-1 cell line treated with NaBu appeared more differentiated, in a dose-dependent manner. The EGFR expression of NaBu-treated PANC-1 cells was decreased in a dose-dependent manner. Ezrin is a cytosolic molecule that cross-links the plasma membrane to actin filaments and has functions related to cell motility, signal transduction, cell-cell and cell-matrix recognition, invasion and metastasis. Both membranous ezrin expression of PANC-1 cells and mRNA expression of ezrin were decreased[97].

A novel bioconjugate (HA-But) obtained by the esterification of butyric acid (BA) with hyaluronic acid (HA), the main constituent of the ECM, which selectively recognizes transmembrane receptor CD44, was developed. All HA-But treated cell lines, including the pancreatic cancer cell line MiaPaCa, were responsive to the antiproliferative effect of HA-But in a dose-dependent manner with cell growth inhibition higher than that observed in the presence of BA alone. Like BA, HA-But induced hyperacetylation of histone H4, a dose-dependent overexpression of some G1/S transition-related proteins, including the CdkIs p27 and p21, and the block of cell growth in the G0/G1 phase of the cell cycle[98]. In another study, HA-But induced a dose-dependent inhibitory effect with an almost complete suppression of MIA PaCa-2 cell growth at the highest concentration used. A decrease in the number of cells in S phase and a concomitant increase of those in G0/G1 or G2/M phase were noted. HA-But decreased cyclin D1 and mut-p53 and increased p27 and p21 protein levels. Additionally, HA-But slightly increased the level of Bax and caspase-7, slightly decreased Bcl-2, but strongly decreased survivin protein levels, providing to be active on both Bcl-2 and survivin-mediated apoptosis pathways[99].

Benzamides

MS-275: This synthetic benzamide derivative 3-pyridylmethyl-N-{4-[(2-aminophenyl)carbamoyl]benzyl}carbamate inhibits HDACs, and has anti-tumor activity in many preclinical models. The first clinical trial with this agent in 2005 included patients with advanced solid tumors or lymphoma. At high concentrations of MS-275, there is a marked induction of reactive oxygen species, mitochondrial damage, caspase activation and apoptosis. Treatment of sensitive tumor cell lines with MS-275 induces gelsolin, a maturation marker, and produces a change in the cell cycle distribution with a decrease in S phase and an accumulation of cells G_1. The *in vivo* therapeutic efficacy of MS-275 has been demonstrated in a variety of human tumor xenograft models[100].

The addition of MS-27-275 to cell cultures resulted in the accumulation of hyperacetylated H4 molecules. MS-27-275 transcriptionally induced p21 and gelsolin (tumor suppressor) through acetylation of histones affecting cell cycle progression. In addition, pRb molecules were reduced. The response of Capan-1 cell line, presenting a p53 mutation, to MS-27-275 treatment was moderate, although expression of gelsolin was induced. The gelsolin induction by MS-27-275 seemed to have no correlation with the sensitivity of the cells to the treatment. Additionally, p21 induction was considered crucial for the action of MS-27-275[101].

TSA, NaBu and MS-275 inhibited the growth of the NET cell lines CM and BON in a dose-dependent manner. In both cell lines, HDAC inhibition resulted in a dose-dependent increase of caspase-3 enzyme activity without affecting cell membrane integrity or exerting immediate necrotic effects. Treatment with HDACIs resulted in cell cycle arrest of the NET cells at G1, thereby decreasing those in the S phase. MS-275 treatment of CM and BON cells resulted in a dose-dependent decrease of Bcl-2, whereas Bax remained unaffected in both cell lines. Cyclin D1 was also downregulated in CM and BON cells by MS-275 treatment, while p21 and p27 were markedly increased[102].

MGCD0103: MGCD0103 is an isotype-specific aminophenylbenzamide that inhibits HDAC classes I and Ⅳ,

Table 2 Studies of histone deacetylase inhibitors and different pancreatic cancer cell lines in xenograft models

HDAC inhibitor	Pancreatic cancer cell line	Time schedule	Dosage schedule	Results	Ref.
TSA (+ gemcitabine)	T3M4	q28 d	0.25 mg/kg *ip*, biweekly	50% tumor weight reduction 3-fold H4 increase	[33]
SAHA (+ bortezomib)	L3.6pl	q21 d	50 mg/kg *ip*, daily	About 70% tumor weight reduction aggresome disruption	[61]
SAHA (+ Zebularine)	Panc-89, YAP C	(1) q7 d or q14 d (2) q7 d	(1) 50 mg/kg *ip*, daily (2) 50 mg/kg *ip*, daily	Tumor growth inhibition Upregulation of CK7, CK20 Downregulation of CK8, Vimentin, chromogranin-A	[55]
FK228	CAPAN-1	q14 d-q21 d	1.5 mg/kg *ip*, biweekly	50% tumor growth inhibition	[108]
MS-275	CAPAN-1	q28 d	(1) 12.3 mg/kg per os (2) 24.5 mg/kg per os (3) 49 mg/kg per os, 5 × weekly	Moderate growth inhibitory effect	[101]
	CAPAN-1, MiaPaca, Panc-1, Panc-15	Not defined	per os once daily (dosage not defined)	Mixed response: moderate growth inhibitory effect/ resistant to inhibition	[109]
NVP-LBH589 (+ gemcitabine)	HPAF-2, L3.6pl	q28 d	25 mg/kg *ip*, 5 × weekly	63% tumor weight reduction (HPAF-2) About 80% tumor weight reduction (L3.6pl) MIB-1 slight reduction TUNEL slight induction (HPAF-2)	[113]

HDAC: Histone deacetylase; TSA: Trichostatin A; HPAF: Suberoylanilide hydroxamic acid; TUNEL: Transferase dUTP nick end labeling.

with almost no class Ⅱ effect. MGCD0103 is well-tolerated and exhibits favorable pharmacokinetic and pharmacodynamic profiles, demonstrating target inhibition and clinical responses. It induces cell death and autophagy, synergizes with proteasomal inhibitors and affects non-histone targets, such as microtubules[103].

A comparative study in order to estimate the pharmacological properties of second generation HDACIs with the hydroxamate and benzamide head group, namely SAHA, LAQ824/LBH589, CI-994, MS-275 and MGCD0103 was carried out. SAHA and LAQ824/LBH589 seemed to behave as quite unselective HDACIs, while the benzamides CI-994, MS275 and MGCD0103 were more selective HDAC1 and HDAC3 inhibitors. All the compounds induced histone H3 hyperacetylation, as well as cell differentiation and apoptosis and inhibited proliferation. A broad cytotoxicity was seen across different tumor cell lines, among them the pancreatic lines AsPC-1, BxPc3 and Panc-1, with LAQ824/LBH589 being the most potent agents[104,105]. The inhibitory activities of MGCD0103, MS-275 and SAHA were also compared using a panel of cancer cell lines, among them Panc-1. Although the measured IC50 values varied between cell lines, MGCD0103 was always more potent than the comparator molecules in all cases examined, being at least 7-fold more potent than SAHA in PANC-1 pancreatic cancer cells[106].

IN VIVO EXPERIMENTAL STUDIES

The data available so far regarding the different classes of HDACIs used in *in vivo* animal studies of pancreatic cancer are presented in the following section and are listed in Table 2.

Hydroxamic acids

TSA: *In vivo* studies on xenografts of pancreatic cancer line T3M4 in nude mice, showed that the combination of TSA (0.25 mg/kg) and gemcitabine (2.5 mg/kg), given biweekly for 4 wk, led to a reduction in the mean tumor weight by about 50% compared to control or single drug treatments. Additionally, TSA treatment resulted in only a 3-fold increase of H4 acetylation levels *in vivo* compared to the 18-fold increase noted *in vitro*. None of the treatments produced any toxicity, as indicated by lack of change in body weight, and none of the animals developed ulcerating tumors[33]. Finally, TSA exerted an inhibitory effect on the DMBA-induced carcinogenesis model and the growth of pancreatic ductal adenocarcinoma in rats, by upregulating *KiSS-1*, a metastasis suppressor gene located on 1q32, which is considered to exert an important role in inhibiting the invasion and metastasis of pancreatic cancer[107].

SAHA: Effects of bortezomib and SAHA in orthotopic human pancreatic tumors (cell line L3.6pl) were investigated. Tumors were treated biweekly with 1 mg/kg bortezomib, daily with 50 mg/kg SAHA, or a combination of the two agents for 21 d. *In vivo* data showed aggresome disruption by the combination and reduction of pancreatic tumor weight, with minimal toxicity noted, what there was being related to bortezomib[61]. Another experiment in mice presented tumor suppression when treated with SAHA and the DNA methyltransferase inhibitor Zebularine. The first experimental setting included a single intraperitoneal (*ip*) bolus injection with SAHA (50 mg/kg), Zebularine (1 g/kg) or the combination of both agents, and the animals were sacrificed after 7 or 14 d. For daily treatment, animals received the same doses of drugs over a time-course of 1 wk. Gemcitabine as a control therapy was administered *ip* every fourth day. The hypermethylated cell line Panc-89 was more susceptible to SAHA than YAP C. No adverse effects were noted and all animals

survived. Furthermore, the expression of CK7, which is a marker associated with pancreatic cancer cell differentiation, was higher in Zebularine and combined Zebularine/SAHA-treated xenografts, as well as that of the glandular differentiation marker CK20. The expression of CK8, Vimentin and chromogranin-A was lower or constant in Zebularine and Zebularine/SAHA-treated animals[55].

Cyclic peptides

FK228 (FR901228, depsipeptide): More than 90% of human pancreatic cancers are associated with oncogenic mutations of RAS, in particular K-RAS at codon 12. The Tyr-kinase inhibitors, PP1 and AG, block the RAS mutation-induced activation of PAK1, which is the Rac/CDC42-dependent Ser/Thr kinase. PAK1 is essential for RAS-transformation. Based on these data, the therapeutic potential of either FK228, the combination of these two Tyr-kinase inhibitors or GL-2003, a water-soluble derivative of AG 879, on human pancreatic cancer (Capan-1) xenograft in mice, was examined. Capan-1 cells were injected sub-cutaneously (*sc*) into several groups of nude mice. Each group was treated *ip* with either FK228 (1.5 mg/kg), GL-2003 (20 mg/kg), a combination of PP1 and AG 879 (20 mg/kg of each drug), a combination of PP1 and GL-2003 (20 mg/kg of each drug), or vehicle alone (0.1 mL of 1% DMSO in PBS) as the control, twice a week for 2-3 wk. No adverse effects were detected. The most effective combination was that of GL-2003/PP1 that suppressed cell growth by around 80%, while FK228 alone showed only 50% inhibition. The synergy between GL-2003 and PP1 in blocking the RAS-induced PAK1 activation was not observed *in vitro*[108].

Benzamides

MS-275: MS-275 administered orally, once daily, 5 d per week for 4 wk, strongly inhibited the growth in 7 out of 8 tumor cell lines implanted into nude mice, although most of these did not respond to 5-FU. Tumors were passaged several times before starting *in vivo* antitumor testing, a tumor lump (2-3 mm in diameter) was transplanted *sc* into the flank of a nude mouse, and the therapeutic efficacy of MS-27-275 was examined. MS-27-275 at 49 mg/kg showed a moderate effect against the only pancreatic Capan-1 tumor. The drug at 24.5 mg/kg and 12.3 mg/kg also showed significant effects against these tumors. As the dose of 49 mg/kg was the maximum tolerated one in this administration schedule, and apparent signs of toxicity, such as weight loss and poor appearance, were reported. The maximum dose of the drug was lowered to 24.5 mg/kg, at which no gross weight loss was observed[101]. Additionally, the pancreatic carcinoma cell lines Capan1, MiaPaCa, Panc1 and Panc15 were grown as xenografts in nude mice and afterwards, treated per os once daily with MS-275. For most cell lines used, antitumor activity and dose-dependent response of MS-275 *in vivo* were observed. While various tumor cell lines from other malignancies showed an almost complete response, in pancreatic cell lines a mixed response was achieved, with half of the models tested to present either a moderate growth inhibitory effect or resistance to treatment[109].

CI-994: CI-994 or N-acetyldinaline[4-(acetylamino)-N-(2-amino-phenyl) benzamide] is a novel oral compound with a wide spectrum of antitumor activity in preclinical models. The mechanism of action may involve inhibition of histone deacetylation and cell cycle arrest. CI-994 is currently undergoing clinical trials. Although several changes in cellular metabolism induced by the drug have been characterized, the primary molecular mechanism of its antitumor activity remains unknown[110].

CI-994 was previously identified as having cytotoxic and cytostatic activity against several murine and human xenograft tumor models. CI-994 had activity against 8/8 solid tumors tested among them pancreatic adenocarcinoma 02 and 03[111]. Notably, CI-994 was active against a Pan-02 pancreatic tumor of C57BL/6 mouse origin[112].

Other HDACIs

Pancreatic tumors were induced in nude mice by *sc* injection of HPAF-2 and L3.6pl cells. Animal groups received either NVP-LBH589 (25 mg/kg, 5 × weekly) or gemcitabine (5 mg/kg, 1 × weekly) or a combination of both (NVP-LBH589 at 25 mg/kg, 5 × weekly plus gemcitabine at 5 mg/kg, 1 × weekly) *ip*, whereas the control group received placebo only, for 28 consecutive days. Three days after commencement of NVP-LBH589 or combination treatment, HPAF-2 cell tumors showed a significantly reduced volume compared with the control. Combination therapy was significantly more efficient than gemcitabine treatment alone and significantly more efficient than NVP-LBH589 therapy alone, in both cell lines. Regarding side effects, weight loss was 6% and 25% for the combination treatment in HPAF-2 and L3.6pl cell tumor bearing mice, respectively. Treatment with NVP-LBH589 and the combination slightly reduced proliferation (Ki-67 index) and slightly induced apoptosis markers in HPAF-2 cell bearing mice, whereas proliferation was not decreased and apoptosis only slightly increased in L3.6pl cell bearing mice[113].

CONCLUSION

In this review, the recent advances in the understanding and clinical development of HDACIs were discussed. The exact mechanism and the molecular basis for the antitumor effects of these new drugs are complex and not completely understood. HDACIs regulate the acetylation of histones and many non-histone proteins that are involved in gene expression, cell proliferation, migration and death. HDACIs have been shown to induce differentiation and cell cycle arrest, activate the extrinsic or intrinsic pathways of apoptosis and to inhibit invasion, migration and angiogenesis in different cancer cell lines. Normal cells are relatively more resistant to HDACIs-induced cell death. Although not completely elucidated, the main mechanisms by which HDACIs act in pancre-

atic cancer are common. The cdk inhibitor p21 is one of the most commonly induced genes in various pancreatic cancer cell lines by all HDACIs tested. Transcriptional induction of p21 is associated with G1 cell cycle arrest and growth inhibition. Cell cycle arrest and growth inhibition is also correlated with transcriptional activation of other cell cycle regulatory genes such as p16, p27, cyclin E and gelsolin, while inhibition of cyclins A, B1, D1 and D2 were also noted in many cancer cell lines. The pro-apoptotic proteins Bax, Bad and Bim were upregulated, among others, whereas anti-apoptotic proteins, such as Bcl-2, Bcl-XL and survivin, were downregulated. HDACIs reduced the expression of angiogenetic factors, such as VEGF receptors -1 and -2, and affected the expression of a panel of metastasis promoting genes (Table 1).

Additionally, these drugs exhibited antiproliferative effects in cancer animal models. Various pancreatic carcinoma cell lines were grown as xenografts in nude mice and treated with HDACIs (Table 2). For most lines used, anti-tumor activity and dose-dependent response were observed, with reduction of cell proliferation (Ki-67 index) and induction of apoptosis (transferase dUTP nick end labeling test). The reduction of pancreatic tumor weight was achieved with minimal toxicity.

These results support the efficiency that HDACIs presented *in vitro;* however, more studies and well-controlled experiments are required to obtain stronger *in vivo* data. Furthermore, phase Ⅱ/Ⅲ trials including patients with pancreatic cancer are needed to determine the clinical efficacy of these new drugs.

REFERENCES

1 **Raimondi S**, Maisonneuve P, Lowenfels AB. Epidemiology of pancreatic cancer: an overview. *Nat Rev Gastroenterol Hepatol* 2009; **6**: 699-708 [PMID: 19806144 DOI: 10.1038/nrgastro.2009.17]

2 **Hidalgo M**. Pancreatic cancer. *N Engl J Med* 2010; **362**: 1605-1617 [PMID: 20427809 DOI: 10.1056/NEJMra0901557]

3 **Ries LAG**, Eisner MP, Kosary CL, Hankey BF, Miller BA, Clegg L, Mariotto A, Fay MP, Feuer EJ, Edwards BK. SEER Cancer Statistics Review, 1975-2000. National Cancer Institute, 2003

4 **Micke O**, Hesselmann S, Bruns F, Horst E, Devries A, Schüller P, Willich N, Schäfer U. Results and follow-up of locally advanced cancer of the exocrine pancreas treated with radiochemotherapy. *Anticancer Res* 2005; **25**: 1523-1530 [PMID: 16033054]

5 **Riess H**, Helm A, Niedergethmann M, Schmidt-Wolf I, Moik M, Hammer C, Zippel K. A randomised, prospective, multicenter, phase iii trial of gemcitabine, 5-fluorouracil (5-fu), folinic acid vs. gemcitabine alone in patients with advanced pancreatic cancer. *J Clin Oncol* 2005; **23**: 4009

6 **Rocha Lima CM**, Green MR, Rotche R, Miller WH, Jeffrey GM, Cisar LA, Morganti A, Orlando N, Gruia G, Miller LL. Irinotecan plus gemcitabine results in no survival advantage compared with gemcitabine monotherapy in patients with locally advanced or metastatic pancreatic cancer despite increased tumor response rate. *J Clin Oncol* 2004; **22**: 3776-3783 [PMID: 15365074 DOI: 10.1200/JCO.2004.12.082]

7 **Poplin E**, Feng Y, Berlin J, Rothenberg ML, Hochster H, Mitchell E, Alberts S, O'Dwyer P, Haller D, Catalano P, Cella D, Benson AB. Phase III, randomized study of gemcitabine and oxaliplatin versus gemcitabine (fixed-dose rate infusion) compared with gemcitabine (30-minute infusion) in patients with pancreatic carcinoma E6201: a trial of the Eastern Cooperative Oncology Group. *J Clin Oncol* 2009; **27**: 3778-3785 [PMID: 19581537 DOI: 10.1200/JCO.2008.20.9007]

8 **Oettle H**, Richards D, Ramanathan RK, van Laethem JL, Peeters M, Fuchs M, Zimmermann A, John W, Von Hoff D, Arning M, Kindler HL. A phase III trial of pemetrexed plus gemcitabine versus gemcitabine in patients with unresectable or metastatic pancreatic cancer. *Ann Oncol* 2005; **16**: 1639-1645 [PMID: 16087696 DOI: 10.1093/annonc/mdi309]

9 **Abou-Alfa GK**, Letourneau R, Harker G, Modiano M, Hurwitz H, Tchekmedyian NS, Feit K, Ackerman J, De Jager RL, Eckhardt SG, O'Reilly EM. Randomized phase III study of exatecan and gemcitabine compared with gemcitabine alone in untreated advanced pancreatic cancer. *J Clin Oncol* 2006; **24**: 4441-4447 [PMID: 16983112 DOI: 10.1200/JCO.2006.07.0201]

10 **Heinemann V**, Quietzsch D, Gieseler F, Gonnermann M, Schönekäs H, Rost A, Neuhaus H, Haag C, Clemens M, Heinrich B, Vehling-Kaiser U, Fuchs M, Fleckenstein D, Gesierich W, Uthgenannt D, Einsele H, Holstege A, Hinke A, Schalhorn A, Wilkowski R. Randomized phase III trial of gemcitabine plus cisplatin compared with gemcitabine alone in advanced pancreatic cancer. *J Clin Oncol* 2006; **24**: 3946-3952 [PMID: 16921047 DOI: 10.1200/JCO.2005.05.1490]

11 **Cunningham D**, Chau I, Stocken DD, Valle JW, Smith D, Steward W, Harper PG, Dunn J, Tudur-Smith C, West J, Falk S, Crellin A, Adab F, Thompson J, Leonard P, Ostrowski J, Eatock M, Scheithauer W, Herrmann R, Neoptolemos JP. Phase III randomized comparison of gemcitabine versus gemcitabine plus capecitabine in patients with advanced pancreatic cancer. *J Clin Oncol* 2009; **27**: 5513-5518 [PMID: 19858379 DOI: 10.1200/JCO.2009.24.2446]

12 **Conroy T**, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, Adenis A, Raoul JL, Gourgou-Bourgade S, de la Fouchardière C, Bennouna J, Bachet JB, Khemissa-Akouz F, Péré-Vergé D, Delbaldo C, Assenat E, Chauffert B, Michel P, Montoto-Grillot C, Ducreux M. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med* 2011; **364**: 1817-1825 [PMID: 21561347 DOI: 10.1056/NEJMoa1011923]

13 **Moore MJ**, Goldstein D, Hamm J, Figer A, Hecht JR, Gallinger S, Au HJ, Murawa P, Walde D, Wolff RA, Campos D, Lim R, Ding K, Clark G, Voskoglou-Nomikos T, Ptasynski M, Parulekar W. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol* 2007; **25**: 1960-1966 [PMID: 17452677 DOI: 10.1200/JCO.2006.07.9525]

14 **Rocha-Lima CM**. New directions in the management of advanced pancreatic cancer: a review. *Anticancer Drugs* 2008; **19**: 435-446 [PMID: 18418211]

15 **Bernstein BE**, Meissner A, Lander ES. The mammalian epigenome. *Cell* 2007; **128**: 669-681 [PMID: 17320505 DOI: 10.1016/j.cell.2007.01.033]

16 **Kouraklis G**, Theocharis S. Histone acetylation and cancer. *Acta Oncol* 2003; **42**: 792 [PMID: 14690169]

17 **Gregoretti IV**, Lee YM, Goodson HV. Molecular evolution of the histone deacetylase family: functional implications of phylogenetic analysis. *J Mol Biol* 2004; **338**: 17-31 [PMID: 15050820 DOI: 10.1016/j.jmb.2004.02.006]

18 **Kristensen LS**, Nielsen HM, Hansen LL. Epigenetics and cancer treatment. *Eur J Pharmacol* 2009; **625**: 131-142 [PMID: 19836388 DOI: 10.1016/j.ejphar.2009.10.011]

19 **Marks PA**, Xu WS. Histone deacetylase inhibitors: Potential in cancer therapy. *J Cell Biochem* 2009; **107**: 600-608 [PMID: 19459166 DOI: 10.1002/jcb.22185]

20 **Mai A**, Altucci L. Epi-drugs to fight cancer: from chemistry to cancer treatment, the road ahead. *Int J Biochem Cell Biol* 2009; **41**: 199-213 [PMID: 18790076 DOI: 10.1016/j.biocel.2008.08.020]

21 **Xu WS**, Parmigiani RB, Marks PA. Histone deacetylase inhibitors: molecular mechanisms of action. *Oncogene* 2007; **26**: 5541-5552 [PMID: 17694093 DOI: 10.1038/sj.onc.1210620]

22 **Kouraklis G**, Theocharis S. Histone deacetylase inhibitors: a novel target of anticancer therapy (review). *Oncol Rep* 2006; **15**: 489-494 [PMID: 16391874]

23 **Kouraklis G**, Theocharis S. Histone deacetylase inhibitors and anticancer therapy. *Curr Med Chem Anticancer Agents* 2002; **2**: 477-484 [PMID: 12678732]

24 **Codd R**, Braich N, Liu J, Soe CZ, Pakchung AA. Zn(II)-dependent histone deacetylase inhibitors: suberoylanilide hydroxamic acid and trichostatin A. *Int J Biochem Cell Biol* 2009; **41**: 736-739 [PMID: 18725319 DOI: 10.1016/j.biocel.2008.05.026]

25 **Donadelli M**, Costanzo C, Faggioli L, Scupoli MT, Moore PS, Bassi C, Scarpa A, Palmieri M. Trichostatin A, an inhibitor of histone deacetylases, strongly suppresses growth of pancreatic adenocarcinoma cells. *Mol Carcinog* 2003; **38**: 59-69 [PMID: 14502645 DOI: 10.1002/mc.10145]

26 **Moore PS**, Barbi S, Donadelli M, Costanzo C, Bassi C, Palmieri M, Scarpa A. Gene expression profiling after treatment with the histone deacetylase inhibitor trichostatin A reveals altered expression of both pro- and anti-apoptotic genes in pancreatic adenocarcinoma cells. *Biochim Biophys Acta* 2004; **1693**: 167-176 [PMID: 15363630 DOI: 10.1016/j.bbamcr.2004.07.001]

27 **Bai J**, Sui J, Demirjian A, Vollmer CM, Marasco W, Callery MP. Predominant Bcl-XL knockdown disables antiapoptotic mechanisms: tumor necrosis factor-related apoptosis-inducing ligand-based triple chemotherapy overcomes chemoresistance in pancreatic cancer cells in vitro. *Cancer Res* 2005; **65**: 2344-2352 [PMID: 15781649 DOI: 10.1158/0008-5472.CAN-04-3502]

28 **García-Morales P**, Gómez-Martínez A, Carrato A, Martínez-Lacaci I, Barberá VM, Soto JL, Carrasco-García E, Menéndez-Gutierrez MP, Castro-Galache MD, Ferragut JA, Saceda M. Histone deacetylase inhibitors induced caspase-independent apoptosis in human pancreatic adenocarcinoma cell lines. *Mol Cancer Ther* 2005; **4**: 1222-1230 [PMID: 16093438 DOI: 10.1158/1535-7163.MCT-04-0186]

29 **Ryu JK**, Lee WJ, Lee KH, Hwang JH, Kim YT, Yoon YB, Kim CY. SK-7041, a new histone deacetylase inhibitor, induces G2-M cell cycle arrest and apoptosis in pancreatic cancer cell lines. *Cancer Lett* 2006; **237**: 143-154 [PMID: 16009488 DOI: 10.1016/j.canlet.2005.05.040]

30 **Bai J**, Demirjian A, Sui J, Marasco W, Callery MP. Histone deacetylase inhibitor trichostatin A and proteasome inhibitor PS-341 synergistically induce apoptosis in pancreatic cancer cells. *Biochem Biophys Res Commun* 2006; **348**: 1245-1253 [PMID: 16904634 DOI: 10.1016/j.bbrc.2006.07.185]

31 **Cecconi D**, Donadelli M, Rinalducci S, Zolla L, Scupoli MT, Scarpa A, Palmieri M, Righetti PG. Proteomic analysis of pancreatic endocrine tumor cell lines treated with the histone deacetylase inhibitor trichostatin A. *Proteomics* 2007; **7**: 1644-1653 [PMID: 17443844 DOI: 10.1002/pmic.200600811]

32 **Cecconi D**, Donadelli M, Dalla Pozza E, Rinalducci S, Zolla L, Scupoli MT, Righetti PG, Scarpa A, Palmieri M. Synergistic effect of trichostatin A and 5-aza-2′-deoxycytidine on growth inhibition of pancreatic endocrine tumour cell lines: a proteomic study. *Proteomics* 2009; **9**: 1952-1966 [PMID: 19294695 DOI: 10.1002/pmic.200701089]

33 **Donadelli M**, Costanzo C, Beghelli S, Scupoli MT, Dandrea M, Bonora A, Piacentini P, Budillon A, Caraglia M, Scarpa A, Palmieri M. Synergistic inhibition of pancreatic adenocarcinoma cell growth by trichostatin A and gemcitabine. *Biochim Biophys Acta* 2007; **1773**: 1095-1106 [PMID: 17555830 DOI: 10.1016/j.bbamcr.2007.05.002]

34 **Gahr S**, Ocker M, Ganslmayer M, Zopf S, Okamoto K, Hartl A, Leitner S, Hahn EG, Herold C. The combination of the histone-deacetylase inhibitor trichostatin A and gemcitabine induces inhibition of proliferation and increased apoptosis in pancreatic carcinoma cells. *Int J Oncol* 2007; **31**: 567-576 [PMID: 17671683]

35 **Zhang S**, Cai X, Huang F, Zhong W, Yu Z. Effect of trichostatin a on viability and microRNA expression in human pancreatic cancer cell line BxPC-3. *Exp Oncol* 2008; **30**: 265-268 [PMID: 19112422]

36 **Ouaïssi M**, Cabral S, Tavares J, da Silva AC, Mathieu Daude F, Mas E, Bernard J, Sastre B, Lombardo D, Ouaissi A. Histone deacetylase (HDAC) encoding gene expression in pancreatic cancer cell lines and cell sensitivity to HDAC inhibitors. *Cancer Biol Ther* 2008; **7**: 523-531 [PMID: 18296916]

37 **Ammanamanchi S**, Freeman JW, Brattain MG. Acetylated sp3 is a transcriptional activator. *J Biol Chem* 2003; **278**: 35775-35780 [PMID: 12837748 DOI: 10.1074/jbc.M305961200]

38 **Zhao S**, Venkatasubbarao K, Li S, Freeman JW. Requirement of a specific Sp1 site for histone deacetylase-mediated repression of transforming growth factor beta Type II receptor expression in human pancreatic cancer cells. *Cancer Res* 2003; **63**: 2624-2630 [PMID: 12750289]

39 **Huang W**, Zhao S, Ammanamanchi S, Brattain M, Venkatasubbarao K, Freeman JW. Trichostatin A induces transforming growth factor beta type II receptor promoter activity and acetylation of Sp1 by recruitment of PCAF/p300 to a Sp1.NF-Y complex. *J Biol Chem* 2005; **280**: 10047-10054 [PMID: 15647279 DOI: 10.1074/jbc.M408680200]

40 **Bhakat KK**, Mitra S. Regulation of the human O(6)-methylguanine-DNA methyltransferase gene by transcriptional coactivators cAMP response element-binding protein-binding protein and p300. *J Biol Chem* 2000; **275**: 34197-34204 [PMID: 10942771 DOI: 10.1074/jbc.M005447200]

41 **Sato N**, Fukushima N, Maitra A, Matsubayashi H, Yeo CJ, Cameron JL, Hruban RH, Goggins M. Discovery of novel targets for aberrant methylation in pancreatic carcinoma using high-throughput microarrays. *Cancer Res* 2003; **63**: 3735-3742 [PMID: 12839967]

42 **Cecconi D**, Scarpa A, Donadelli M, Palmieri M, Hamdan M, Astner H, Righetti PG. Proteomic profiling of pancreatic ductal carcinoma cell lines treated with trichostatin-A. *Electrophoresis* 2003; **24**: 1871-1878 [PMID: 12783462 DOI: 10.1002/elps.200305430]

43 **Marengo E**, Robotti E, Cecconi D, Hamdan M, Scarpa A, Righetti PG. Identification of the regulatory proteins in human pancreatic cancers treated with Trichostatin A by 2D-PAGE maps and multivariate statistical analysis. *Anal Bioanal Chem* 2004; **379**: 992-1003 [PMID: 15257427 DOI: 10.1007/s00216-004-2707-x]

44 **Ohike N**, Maass N, Mundhenke C, Biallek M, Zhang M, Jonat W, Lüttges J, Morohoshi T, Klöppel G, Nagasaki K. Clinicopathological significance and molecular regulation of maspin expression in ductal adenocarcinoma of the pancreas. *Cancer Lett* 2003; **199**: 193-200 [PMID: 12969792]

45 **Sato N**, Fukushima N, Matsubayashi H, Goggins M. Identification of maspin and S100P as novel hypomethylation targets in pancreatic cancer using global gene expression profiling. *Oncogene* 2004; **23**: 1531-1538 [PMID: 14716296 DOI: 10.1038/sj.onc.1207269]

46 **Sato N**, Maitra A, Fukushima N, van Heek NT, Matsubayashi H, Iacobuzio-Donahue CA, Rosty C, Goggins M. Frequent hypomethylation of multiple genes overexpressed in pancreatic ductal adenocarcinoma. *Cancer Res* 2003; **63**: 4158-4166 [PMID: 12874021]

47 **Sato N**, Matsubayashi H, Abe T, Fukushima N, Goggins M. Epigenetic down-regulation of CDKN1C/p57KIP2 in pancreatic ductal neoplasms identified by gene expression profiling. *Clin Cancer Res* 2005; **11**: 4681-4688 [PMID: 16000561 DOI: 10.1158/1078-0432.CCR-04-2471]

48 **Vincent A**, Perrais M, Desseyn JL, Aubert JP, Pigny P, Van Seuningen I. Epigenetic regulation (DNA methylation, histone modifications) of the 11p15 mucin genes (MUC2,

MUC5AC, MUC5B, MUC6) in epithelial cancer cells. *Oncogene* 2007; **26**: 6566-6576 [PMID: 17471237 DOI: 10.1038/sj.onc.1210479]
49 **Yamada N**, Hamada T, Goto M, Tsutsumida H, Higashi M, Nomoto M, Yonezawa S. MUC2 expression is regulated by histone H3 modification and DNA methylation in pancreatic cancer. *Int J Cancer* 2006; **119**: 1850-1857 [PMID: 16721789 DOI: 10.1002/ijc.22047]
50 **Vincent A**, Ducourouble MP, Van Seuningen I. Epigenetic regulation of the human mucin gene MUC4 in epithelial cancer cell lines involves both DNA methylation and histone modifications mediated by DNA methyltransferases and histone deacetylases. *FASEB J* 2008; **22**: 3035-3045 [PMID: 18492726 DOI: 10.1096/fj.07-103390]
51 **Lee KH**, Lotterman C, Karikari C, Omura N, Feldmann G, Habbe N, Goggins MG, Mendell JT, Maitra A. Epigenetic silencing of MicroRNA miR-107 regulates cyclin-dependent kinase 6 expression in pancreatic cancer. *Pancreatology* 2009; **9**: 293-301 [PMID: 19407485 DOI: 10.1159/000186051]
52 **Piacentini P**, Donadelli M, Costanzo C, Moore PS, Palmieri M, Scarpa A. Trichostatin A enhances the response of chemotherapeutic agents in inhibiting pancreatic cancer cell proliferation. *Virchows Arch* 2006; **448**: 797-804 [PMID: 16568310 DOI: 10.1007/s00428-006-0173-x]
53 **Lee S**, Shinji C, Ogura K, Shimizu M, Maeda S, Sato M, Yoshida M, Hashimoto Y, Miyachi H. Design, synthesis, and evaluation of isoindolinone-hydroxamic acid derivatives as histone deacetylase (HDAC) inhibitors. *Bioorg Med Chem Lett* 2007; **17**: 4895-4900 [PMID: 17588744 DOI: 10.1016/j.bmcl.2007.06.038]
54 **Arnold NB**, Arkus N, Gunn J, Korc M. The histone deacetylase inhibitor suberoylanilide hydroxamic acid induces growth inhibition and enhances gemcitabine-induced cell death in pancreatic cancer. *Clin Cancer Res* 2007; **13**: 18-26 [PMID: 17200334 DOI: 10.1158/1078-0432.CCR-06-0914]
55 **Neureiter D**, Zopf S, Leu T, Dietze O, Hauser-Kronberger C, Hahn EG, Herold C, Ocker M. Apoptosis, proliferation and differentiation patterns are influenced by Zebularine and SAHA in pancreatic cancer models. *Scand J Gastroenterol* 2007; **42**: 103-116 [PMID: 17190770 DOI: 10.1080/00365520600874198]
56 **Kumagai T**, Wakimoto N, Yin D, Gery S, Kawamata N, Takai N, Komatsu N, Chumakov A, Imai Y, Koeffler HP. Histone deacetylase inhibitor, suberoylanilide hydroxamic acid (Vorinostat, SAHA) profoundly inhibits the growth of human pancreatic cancer cells. *Int J Cancer* 2007; **121**: 656-665 [PMID: 17417771 DOI: 10.1002/ijc.22558]
57 **Martin AP**, Park MA, Mitchell C, Walker T, Rahmani M, Thorburn A, Häussinger D, Reinehr R, Grant S, Dent P. BCL-2 family inhibitors enhance histone deacetylase inhibitor and sorafenib lethality via autophagy and overcome blockade of the extrinsic pathway to facilitate killing. *Mol Pharmacol* 2009; **76**: 327-341 [PMID: 19483105 DOI: 10.1124/mol.109.056309]
58 **Zhang G**, Park MA, Mitchell C, Hamed H, Rahmani M, Martin AP, Curiel DT, Yacoub A, Graf M, Lee R, Roberts JD, Fisher PB, Grant S, Dent P. Vorinostat and sorafenib synergistically kill tumor cells via FLIP suppression and CD95 activation. *Clin Cancer Res* 2008; **14**: 5385-5399 [PMID: 18765530 DOI: 10.1158/1078-0432.CCR-08-0469]
59 **Park MA**, Zhang G, Martin AP, Hamed H, Mitchell C, Hylemon PB, Graf M, Rahmani M, Ryan K, Liu X, Spiegel S, Norris J, Fisher PB, Grant S, Dent P. Vorinostat and sorafenib increase ER stress, autophagy and apoptosis via ceramide-dependent CD95 and PERK activation. *Cancer Biol Ther* 2008; **7**: 1648-1662 [PMID: 18787411]
60 **Chun SG**, Zhou W, Yee NS. Combined targeting of histone deacetylases and hedgehog signaling enhances cytoxicity in pancreatic cancer. *Cancer Biol Ther* 2009; **8**: 1328-1339 [PMID: 19421011]
61 **Nawrocki ST**, Carew JS, Pino MS, Highshaw RA, Andtbacka RH, Dunner K, Pal A, Bornmann WG, Chiao PJ, Huang P, Xiong H, Abbruzzese JL, McConkey DJ. Aggresome disruption: a novel strategy to enhance bortezomib-induced apoptosis in pancreatic cancer cells. *Cancer Res* 2006; **66**: 3773-3781 [PMID: 16585204 DOI: 10.1158/0008-5472.CAN-05-2961]
62 **Sato N**, Fukushima N, Chang R, Matsubayashi H, Goggins M. Differential and epigenetic gene expression profiling identifies frequent disruption of the RELN pathway in pancreatic cancers. *Gastroenterology* 2006; **130**: 548-565 [PMID: 16472607 DOI: 10.1053/j.gastro.2005.11.008]
63 **Deorukhkar A**, Park H, Shentu S, Diagaradjane P, Krishnan S. Vorinostat radiosensitizes pancreatic cancers by inhibiting DNA repair and radiation-induced EGFR and NF-KB signaling. *Pancreas* 2007; **35**: 398-399
64 **Kajiwara Y**, Panchabhai S, Liu DD, Kong M, Lee JJ, Levin VA. Melding a New 3-Dimensional Agarose Colony Assay with the E(max) Model to Determine the Effects of Drug Combinations on Cancer Cells. *Technol Cancer Res Treat* 2009; **8**: 163-176 [PMID: 19334798]
65 **Kumagai T**, Akagi T, Desmond JC, Kawamata N, Gery S, Imai Y, Song JH, Gui D, Said J, Koeffler HP. Epigenetic regulation and molecular characterization of C/EBPalpha in pancreatic cancer cells. *Int J Cancer* 2009; **124**: 827-833 [PMID: 19035457 DOI: 10.1002/ijc.23994]
66 **Lehmann A**, Denkert C, Budczies J, Buckendahl AC, Darb-Esfahani S, Noske A, Müller BM, Bahra M, Neuhaus P, Dietel M, Kristiansen G, Weichert W. High class I HDAC activity and expression are associated with RelA/p65 activation in pancreatic cancer in vitro and in vivo. *BMC Cancer* 2009; **9**: 395 [PMID: 19912635 DOI: 10.1186/1471-2407-9-395]
67 **Grant C**, Rahman F, Piekarz R, Peer C, Frye R, Robey RW, Gardner ER, Figg WD, Bates SE. Romidepsin: a new therapy for cutaneous T-cell lymphoma and a potential therapy for solid tumors. *Expert Rev Anticancer Ther* 2010; **10**: 997-1008 [PMID: 20645688 DOI: 10.1586/era.10.88]
68 **Sato N**, Ohta T, Kitagawa H, Kayahara M, Ninomiya I, Fushida S, Fujimura T, Nishimura G, Shimizu K, Miwa K. FR901228, a novel histone deacetylase inhibitor, induces cell cycle arrest and subsequent apoptosis in refractory human pancreatic cancer cells. *Int J Oncol* 2004; **24**: 679-685 [PMID: 14767553]
69 **Lundqvist A**, Abrams SI, Schrump DS, Alvarez G, Suffredini D, Berg M, Childs R. Bortezomib and depsipeptide sensitize tumors to tumor necrosis factor-related apoptosis-inducing ligand: a novel method to potentiate natural killer cell tumor cytotoxicity. *Cancer Res* 2006; **66**: 7317-7325 [PMID: 16849582 DOI: 10.1158/0008-5472.CAN-06-0680]
70 **Wu LP**, Wang X, Li L, Zhao Y, Lu S, Yu Y, Zhou W, Liu X, Yang J, Zheng Z, Zhang H, Feng J, Yang Y, Wang H, Zhu WG. Histone deacetylase inhibitor depsipeptide activates silenced genes through decreasing both CpG and H3K9 methylation on the promoter. *Mol Cell Biol* 2008; **28**: 3219-3235 [PMID: 18332107 DOI: 10.1128/MCB.01516-07]
71 **Göttlicher M**, Minucci S, Zhu P, Krämer OH, Schimpf A, Giavara S, Sleeman JP, Lo Coco F, Nervi C, Pelicci PG, Heinzel T. Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. *EMBO J* 2001; **20**: 6969-6978 [PMID: 11742974 DOI: 10.1093/emboj/20.24.6969]
72 **Duenas-Gonzalez A**, Candelaria M, Perez-Plascencia C, Perez-Cardenas E, de la Cruz-Hernandez E, Herrera LA. Valproic acid as epigenetic cancer drug: preclinical, clinical and transcriptional effects on solid tumors. *Cancer Treat Rev* 2008; **34**: 206-222 [PMID: 18226465 DOI: 10.1016/j.ctrv.2007.11.003]
73 **Erhuma M**, Köbel M, Mustafa T, Wulfänger J, Dralle H, Hoang-Vu C, Langner J, Seliger B, Kehlen A. Expression of neutral endopeptidase (NEP/CD10) on pancreatic tumor cell lines, pancreatitis and pancreatic tumor tissues. *Int J Cancer* 2007; **120**: 2393-2400 [PMID: 17294442 DOI: 10.1002/ijc.22252]

74 **Jones J**, Bentas W, Blaheta RA, Makarevic J, Hudak L, Wedel S, Probst M, Jonas D, Juengel E. Modulation of adhesion and growth of colon and pancreatic cancer cells by the histone deacetylase inhibitor valproic acid. *Int J Mol Med* 2008; **22**: 293-299 [PMID: 18698487]

75 **Fritsche P**, Seidler B, Schüler S, Schnieke A, Göttlicher M, Schmid RM, Saur D, Schneider G. HDAC2 mediates therapeutic resistance of pancreatic cancer cells via the BH3-only protein NOXA. *Gut* 2009; **58**: 1399-1409 [PMID: 19528037 DOI: 10.1136/gut.2009.180711]

76 **Pineau T**, Hudgins WR, Liu L, Chen LC, Sher T, Gonzalez FJ, Samid D. Activation of a human peroxisome proliferator-activated receptor by the antitumor agent phenylacetate and its analogs. *Biochem Pharmacol* 1996; **52**: 659-667 [PMID: 8759039]

77 **Svechnikova I**, Almqvist PM, Ekström TJ. HDAC inhibitors effectively induce cell type-specific differentiation in human glioblastoma cell lines of different origin. *Int J Oncol* 2008; **32**: 821-827 [PMID: 18360709]

78 **Egger G**, Aparicio AM, Escobar SG, Jones PA. Inhibition of histone deacetylation does not block resilencing of p16 after 5-aza-2′-deoxycytidine treatment. *Cancer Res* 2007; **67**: 346-353 [PMID: 17210717 DOI: 10.1158/0008-5472.CAN-06-2845]

79 **Saito Y**, Liang G, Egger G, Friedman JM, Chuang JC, Coetzee GA, Jones PA. Specific activation of microRNA-127 with downregulation of the proto-oncogene BCL6 by chromatin-modifying drugs in human cancer cells. *Cancer Cell* 2006; **9**: 435-443 [PMID: 16766263 DOI: 10.1016/j.ccr.2006.04.020]

80 **Ammerpohl O**, Trauzold A, Schniewind B, Griep U, Pilarsky C, Grutzmann R, Saeger HD, Janssen O, Sipos B, Kloppel G, Kalthoff H. Complementary effects of HDAC inhibitor 4-PB on gap junction communication and cellular export mechanisms support restoration of chemosensitivity of PDAC cells. *Br J Cancer* 2007; **96**: 73-81 [PMID: 17164759 DOI: 10.1038/sj.bjc.6603511]

81 **Davie JR**. Inhibition of histone deacetylase activity by butyrate. *J Nutr* 2003; **133**: 2485S-2493S [PMID: 12840228]

82 **Mullins TD**, Kern HF, Metzgar RS. Ultrastructural differentiation of sodium butyrate-treated human pancreatic adenocarcinoma cell lines. *Pancreas* 1991; **6**: 578-587 [PMID: 1946315]

83 **Gaschott T**, Maassen CU, Stein J. Tributyrin, a butyrate precursor, impairs growth and induces apoptosis and differentiation in pancreatic cancer cells. *Anticancer Res* 2001; **21**: 2815-2819 [PMID: 11724360]

84 **Zhang JS**, Wang L, Huang H, Nelson M, Smith DI. Keratin 23 (K23), a novel acidic keratin, is highly induced by histone deacetylase inhibitors during differentiation of pancreatic cancer cells. *Genes Chromosomes Cancer* 2001; **30**: 123-135 [PMID: 11135429]

85 **Natoni F**, Diolordi L, Santoni C, Gilardini Montani MS. Sodium butyrate sensitises human pancreatic cancer cells to both the intrinsic and the extrinsic apoptotic pathways. *Biochim Biophys Acta* 2005; **1745**: 318-329 [PMID: 16109447 DOI: 10.1016/j.bbamcr.2005.07.003]

86 **McIntyre LJ**, Kim YS. Effects of sodium butyrate and dimethylsulfoxide on human pancreatic tumor cell lines. *Eur J Cancer Clin Oncol* 1984; **20**: 265-271 [PMID: 6200329]

87 **Bensaadi N**, Clemente F, Vaysse N. Modulation of enzymatic activities during spontaneous and induced differentiation in a human pancreatic adenocarcinoma cell line CAPAN-1. *Int J Pancreatol* 1989; **4**: 391-406 [PMID: 2543714]

88 **Bloom EJ**, Siddiqui B, Hicks JW, Kim YS. Effect of sodium butyrate, a differentiating agent, on cell surface glycoconjugates of a human pancreatic cell line. *Pancreas* 1989; **4**: 59-64 [PMID: 2717602]

89 **Parekh D**, Ishizuka J, Townsend CM, Haber BE, Beauchamp RD, Rajaraman S, Karp G, Hsieh J, Thompson JC. Differential effects of sodium butyrate and hexamethylene bisacetamide on growth and secretion of cultured human endocrine tumor cells. *Arch Surg* 1991; **126**: 467-472 [PMID: 1706915]

90 **Estival A**, Clerc P, Vaysse N, Tam JP, Clemente F. Decreased expression of transforming growth factor alpha during differentiation of human pancreatic cancer cells. *Gastroenterology* 1992; **103**: 1851-1859 [PMID: 1451978]

91 **Ide Y**, Miyoshi E, Nakagawa T, Gu J, Tanemura M, Nishida T, Ito T, Yamamoto H, Kozutsumi Y, Taniguchi N. Aberrant expression of N-acetylglucosaminyltransferase-IVa and IVb (GnT-IVa and b) in pancreatic cancer. *Biochem Biophys Res Commun* 2006; **341**: 478-482 [PMID: 16434023 DOI: 10.1016/j.bbrc.2005.12.208]

92 **el-Deriny SE**, O'Brien MJ, Christensen TG, Kupchik HZ. Ultrastructural differentiation and CEA expression of butyrate-treated human pancreatic carcinoma cells. *Pancreas* 1987; **2**: 25-33 [PMID: 3575313]

93 **Corra S**, Kazakoff K, Mogaki M, Cano M, Pour PM. Modification of antigen expression in human and hamster pancreatic cancer cell lines induced by sodium butyrate. *Teratog Carcinog Mutagen* 1993; **13**: 199-215 [PMID: 7905674]

94 **Miszczuk-Jamska B**, Merten M, Renaud W, Guy-Crotte O, Figarella C. Trypsinogen expression by two human pancreatic cell lines CFPAC-1 and CAPAN-1. Modulation during spontaneous and induced cell growth. *Int J Pancreatol* 1994; **16**: 61-69 [PMID: 7806913]

95 **Egawa N**, Maillet B, VanDamme B, De Grève J, Klöppel G. Differentiation of pancreatic carcinoma induced by retinoic acid or sodium butyrate: a morphological and molecular analysis of four cell lines. *Virchows Arch* 1996; **429**: 59-68 [PMID: 8865855]

96 **Farrow B**, Rychahou P, O'Connor KL, Evers BM. Butyrate inhibits pancreatic cancer invasion. *J Gastrointest Surg* 2003; **7**: 864-870 [PMID: 14592659]

97 **Yeh TS**, Tseng JH, Liu NJ, Chen TC, Jan YY, Chen MF. Significance of cellular distribution of ezrin in pancreatic cystic neoplasms and ductal adenocarcinoma. *Arch Surg* 2005; **140**: 1184-1190 [PMID: 16365240 DOI: 10.1001/archsurg.140.12.1184]

98 **Speranza A**, Pellizzaro C, Coradini D. Hyaluronic acid butyric esters in cancer therapy. *Anticancer Drugs* 2005; **16**: 373-379 [PMID: 15746573]

99 **Pellizzaro C**, Speranza A, Zorzet S, Crucil I, Sava G, Scarlata I, Cantoni S, Fedeli M, Coradini D. Inhibition of human pancreatic cell line MIA PaCa2 proliferation by HA-But, a hyaluronic butyric ester: a preliminary report. *Pancreas* 2008; **36**: e15-e23 [PMID: 18437074 DOI: 10.1097/MPA.0b013e31816705bc]

100 **Hess-Stumpp H**, Bracker TU, Henderson D, Politz O. MS-275, a potent orally available inhibitor of histone deacetylases--the development of an anticancer agent. *Int J Biochem Cell Biol* 2007; **39**: 1388-1405 [PMID: 17383217 DOI: 10.1016/j.biocel.2007.02.009]

101 **Saito A**, Yamashita T, Mariko Y, Nosaka Y, Tsuchiya K, Ando T, Suzuki T, Tsuruo T, Nakanishi O. A synthetic inhibitor of histone deacetylase, MS-27-275, with marked in vivo antitumor activity against human tumors. *Proc Natl Acad Sci USA* 1999; **96**: 4592-4597 [PMID: 10200307]

102 **Baradari V**, Huether A, Höpfner M, Schuppan D, Scherübl H. Antiproliferative and proapoptotic effects of histone deacetylase inhibitors on gastrointestinal neuroendocrine tumor cells. *Endocr Relat Cancer* 2006; **13**: 1237-1250 [PMID: 17158768 DOI: 10.1677/erc.1.01249]

103 **Boumber Y**, Younes A, Garcia-Manero G. Mocetinostat (MGCD0103): a review of an isotype-specific histone deacetylase inhibitor. *Expert Opin Investig Drugs* 2011; **20**: 823-829 [PMID: 21554162 DOI: 10.1517/13543784.2011.577737]

104 **Beckers T**, Burkhardt C, Wieland H, Gimmnich P, Ciossek T, Maier T, Sanders K. Distinct pharmacological properties of second generation HDAC inhibitors with the benzamide or hydroxamate head group. *Int J Cancer* 2007; **121**: 1138-1148 [PMID: 17455259 DOI: 10.1002/ijc.22751]

105 **Fournel M**, Bonfils C, Hou Y, Yan PT, Trachy-Bourget MC, Kalita A, Liu J, Lu AH, Zhou NZ, Robert MF, Gillespie J, Wang JJ, Ste-Croix H, Rahil J, Lefebvre S, Moradei O, Delorme D, Macleod AR, Besterman JM, Li Z. MGCD0103, a novel isotype-selective histone deacetylase inhibitor, has broad spectrum antitumor activity in vitro and in vivo. *Mol Cancer Ther* 2008; **7**: 759-768 [PMID: 18413790 DOI: 10.1158/1535-7163.MCT-07-2026]

106 **Bonfils C**, Kalita A, Dubay M, Siu LL, Carducci MA, Reid G, Martell RE, Besterman JM, Li Z. Evaluation of the pharmacodynamic effects of MGCD0103 from preclinical models to human using a novel HDAC enzyme assay. *Clin Cancer Res* 2008; **14**: 3441-3449 [PMID: 18519775 DOI: 10.1158/1078-0432.CCR-07-4427]

107 **Liang S**, Yang ZL. Expression of KiSS-1mRNA in pancreatic ductal adenocarcinoma and non-cancerous pancreatic tissues in SD rats. *Zhongnan Daxue Xuebao Yixueban* 2007; **32**: 109-113 [PMID: 17344598]

108 **Hirokawa Y**, Levitzki A, Lessene G, Baell J, Xiao Y, Zhu H, Maruta H. Signal therapy of human pancreatic cancer and NF1-deficient breast cancer xenograft in mice by a combination of PP1 and GL-2003, anti-PAK1 drugs (Tyr-kinase inhibitors). *Cancer Lett* 2007; **245**: 242-251 [PMID: 16540233 DOI: 10.1016/j.canlet.2006.01.018]

109 **Hess-Stumpp H**, Apetri E, Hoffmann J. MS-275, a potent orally active inhibitor of histone deacetylases, is efficacious in a wide range of experimental tumors: In vivo efficacy data. *Proc Am Ass Cancer Res* 2005; **46**: 607

110 **Kraker AJ**, Mizzen CA, Hartl BG, Miin J, Allis CD, Merriman RL. Modulation of histone acetylation by [4-(acetylamino)-N-(2-amino-phenyl) benzamide] in HCT-8 colon carcinoma. *Mol Cancer Ther* 2003; **2**: 401-408 [PMID: 12700284]

111 **LoRusso PM**, Demchik L, Foster B, Knight J, Bissery MC, Polin LM, Leopold WR, Corbett TH. Preclinical antitumor activity of CI-994. *Invest New Drugs* 1996; **14**: 349-356 [PMID: 9157069]

112 **Corbett TH**, Roberts BJ, Leopold WR, Peckham JC, Wilkoff LJ, Griswold DP, Schabel FM. Induction and chemotherapeutic response of two transplantable ductal adenocarcinomas of the pancreas in C57BL/6 mice. *Cancer Res* 1984; **44**: 717-726 [PMID: 6692374]

113 **Haefner M**, Bluethner T, Niederhagen M, Moebius C, Wittekind C, Mossner J, Caca K, Wiedmann M. Experimental treatment of pancreatic cancer with two novel histone deacetylase inhibitors. *World J Gastroenterol* 2008; **14**: 3681-3692 [PMID: 18595135]

P- Reviewer Michl P **S- Editor** Cheng JX **L- Editor** Stewart GJ **E- Editor** Li JY



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i6.829

World J Gastroenterol 2013 February 14; 19(6): 829-837
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

REVIEW

Diagnosis and management of insulinoma

Takehiro Okabayashi, Yasuo Shima, Tatsuaki Sumiyoshi, Akihito Kozuki, Satoshi Ito, Yasuhiro Ogawa, Michiya Kobayashi, Kazuhiro Hanazaki

Takehiro Okabayashi, Yasuo Shima, Tatsuaki Sumiyoshi, Akihito Kozuki, Department of Surgery, Kochi Health Sciences Center, Kochi 781-8555, Japan
Satoshi Ito, Yasuhiro Ogawa, Department of Radiology, Kochi Medical School, Nankoku 783-8505, Japan
Michiya Kobayashi, Kazuhiro Hanazaki, Department of Surgery, Kochi Medical School, Nankoku 783-8505, Japan
Author contributions: Okabayashi T and Hanazaki K designed research; Sumiyoshi T, Kozuki A and Ito S contributed analytic tools; Shima Y, Ogawa Y and Kobayashi M supervised the paper; and Okabayashi T wrote the paper.
Supported by Kochi Organization for Medical Reformation and Renewal Fund
Correspondence to: Dr. Takehiro Okabayashi, MD, PhD, Department of Surgery, Kochi Health Sciences Center, 2125-1 Ike, Kochi 781-8555, Japan. takehiro_okabayashi@khsc.or.jp
Telephone: +81-88-8373000 Fax: +81-88-8376766
Received: August 13, 2012 Revised: November 12, 2012
Accepted: November 14, 2012
Published online: February 14, 2013

Abstract

Insulinomas, the most common cause of hypoglycemia related to endogenous hyperinsulinism, occur in 1-4 people per million of the general population. Common autonomic symptoms of insulinoma include diaphoresis, tremor, and palpitations, whereas neuroglycopenic symptoms include confusion, behavioural changes, personality changes, visual disturbances, seizure, and coma. Diagnosis of suspected cases is based on standard endocrine tests, especially the prolonged fasting test. Non-invasive imaging procedures, such as computed tomography and magnetic resonance imaging, are used when a diagnosis of insulinoma has been made to localize the source of pathological insulin secretion. Invasive modalities, such as endoscopic ultrasonography and arterial stimulation venous sampling, are highly accurate in the preoperative localization of insulinomas and have frequently been shown to be superior to non-invasive localization techniques. The range of techniques available for the localization of insulinomas means that blind resection can be avoided. Intraoperative manual palpation of the pancreas by an experienced surgeon and intraoperative ultrasonography are both sensitive methods with which to finalize the location of insulinomas. A high proportion of patients with insulinomas can be cured with surgery. In patients with malignant insulinomas, an aggressive medical approach, including extended pancreatic resection, liver resection, liver transplantation, chemoembolization, or radiofrequency ablation, is recommended to improve both survival and quality of life. In patients with unresectable or uncontrollable insulinomas, such as malignant insulinoma of the pancreas, several techniques should be considered, including administration of ocreotide and/or continuous glucose monitoring, to prevent hypoglycemic episodes and to improve quality of life.

© 2013 Baishideng. All rights reserved.

Key words: Pancreas; Insulinoma; Neuroendocrine pancreatic tumor; Diagnosis; Management; Continuous blood glucose monitoring

Okabayashi T, Shima Y, Sumiyoshi T, Kozuki A, Ito S, Ogawa Y, Kobayashi M, Hanazaki K. Diagnosis and management of insulinoma. *World J Gastroenterol* 2013; 19(6): 829-837 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i6/829.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i6.829

INTRODUCTION

Insulinomas are the most common functioning endocrine neoplasm of the pancreas[1-4]. They are insulin-secreting tumors of pancreatic origin that cause hypoglycemia[5-7]. Insulinomas occur in 1-4 people per million in the general population and represent 1%-2% of all pancreatic neoplasms[8-10]. Insulinomas can occur at any age and have an equal gender distribution. As many as 90% of insulinomas have been reported to be benign, 90% are solitary, > 90% occur at intrapancreatic sites, and 90% are < 2 cm in

diameter[10-13]. Insulinomas are evenly distributed over the entire pancreas. Most insulinomas are located in the pancreas or are attached directly to the pancreas. Extrapancreatic insulinomas causing hypoglycemia are extremely rare (incidence < 2%); extrapancreatic insulinomas are most commonly found in the duodenal wall[8]. The etiology and pathogenesis of insulinomas are not known.

Following biological and biochemical confirmation of an insulinoma, preoperative localization is sought using computed tomography (CT)[14-16], magnetic resonance imaging (MRI)[16-19], endoscopic ultrasonography (EUS)[20-22], intra-arterial calcium stimulation test with hepatic venous sampling[23], and/or angiography and arterial stimulation venous sampling (ASVS)[24-28]. Surgical resection is the primary treatment modality for insulinomas, and so accurate localization of the tumor before or during surgery is important. Intraoperative manual palpation of the pancreas by an experienced surgeon and intraoperative ultrasonography are both sensitive methods with which to localize insulinomas, supporting the argument by some surgeons that preoperative localization of the tumors is not necessary[29-33]. The present review describes some of the latest findings regarding the clinical diagnosis and medical management of insulinomas in the adult population that are not associated with either multiple endocrine neoplasms or von Hippel-Lindau disease.

CLINICAL SIGNS

Insulinomas are the most common cause of hypoglycemia related to endogenous hyperinsulinism. The episodic nature of the hypoglycemic attack is due to the intermittent secretion of insulin by the tumor[8]. Common autonomic symptoms of an insulinoma include diaphroresis, tremor, and palpitations, whereas neuroglycopenenic symptoms include confusion, behavioral changes, personality changes, visual disturbances, seizures and coma[34,35].

Diagnosis of insulinomas can be challenging. Although it was originally considered that symptoms only became evident in the fasting state or following exercise, it is now known that patients with an insulinoma can also present with postprandial symptoms[36,37]. The classical diagnosis of insulinoma depends on satisfying the criteria of Whipple's triad, which remains the cornerstone of the screening process: (1) hypoglycemia (plasma glucose < 50 mg/dL); (2) neuroglycopenic symptoms; and (3) prompt relief of symptoms following the administration of glucose (Table 1)[38]. In adults with symptoms of neuroglycopenia or documented low blood glucose levels, the gold standard for biochemical diagnosis remains measurement of plasma glucose, insulin, C-peptide, and proinsulin during a 72-h fast (Table 1). This prolonged fasting test can detect up to 99% of insulinomas[39]. Endogenous hypoglycemia due to insulinomas was previously based on findings of abnormal serum levels of insulin, C-peptide, and, more recently, proinsulin at the time of fasting hypoglycemia. To date, there is some general agreement regarding the diagnostic thresholds that must be reached for insulin, C-peptide, and proinsulin levels to be considered abnormal. Several years ago, ratios calculated from insulin and blood glucose levels were used, with the insulin/C-peptide ratio in patients diagnosed with insulinoma reported to be < 1.0[40,41]. It is of note that a normal insulin level does not exclude the disease, because the absolute insulin level is not elevated in all patients with insulinoma. In addition, because the proportion of proinsulin secreted by insulinoma cells is generally higher than that secreted by normal β-cells, high proinsulin levels have been suggested as being diagnostic of insulinoma, regardless of concomitant blood glucose levels[42]. The availability of proinsulin assays has led to the use of serum proinsulin thresholds as a diagnostic tool: it has been recommended that a cut-off level of 20 pmol/L proinsulin at the time of hypoglycemia < 45 mg/dL is indicative of the presence of an insulinoma (Table 1)[42-44].

Table 1 Diagnosis of insulinoma

Classical diagnosis
Hypoglycemia (plasma glucose < 50 mg/dL)
Neuroglycopenic symptoms
Prompt relief of symptoms following the administration of glucose
Present consensus
At the time of hypoglycemia during a 72-h fasting test:
5 mIU/L (36 pmol/L) insulin threshold
0.6 ng/mL (0.2 nmol/L) C-peptide threshold
Insulin/C-peptide ratio < 1.0
20 pmol/L proinsulin cut-off level
Absence of sulfonylurea (metabolites) in the plasma or urine

Delays in the diagnosis of insulinoma are common because the symptoms usually precede detection of a tumor and there may be misattribution of the symptoms to psychiatric, cardiac, or neurological disorders[45]. Once a diagnosis of insulinoma is considered, it is important that patients are managed in a timely and safe manner. As a general rule, patients with insulinoma can be cured by surgical resection of the tumor. The need for preoperative localization of insulinomas and the methods used remain contentious. In the past, confirmation of the presence of the Whipple triad (symptoms known or likely to be caused by hypoglycemia; a low plasma glucose measured at the time of the symptoms; relief of symptoms when the glucose is raised to normal) usually meant that a patient was led directly to surgery[46,47]. However, at present, most agree that knowledge of the site of the tumor before surgery is helpful in that it allows one to determine not only whether enucleation (the surgical removal of a mass) of the neoplasm or pancreatic resection is likely to be required, but also whether the tumor is amenable to removal *via* a laparoscopic approach. Preoperative localization should also mean that the operation itself can be performed more quickly, thereby reducing associated morbidity and mortality[27]. It is important to remember that most tumors are intrapancreatic, 90% are solitary, 90% are < 2 cm in diameter, and the tumors are distributed equally within the head, body and tail of the pancreas.



Figure 1 Computed tomography of insulinoma of the pancreas. Typically, insulinomas (arrow) are hypervascular and, as a result, demonstrate a greater degree of enhancement than normal pancreatic parenchyma during the arterial and capillary phases of contrast bolus.

NON-INVASIVE IMAGING

A number of non-invasive techniques are available for the localization of a suspected insulinoma, including transabdominal ultrasonography, CT and/or MRI. The sensitivity of transabdominal ultrasonography in the localization of insulinomas is poor (ranging from 9% to 64%)[48]. However, insulinomas demonstrate characteristic features when imaged with both CT and MRI and the sensitivity of these techniques has been reported to be 33%-64% and 40%-90%, respectively[10,49]. The sensitivity and specificity of MRI is generally superior to that of CT, as is the detection of extrapancreatic extensions[16].

CT is a safe and simple procedure to perform that is operator independent. CT visualizes the exact location of an insulinoma, its relationship to vital structures, and the presence of metastases[49]. Typically, insulinomas are hypervascular and, as a result, demonstrate a greater degree of enhancement than normal pancreatic parenchyma during the arterial and capillary phases of contrast bolus (Figure 1)[16]. An atypical CT appearance of insulinomas is occasionally encountered and can include hypovascular and hypodense lesions post-contrast, hyperdense lesions precontrast, cystic masses, and calcified masses[16]. Calcification, when it occurs, tends to be discrete and nodular, and is more common in malignant than benign tumors[50,51]. Technical advances have improved the quality of CT, with a recent study reporting that using a multidetector CT enabled visualization of 94.4% of insulinomas[52]. CT is currently accepted as the first-line investigation for the visualization of insulinomas.

Currently, there is strong evidence emerging for the use of MRI in the imaging of insulinomas, and investigators have shown a high sensitivity for MRI in the detection of insulinomas[16,49]. Like CT, MRI is safe, non-invasive, rapid, and facilitates the detection of metastases. Insulinomas generally demonstrate low signal intensity on T_1-weighted images and high signal intensity on T_2-weighted images (Figure 2)[50]. However, limitations in the use of MRI in the detection of insulinomas include the standard contraindications for MRI. The modern MRI system allows rapid triphasic, breath-held T_1 rapid gadolium-enhanced sequences, and/or diffusion-weighted imaging[19,49]. These sequences significantly reduce motion artifacts and enable accurate assessment of the pancreas in both the arterial and venous phase[49]. MRI has all the advantages of CT and recent evidence suggests that it may be the more sensitive tool. In current practice, MRI is a second-line investigation for the localization of insulinomas, but it could potentially take over from CT in the future as it becomes more widely available and expertise improves.

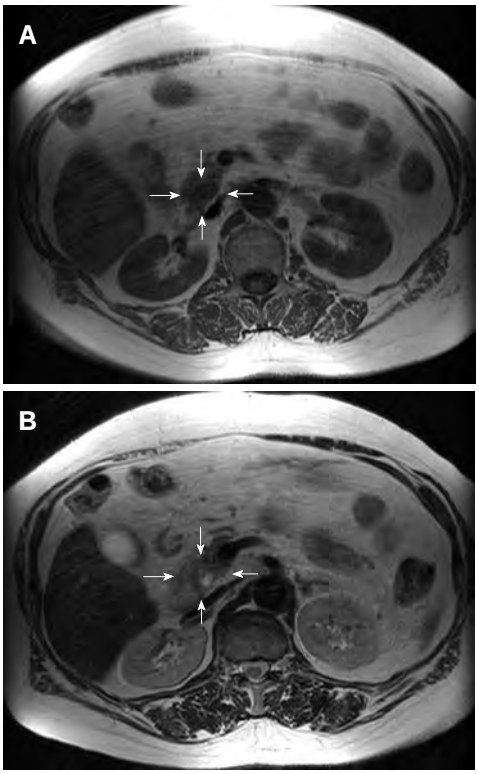


Figure 2 Magnetic resonance imaging of insulinoma of the pancreas. Insulinomas (arrows) generally demonstrate low signal intensity on T1-weighted images (A) and high signal intensity on T2-weighted images (B).

INVASIVE DIAGNOSTIC MODALITIES

The diagnostic procedure in cases of suspected insulinoma is based on standard endocrine examinations, especially the prolonged fasting test. Non-invasive imaging procedures are used to localize the source of pathological insulin secretion after a diagnosis of insulinoma has been established[20]. Invasive modalities, such as EUS and ASVS, have been shown to be highly accurate in the preoperative localization of insulinomas, and have frequently been shown to be superior to non-invasive localization techniques.

EUS is currently the test of choice in most Western centers, with reported detection rates of 86.6%-92.3%[10,46]. The appearance of insulinomas on EUS is quite characteristic, with most tumors homogeneously hypoechoic, rounded in shape, and with distinct margins (Figure 3).

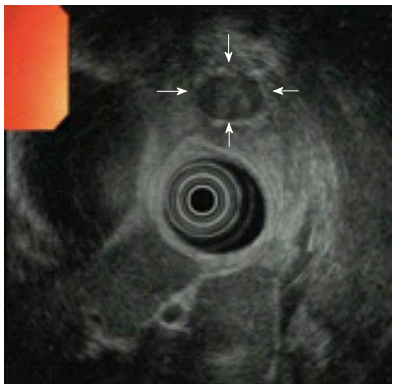


Figure 3 Endoscopic ultrasound features of insulinoma of the pancreas. The appearance of insulinomas (arrows) on endoscopic ultrasonography is quite characteristic, with most tumors homogeneously hypoechoic, rounded in shape, and with distinct margins.

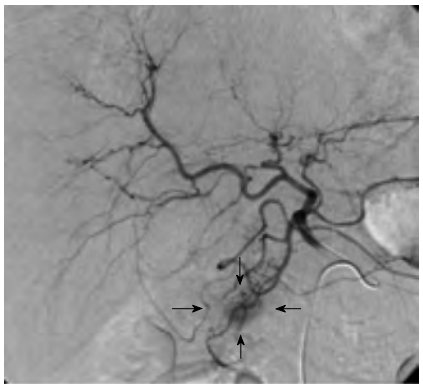


Figure 4 Angiography and arterial stimulation venous sampling. Using arterial stimulation venous sampling, insulinomas (arrows) are seen as well-defined, round or oval vascular blushes that are of increased vascularity compared with the surrounding normal pancreatic parenchyma.

Although EUS is a highly reliable procedure for the preoperative localization of insulinomas, there are several problems associated with the detection of these tumors using EUS. First, EUS may yield both false-positive and false-negative results, with the quality of the EUS findings largely dependent on the examiner's experience[22]. Second, some insulinomas are missed by preoperative EUS because they are completely isoechoic. A low body mass index, female gender, and young age may be risk factors for negative imaging[20]. Third, the sensitivity of EUS for insulinomas depends on the location and size of the tumor; sensitivity is greatest for tumors in the head of the pancreas and lowest for those in the tail of the pancreas or those that are extrapancreatic[10]. Once the site of the tumor has been determined, fine-needle aspiration (FNA) of the pancreas allows for a preoperative diagnosis of insulinoma. Advances in EUS have made EUS-guided FNA particularly useful in the diagnosis of insulinomas, because most functioning tumors are small. EUS-guided FNA is becoming increasingly popular, and it seems likely that it will eventually become the standard for the diagnosis and staging of pancreatic tumors[9].

There can be little doubt that angiography combined with ASVS should not precede non-invasive investigations, such as CT and MRI, but it remains a highly sensitive technique for the precise localization of insulinomas and will usually provide more information than EUS[27]. Morphological imaging modalities do not reflect hormonal function; however, the addition of ASVS helps regionalize a tumor by verifying hormonal function[24]. The use of ASVS allows for a more accurate surgical approach and can minimize the likelihood of re-operation[24]. For atypical insulinomas, preoperative localization of insulinomas by ASVS is particularly important. The accuracy of ASVS in localizing insulinomas has been reported to range from 94% to 100%[23,28]. Using ASVS, insulinomas are seen as well-defined, round or oval vascular blushes that are of increased vascularity compared with the surrounding normal pancreatic parenchyma (Figure 4). Insulinomas are visualized during the early arterial phase and persist for a variable length of time into the venous phase of the run. The localization of an insulinoma by ASVS relies on the fact that a hyperosmolar concentration of calcium in the vessels supplying the tumor will cause degranulation of cells within the neoplasm, releasing insulin into the portal venous system, which results in a detectable rise in insulin in venous samples obtained from the hepatic vein[27]. The splenic, gastroduodenal, superior mesenteric, and proper hepatic arteries are the vessels most commonly studied during ASVS; an increase in insulin concentrations in the hepatic vein will localize the insulinoma to the body/tail of the pancreas, an anterosuperior site of the pancreatic head, and postero-inferior site of the pancreatic head, respectively. An increase in insulin concentrations after injection of calcium into the proper hepatic artery suggests that hepatic metastases may be present. Changes in serum insulin levels plotted as a function of time after calcium injection indicate that insulin concentrations are markedly elevated only in the feeding arteries of the insulinoma (Figure 5).

The range of imaging modalities now available means that blind resection for insulinoma can be avoided because of accurate preoperative and intraoperative localization of the tumor[53]. Manual palpation of the pancreas by an experienced surgeon and ultrasonography are both sensitive methods for the intraoperative detection of the site of insulinomas[29-31]. The sensitivity of these two methods is clinically acceptable and has been reported as 75%-95% and 80%-100%, respectively[29,32,54].

MEDICAL MANAGEMENT OF BENIGN INSULINOMAS

Most patients with benign insulinomas can be cured with surgery, although other techniques for the management of insulinomas, including injection of octreotide, EUS-guided alcohol ablation, radiofrequency ablation (RFA), or embolization of an insulinoma of the pancreas, have been described[55-61].

After identification of an insulinoma, surgery is indicated for all localized tumors. The choice of procedure will depend on the features of the tumor mass, such as

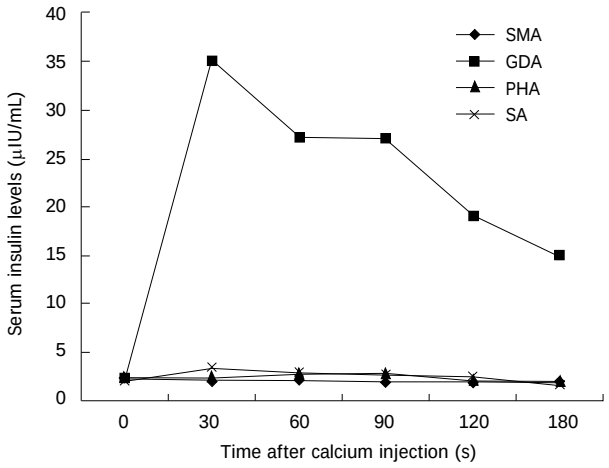


Figure 5 Changes in serum insulin levels. Changes in serum insulin levels plotted as a function of time after calcium injection indicate that insulin concentrations are markedly elevated only in the feeding arteries of the insulinoma. SMA: Superior mesenteric artery; GDA: Gastroduodenal artery; PHA: Proper hepatic artery; SA: Splenic artery.

type, size, and localization. Atypical resection, including enucleation, partial pancreatectomy, or middle pancreatectomy, has the advantage of preserving the pancreatic parenchyma as much as possible, thereby reducing the risk of late exocrine/endocrine insufficiency[62]. To date, laparoscopic resection has often been performed for insulinomas that are benign, small, and/or located in the body or tail of the pancreas[63]. Radical resection should be considered for patients in whom the lesion is not single, not well-capsulated, > 4 cm in diameter, and involves or is near the main pancreatic duct. Lymphadenectomy is not usually performed. Although the cure rate after resection for insulinoma is very high, it is necessary to be aware of the potential for postoperative complications after pancreatic surgery, especially postoperative pancreatic fistula[64-66].

However, there is a considerable risk of morbidity and mortality associated with the surgical management of insulinomas, which precludes surgery in high-risk patients. Alcohol ablation and RFA have been established as minimally invasive procedures in the treatment of primary liver tumors and hepatic metastases. Recently, successful EUS-guided alcohol ablation and CT-guided RFA of pancreatic insulinomas have been reported in humans[56,57]. These two patients were in poor general condition and were experiencing recurrent symptomatic episodes of hypoglycemia. Because it was considered that surgical management for benign insulinoma of the pancreas was impossible in both cases, ablation of the solitary mass was performed. Both patients were discharged without any complications and reported no further hypoglycemic episodes. Embolization of an insulinoma of the pancreas is another non-surgical alternative[55,67,68]. Because angiographically the insulinoma is demonstrated in the arterial phase as a hypervasculated mass, embolization could be performed using flow to direct particles exclusively into the tumor. Although it remains contentious as to whether these procedures are a viable treatment for patients with an insulinoma, they may be offered as an alternative for certain patients, such as those who refuse surgery, those who are of advanced age, those with a poor general condition, those who have already undergone multiple abdominal surgeries, or those with an increased risk of postoperative complications due to other reasons.

Insulinomas are rare endocrine tumors, most of which can be cured by surgery. Medical treatment to normalize blood glucose is useful during the preoperative period, as well as for patients who cannot be cured by surgery, such as those with diffuse β-cell disease, multiple insulinomas, unresectable malignant insulinoma, those in whom surgery is contraindicated, or patients who refuse surgery[59]. Octreotide is a somatostatin analog that inhibits insulin secretion and the peripheral action of many gastrointestinal hormones, primarily *via* activation of somatostatin sst_2 receptors. Octreotide has been used for the treatment of insulinoma, with successful control of blood glucose levels[60,61]. In addition, ocreotide may have an antiproliferative effect, as well as a moderate antitumoral action, on pancreatic endocrine tumors[69]. Therapy may be initiated with short-acting ocreotide two to four times daily, or 20-30 mg long-acting ocreotide every 4 wk[70]. Initiation of therapy with short-acting ocreotide can be used to assess systemic tolerability, particularly any gastrointestinal side-effects[71]. Thus, pharmacotherapy with somatostatin to control hypoglycemia represents a feasible option for the non-surgical management of insulinomas.

MEDICAL MANAGEMENT OF MALIGNANT INSULINOMAS

To be considered malignant, insulinomas must show evidence of local invasion into the surrounding soft tissue or there must be verification of lymph node or liver metastasis[72]. The reported incidence of malignant insulinomas ranges between 7% and 10%[72-74], and the 10-year survival has been reported to be 29%[2]. The major sites of metastasis or recurrence are the liver and regional lymph nodes. Aggressive surgical resection is recommended because these tumors are much less virulent than their malignant ductal exocrine counterparts, in which there are severe hormonal symptoms that cannot be controlled by medical treatment. RFA can be used to reduce the tumor mass in the liver, thereby reducing hormonal symptoms[58,75]. Selective embolization alone or in combination with intra-arterial chemotherapy is an established procedure to reduce both hormonal symptoms and liver metastases. Although experience is limited, liver transplantation for multiple liver metastases of malignant insulinomas may be considered in patients with no extrahepatic metastases[76,77]. Aggressive sequential multimodal therapy (chemoembolization, RFA, liver resection, liver transplantation) can prolong the survival of patients with sporadic malignant insulinoma, even in the presence of liver metastases.

Malignant insulinomas remain extremely rare tumors. In many patients with malignant insulinomas, the tumors are unresectable and medical treatment therapy is limited

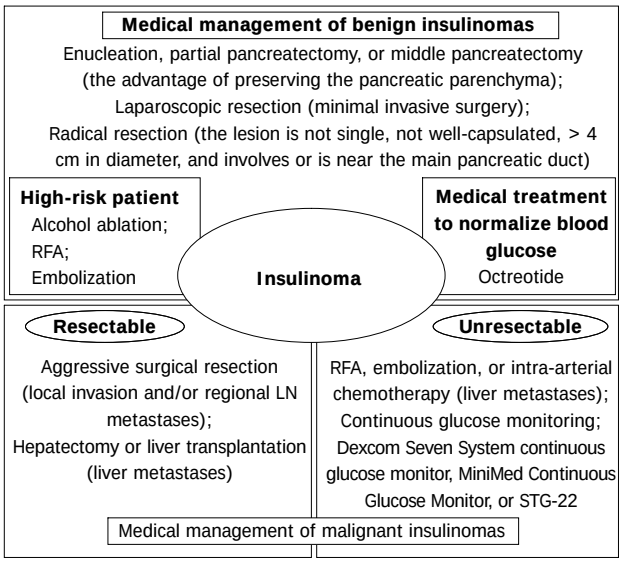


Figure 6 In patients who have unresectable or uncontrollable malignant insulinomas of the pancreas, several strategies need to be considered to both control hypoglycemic episodes and improve quality of life, including administration of ocreotide and continuous glucose monitoring. RFA: Radiofrequency ablation; LN: Lymph node.

in its ability to prevent hypoglycemic episodes[78,79]. Continuous glucose monitoring in patients with insulinomas can detect hypoglycemia, monitor responses to medical therapy, and confirm a cure postoperatively. In the literature, continuous glucose monitoring has been reported using a Dexcom Seven System continuous glucose monitor (Dexcom, San Diego, CA, United States) or the MiniMed Continuous Glucose Monitor (CGM Medtronic MiniMed, Northridge, CA, United States)[78,79]. These studies reported that continuous glucose monitoring is a useful addition to the armamentarium for the prevention of hypoglycemia. These techniques are considered an effective adjunct to therapy to reduce hypoglycemic episodes by alerting patients to low glucose concentrations before they develop neuroglycopenic symptoms; however, patients should respond promptly to oral glucose intake after hypoglycemia has been detected by these machines. In patients with a poorer general condition, malignant insulinomas that are unresectable, and uncontrolled hypoglycemia, it is proposed that blood glucose concentrations are monitored using the STG-22 (Nikkiso Co., Tokyo, Japan). The STG-22 is a reliable and accurate device for the measurement of blood glucose concentrations compared with the ABL 800FLEX machine (Radiometer Medical ApS, Brønshøj, Denmark) that is recommended by the National Committee for Clinical Laboratory Standards[80,81]. The STG-22 closed-loop glycemic control system is composed of a glucose sensor for the detection and/or monitoring of glucose and pumps for infusing an appropriate amount of insulin or glucose. The insulin and glucose pumps are computer regulated based on a target blood glucose value that is defined prior to initiation of the system[82,83]. It is has been proven clinically that the STG-22 device is safe and beneficial for maintaining glycemic control without hypoglycemic episodes in surgical patients[84-86] (Figure 6).

CONCLUSION

Insulinomas are the most common neuroendocrine tumors of the pancreas and cause hypoglycemia related to endogenous hyperinsulinism. More than 90% of insulinomas are benign and usually small, well-encapsulated, solitary tumors. Surgical resection is the treatment of choice for insulinomas and offers the only chance for cure. Most insulinomas can be identified intraoperatively by an experienced surgeon. However, what should be done in an operating theater when an insulinoma cannot be identified? Based on information presented in this review, we recommended the use of various non-invasive and invasive imaging modalities when the tumor cannot be detected using conventional diagnostic procedures. Blind surgical resection of the pancreas should not be undertaken in any patient who suffers from hypoglycemic episodes due to an insulinoma. Identifying the location of the insulinoma enables the surgeon to proceed with surgery uninterrupted, minimizing time in the operating theater, reducing the likelihood for re-operation, limiting perioperative complications, and ensuring, in most cases, a successful outcome[38].

In patients with malignant insulinomas, aggressive surgical resection, including extended pancreatic resection, liver resection, and/or liver transplantation, should be attempted when possible to improve patient survival[58,75-77]. Furthermore, aggressive secondary treatments may be indicated, such as chemoembolization or RFA for liver metastases from malignant insulinomas of the pancreas to control hypoglycemia. In patients who have unresectable or uncontrollable malignant insulinomas of the pancreas, several strategies need to be considered to both control hypoglycemic episodes and improve quality of life, including administration of ocreotide and continuous glucose monitoring. Insulinomas of the pancreas remain rare and may occur simply by chance. To refine the diagnosis and management of these tumors, epidemiologic and pathologic data should continue to be collected.

ACKNOWLEDGMENTS

This study was made possible with the support of Satoshi Ito and Kazuhiro Hanazaki (Kochi Medical School).

REFERENCES

1 **Moldow RE**, Connelly RR. Epidemiology of pancreatic cancer in Connecticut. *Gastroenterology* 1968; **55**: 677-686 [PMID: 4302500]
2 **Service FJ**, McMahon MM, O'Brien PC, Ballard DJ. Functioning insulinoma--incidence, recurrence, and long-term survival of patients: a 60-year study. *Mayo Clin Proc* 1991; **66**: 711-719 [PMID: 1677058]
3 **Kimura W**, Kuroda A, Morioka Y. Clinical pathology of endocrine tumors of the pancreas. Analysis of autopsy cases. *Dig Dis Sci* 1991; **36**: 933-942 [PMID: 2070707 DOI: 10.1007/

BF01297144]
4 **Lam KY**, Lo CY. Pancreatic endocrine tumour: a 22-year clinico-pathological experience with morphological, immunohistochemical observation and a review of the literature. *Eur J Surg Oncol* 1997; **23**: 36-42 [PMID: 9066745 DOI: 10.1016/S0748-7983(97)80140-0]
5 **Eriksson B**, Oberg K. Neuroendocrine tumours of the pancreas. *Br J Surg* 2000; **87**: 129-131 [PMID: 10671915 DOI: 10.1046/j.1365-2168.2000.01277.x]
6 **Gumbs AA**, Moore PS, Falconi M, Bassi C, Beghelli S, Modlin I, Scarpa A. Review of the clinical, histological, and molecular aspects of pancreatic endocrine neoplasms. *J Surg Oncol* 2002; **81**: 45-53; discussion 54 [PMID: 12210027 DOI: 10.1002/jso.10142]
7 **Mansour JC**, Chen H. Pancreatic endocrine tumors. *J Surg Res* 2004; **120**: 139-161 [PMID: 15172200 DOI: 10.1016/j.jss.2003.12.007]
8 **Oberg K**, Eriksson B. Endocrine tumours of the pancreas. *Best Pract Res Clin Gastroenterol* 2005; **19**: 753-781 [PMID: 16253899 DOI: 10.1016/j.bpg.2005.06.002]
9 **Chang F**, Chandra A, Culora G, Mahadeva U, Meenan J, Herbert A. Cytologic diagnosis of pancreatic endocrine tumors by endoscopic ultrasound-guided fine-needle aspiration: a review. *Diagn Cytopathol* 2006; **34**: 649-658 [PMID: 16900463 DOI: 10.1002/dc.20503]
10 **Sotoudehmanesh R**, Hedayat A, Shirazian N, Shahraeeni S, Ainechi S, Zeinali F, Kolahdoozan S. Endoscopic ultrasonography (EUS) in the localization of insulinoma. *Endocrine* 2007; **31**: 238-241 [PMID: 17906369 DOI: 10.1007/s12020-007-0045-4]
11 **Doi R**, Komoto I, Nakamura Y, Kawamura J, Fujimoto K, Wada M, Saga T, Imamura M. Pancreatic endocrine tumor in Japan. *Pancreas* 2004; **28**: 247-252 [PMID: 15084965 DOI: 10.1097/00006676-200404000-00006]
12 **Mittendorf EA**, Liu YC, McHenry CR. Giant insulinoma: case report and review of the literature. *J Clin Endocrinol Metab* 2005; **90**: 575-580 [PMID: 15522939 DOI: 10.1210/jc.2004-0825]
13 **Kondo T**, Tomita S, Adachi H, Motoshima H, Taketa K, Matsuyoshi A, Tokunaga H, Miyamura N, Araki E. A case of hyperinsulinemia of undetermined origin, successfully treated with long-acting octreotide. *Endocr J* 2005; **52**: 511-517 [PMID: 16284426 DOI: 10.1507/endocrj.52.511]
14 **Chatziioannou A**, Kehagias D, Mourikis D, Antoniou A, Limouris G, Kaponis A, Kavatzas N, Tseleni S, Vlachos L. Imaging and localization of pancreatic insulinomas. *Clin Imaging* 2001; **25**: 275-283 [PMID: 11566091 DOI: 10.1016/S0899-7071(01)00290-X]
15 **Sheth S**, Hruban RK, Fishman EK. Helical CT of islet cell tumors of the pancreas: typical and atypical manifestations. *AJR Am J Roentgenol* 2002; **179**: 725-730 [PMID: 12185053]
16 **Noone TC**, Hosey J, Firat Z, Semelka RC. Imaging and localization of islet-cell tumours of the pancreas on CT and MRI. *Best Pract Res Clin Endocrinol Metab* 2005; **19**: 195-211 [PMID: 15763695 DOI: 10.1016/j.beem.2004.11.013]
17 **Owen NJ**, Sohaib SA, Peppercorn PD, Monson JP, Grossman AB, Besser GM, Reznek RH. MRI of pancreatic neuroendocrine tumours. *Br J Radiol* 2001; **74**: 968-973 [PMID: 11675319]
18 **Thoeni RF**, Mueller-Lisse UG, Chan R, Do NK, Shyn PB. Detection of small, functional islet cell tumors in the pancreas: selection of MR imaging sequences for optimal sensitivity. *Radiology* 2000; **214**: 483-490 [PMID: 10671597]
19 **Anaye A**, Mathieu A, Closset J, Bali MA, Metens T, Matos C. Successful preoperative localization of a small pancreatic insulinoma by diffusion-weighted MRI. *JOP* 2009; **10**: 528-531 [PMID: 19734630]
20 **Kann PH**, Ivan D, Pfützner A, Forst T, Langer P, Schaefer S. Preoperative diagnosis of insulinoma: low body mass index, young age, and female gender are associated with negative imaging by endoscopic ultrasound. *Eur J Endocrinol* 2007; **157**: 209-213 [PMID: 17656600 DOI: 10.1530/EJE-07-0117]
21 **McLean AM**, Fairclough PD. Endoscopic ultrasound in the localisation of pancreatic islet cell tumours. *Best Pract Res Clin Endocrinol Metab* 2005; **19**: 177-193 [PMID: 15763694 DOI: 10.1016/j.beem.2004.11.012]
22 **Kann PH**, Rothmund M, Zielke A. Endoscopic ultrasound imaging of insulinomas: limitations and clinical relevance. *Exp Clin Endocrinol Diabetes* 2005; **113**: 471-474 [PMID: 16151982 DOI: 10.1055/s-2005-865752]
23 **Tseng LM**, Chen JY, Won JG, Tseng HS, Yang AH, Wang SE, Lee CH. The role of intra-arterial calcium stimulation test with hepatic venous sampling (IACS) in the management of occult insulinomas. *Ann Surg Oncol* 2007; **14**: 2121-2127 [PMID: 17431724 DOI: 10.1245/s10434-007-9398-4]
24 **Morita S**, Machida H, Kuwatsuru R, Saito N, Suzuki K, Iihara M, Obara T, Mitsuhashi N. Preoperative localization of pancreatic insulinoma by super selective arterial stimulation with venous sampling. *Abdom Imaging* 2007; **32**: 126-128 [PMID: 16932851 DOI: 10.1007/s00261-006-9040-0]
25 **Guettier JM**, Kam A, Chang R, Skarulis MC, Cochran C, Alexander HR, Libutti SK, Pingpank JF, Gorden P. Localization of insulinomas to regions of the pancreas by intraarterial calcium stimulation: the NIH experience. *J Clin Endocrinol Metab* 2009; **94**: 1074-1080 [PMID: 19190102 DOI: 10.1210/jc.2008-1986]
26 **Chung JC**, Choi SH, Jo SH, Heo JS, Choi DW, Kim YI. Localization and surgical treatment of the pancreatic insulinomas. *ANZ J Surg* 2006; **76**: 1051-1055 [PMID: 17199688 DOI: 10.1111/j.1445-2197.2006.03947.x]
27 **Jackson JE**. Angiography and arterial stimulation venous sampling in the localization of pancreatic neuroendocrine tumours. *Best Pract Res Clin Endocrinol Metab* 2005; **19**: 229-239 [PMID: 15763697 DOI: 10.1016/j.beem.2004.10.002]
28 **Gimm O**, König E, Thanh PN, Brauckhoff M, Karges W, Dralle H. Intra-operative quick insulin assay to confirm complete resection of insulinomas guided by selective arterial calcium injection (SACI). *Langenbecks Arch Surg* 2007; **392**: 679-684 [PMID: 17294212 DOI: 10.1007/s00423-006-0144-3]
29 **Wong M**, Isa SH, Zahiah M, Azmi KN. Intraoperative ultrasound with palpation is still superior to intra-arterial calcium stimulation test in localising insulinoma. *World J Surg* 2007; **31**: 586-592 [PMID: 17322973 DOI: 10.1007/s00268-006-0106-5]
30 **Hashimoto LA**, Walsh RM. Preoperative localization of insulinomas is not necessary. *J Am Coll Surg* 1999; **189**: 368-373 [PMID: 10509462 DOI: 10.1016/S1072-7515(99)00163-5]
31 **Shin LK**, Brant-Zawadzki G, Kamaya A, Jeffrey RB. Intraoperative ultrasound of the pancreas. *Ultrasound Q* 2009; **25**: 39-48; quiz 48 [PMID: 19276960 DOI: 10.1097/RUQ.0b013e3181901ce4]
32 **Ravi K**, Britton BJ. Surgical approach to insulinomas: are pre-operative localisation tests necessary? *Ann R Coll Surg Engl* 2007; **89**: 212-217 [PMID: 17394700 DOI: 10.1308/003588407X179008]
33 **Kalafat H**, Mihmanli I, Saribeyoglu K, Belli A. Intraoperative doppler ultrasound: a reliable diagnostic method in insulinoma. *Hepatogastroenterology* 2007; **54**: 1256-1258 [PMID: 17629082]
34 **Cryer PE**. Mechanisms of hypoglycemia-associated autonomic failure and its component syndromes in diabetes. *Diabetes* 2005; **54**: 3592-3601 [PMID: 16306382 DOI: 10.2337/diabetes.54.12.3592]
35 **Suzuki K**, Miyamoto M, Miyamoto T, Hirata K. Insulinoma with early-morning abnormal behavior. *Intern Med* 2007; **46**: 405-408 [PMID: 17409607 DOI: 10.2169/internalmedicine.46.6071]
36 **Service FJ**. Hypoglycemic disorders. *N Engl J Med* 1995; **332**: 1144-1152 [PMID: 7700289 DOI: 10.1056/NEJM199504273321707]
37 **de Herder WW**, Niederle B, Scoazec JY, Pauwels S, Klop-

pel G, Falconi M, Kwekkeboom DJ, Oberg K, Eriksson B, Wiedenmann B, Rindi G, O'Toole D, Ferone D. Well-differentiated pancreatic tumor/carcinoma: insulinoma. *Neuroendocrinology* 2006; **84**: 183-188 [PMID: 17312378 DOI: 10.1159/000098010]

38 **Rostambeigi N**, Thompson GB. What should be done in an operating room when an insulinoma cannot be found? *Clin Endocrinol* (Oxf) 2009; **70**: 512-515 [PMID: 19170712 DOI: 10.1111/j.1365-2265.2009.03527.x]

39 **Service FJ**, Natt N. The prolonged fast. *J Clin Endocrinol Metab* 2000; **85**: 3973-3974 [PMID: 11095416 DOI: 10.1210/jc.85.11.3973]

40 **Lebowitz MR**, Blumenthal SA. The molar ratio of insulin to C-peptide. An aid to the diagnosis of hypoglycemia due to surreptitious (or inadvertent) insulin administration. *Arch Intern Med* 1993; **153**: 650-655 [PMID: 8439228 DOI: 10.1001/archinte.1993.00410050082011]

41 **van Bon AC**, Benhadi N, Endert E, Fliers E, Wiersinga WM. Evaluation of endocrine tests. D: the prolonged fasting test for insulinoma. *Neth J Med* 2009; **67**: 274-278 [PMID: 19687521]

42 **Vezzosi D**, Bennet A, Fauvel J, Caron P. Insulin, C-peptide and proinsulin for the biochemical diagnosis of hypoglycaemia related to endogenous hyperinsulinism. *Eur J Endocrinol* 2007; **157**: 75-83 [PMID: 17609405 DOI: 10.1530/EJE-07-0109]

43 **Hirshberg B**, Livi A, Bartlett DL, Libutti SK, Alexander HR, Doppman JL, Skarulis MC, Gorden P. Forty-eight-hour fast: the diagnostic test for insulinoma. *J Clin Endocrinol Metab* 2000; **85**: 3222-3226 [PMID: 10999812 DOI: 10.1210/jc.85.9.3222]

44 **Marks V**. Recognition and differential diagnosis of spontaneous hypoglycaemia. *Clin Endocrinol* (Oxf) 1992; **37**: 309-316 [PMID: 1483286 DOI: 10.1111/j.1365-2265.1992.tb02329.x]

45 **Kar P**, Price P, Sawers S, Bhattacharya S, Reznek RH, Grossman AB. Insulinomas may present with normoglycemia after prolonged fasting but glucose-stimulated hypoglycemia. *J Clin Endocrinol Metab* 2006; **91**: 4733-4736 [PMID: 17003090 DOI: 10.1210/jc.2006-1430]

46 **Goh BK**, Ooi LL, Cheow PC, Tan YM, Ong HS, Chung YF, Chow PK, Wong WK, Soo KC. Accurate preoperative localization of insulinomas avoids the need for blind resection and reoperation: analysis of a single institution experience with 17 surgically treated tumors over 19 years. *J Gastrointest Surg* 2009; **13**: 1071-1077 [PMID: 19291334 DOI: 10.1007/s11605-009-0858-y]

47 **Lo CY**, Lam KY, Kung AW, Lam KS, Tung PH, Fan ST. Pancreatic insulinomas. A 15-year experience. *Arch Surg* 1997; **132**: 926-930 [PMID: 9267281 DOI: 10.1001/archsurg.1997.01430320128023]

48 **Tucker ON**, Crotty PL, Conlon KC. The management of insulinoma. *Br J Surg* 2006; **93**: 264-275 [PMID: 16498592 DOI: 10.1002/bjs.5280]

49 **McAuley G**, Delaney H, Colville J, Lyburn I, Worsley D, Govender P, Torreggiani WC. Multimodality preoperative imaging of pancreatic insulinomas. *Clin Radiol* 2005; **60**: 1039-1050 [PMID: 16179163 DOI: 10.1016/j.crad.2005.06.005]

50 **Balci NC**, Semelka RC. Radiologic features of cystic, endocrine and other pancreatic neoplasms. *Eur J Radiol* 2001; **38**: 113-119 [PMID: 11335093 DOI: 10.1016/S0720-048X(01)00296-0]

51 **Kurosaki Y**, Kuramoto K, Itai Y. Hyperattenuating insulinoma at unenhanced CT. *Abdom Imaging* 1996; **21**: 334-336 [PMID: 8661578 DOI: 10.1007/s002619900076]

52 **Gouya H**, Vignaux O, Augui J, Dousset B, Palazzo L, Louvel A, Chaussade S, Legmann P. CT, endoscopic sonography, and a combined protocol for preoperative evaluation of pancreatic insulinomas. *AJR Am J Roentgenol* 2003; **181**: 987-992 [PMID: 14500214]

53 **Davì MV**, Falconi M. Pancreas: Insulinoma--new insights into an old disease. *Nat Rev Endocrinol* 2009; **5**: 300-302 [PMID: 19465896 DOI: 10.1038/nrendo.2009.99]

54 **Ritzel RA**, Isermann B, Schilling T, Knaebel HP, Büchler MW, Nawroth PP. Diagnosis and localization of insulinoma after negative laparotomy by hyperinsulinemic, hypoglycemic clamp and intra-arterial calcium stimulation. *Rev Diabet Stud* 2004; **1**: 42-46 [PMID: 17491664 DOI: 10.1900/RDS.2004.1.42]

55 **Rott G**, Biggemann M, Pfohl M. Embolization of an insulinoma of the pancreas with trisacryl gelatin microspheres as definitive treatment. *Cardiovasc Intervent Radiol* 2008; **31**: 659-662 [PMID: 17922161 DOI: 10.1007/s00270-007-9185-4]

56 **Jürgensen C**, Schuppan D, Neser F, Ernstberger J, Junghans U, Stölzel U. EUS-guided alcohol ablation of an insulinoma. *Gastrointest Endosc* 2006; **63**: 1059-1062 [PMID: 16733126 DOI: 10.1016/j.gie.2005.10.034]

57 **Limmer S**, Huppert PE, Juette V, Lenhart A, Welte M, Wietholtz H. Radiofrequency ablation of solitary pancreatic insulinoma in a patient with episodes of severe hypoglycemia. *Eur J Gastroenterol Hepatol* 2009; **21**: 1097-1101 [PMID: 19685572 DOI: 10.1097/MEG.0b013e328323d70e]

58 **Gillams A**, Cassoni A, Conway G, Lees W. Radiofrequency ablation of neuroendocrine liver metastases: the Middlesex experience. *Abdom Imaging* 2005; **30**: 435-441 [PMID: 15759207 DOI: 10.1007/s00261-004-0258-4]

59 **Vezzosi D**, Bennet A, Courbon F, Caron P. Short- and long-term somatostatin analogue treatment in patients with hypoglycaemia related to endogenous hyperinsulinism. *Clin Endocrinol* (Oxf) 2008; **68**: 904-911 [PMID: 18031316 DOI: 10.1111/j.1365-2265.2007.03136.x]

60 **Kishikawa H**, Okada Y, Hirose A, Tanikawa T, Kanda K, Tanaka Y. Successful treatment of insulinoma by a single daily dose of octreotide in two elderly female patients. *Endocr J* 2006; **53**: 79-85 [PMID: 16543676 DOI: 10.1507/endocrj.53.79]

61 **Katabami T**, Kato H, Shirai N, Naito S, Saito N. Successful long-term treatment with once-daily injection of low-dose octreotide in an aged patient with insulinoma. *Endocr J* 2005; **52**: 629-634 [PMID: 16284444 DOI: 10.1507/endocrj.52.629]

62 **Falconi M**, Bettini R, Boninsegna L, Crippa S, Butturini G, Pederzoli P. Surgical strategy in the treatment of pancreatic neuroendocrine tumors. *JOP* 2006; **7**: 150-156 [PMID: 16407638]

63 **España-Gómez MN**, Velázquez-Fernández D, Bezaury P, Sierra M, Pantoja JP, Herrera MF. Pancreatic insulinoma: a surgical experience. *World J Surg* 2009; **33**: 1966-1970 [PMID: 19629581 DOI: 10.1007/s00268-009-0145-9]

64 **Okabayashi T**, Maeda H, Nishimori I, Sugimoto T, Ikeno T, Hanazaki K. Pancreatic fistula formation after pancreaticoduodenectomy; for prevention of this deep surgical site infection after pancreatic surgery. *Hepatogastroenterology* 2009; **56**: 519-523 [PMID: 19579634]

65 **Okabayashi T**, Kobayashi M, Nishimori I, Sugimoto T, Onishi S, Hanazaki K. Risk factors, predictors and prevention of pancreatic fistula formation after pancreatoduodenectomy. *J Hepatobiliary Pancreat Surg* 2007; **14**: 557-563 [PMID: 18040620 DOI: 10.1007/s00534-007-1242-5]

66 **Okabayashi T**, Hanazaki K, Nishimori I, Sugimoto T, Yoshioka R, Dabanaka K, Kobayashi M, Onishi S. Pancreatic transection using a sharp hook-shaped ultrasonically activated scalpel. *Langenbecks Arch Surg* 2008; **393**: 1005-1008 [PMID: 17973117 DOI: 10.1007/s00423-007-0236-8]

67 **Moore TJ**, Peterson LM, Harrington DP, Smith RJ. Successful arterial embolization of an insulinoma. *JAMA* 1982; **248**: 1353-1355 [PMID: 6287050 DOI: 10.1001/jama.1982.03330110049026]

68 **Uflacker R**. Arterial embolization as definitive treatment for benign insulinoma of the pancreas. *J Vasc Interv Radiol* 1992; **3**: 639-644; discussion 644-646 [PMID: 1446125 DOI: 10.1016/S1051-0443(92)72912-1]

69 **Shojamanesh H**, Gibril F, Louie A, Ojeaburu JV, Bashir S,

Abou-Saif A, Jensen RT. Prospective study of the antitumor efficacy of long-term octreotide treatment in patients with progressive metastatic gastrinoma. *Cancer* 2002; **94**: 331-343 [PMID: 11900219 DOI: 10.1002/cncr.10195]

70 **Oberg K**, Kvols L, Caplin M, Delle Fave G, de Herder W, Rindi G, Ruszniewski P, Woltering EA, Wiedenmann B. Consensus report on the use of somatostatin analogs for the management of neuroendocrine tumors of the gastroenteropancreatic system. *Ann Oncol* 2004; **15**: 966-973 [PMID: 15151956 DOI: 10.1093/annonc/mdh216]

71 **Healy ML**, Dawson SJ, Murray RM, Zalcberg J, Jefford M. Severe hypoglycaemia after long-acting octreotide in a patient with an unrecognized malignant insulinoma. *Intern Med J* 2007; **37**: 406-409 [PMID: 17535385 DOI: 10.1111/j.1445-5994.2007.01371.x]

72 **Grant CS**. Insulinoma. *Best Pract Res Clin Gastroenterol* 2005; **19**: 783-798 [PMID: 16253900 DOI: 10.1016/j.bpg.2005.05.008]

73 **Hirshberg B**, Cochran C, Skarulis MC, Libutti SK, Alexander HR, Wood BJ, Chang R, Kleiner DE, Gorden P. Malignant insulinoma: spectrum of unusual clinical features. *Cancer* 2005; **104**: 264-272 [PMID: 15937909 DOI: 10.1002/cncr.21179]

74 **La Rosa S**, Klersy C, Uccella S, Dainese L, Albarello L, Sonzogni A, Doglioni C, Capella C, Solcia E. Improved histologic and clinicopathologic criteria for prognostic evaluation of pancreatic endocrine tumors. *Hum Pathol* 2009; **40**: 30-40 [PMID: 18715612 DOI: 10.1016/j.humpath.2008.06.005]

75 **Scott A**, Hinwood D, Donnelly R. Radio-frequency ablation for symptom control in a patient with metastatic pancreatic insulinoma. *Clin Endocrinol* (Oxf) 2002; **56**: 557-559 [PMID: 11966750 DOI: 10.1046/j.1365-2265.2002.01506.x]

76 **Le Treut YP**, Delpero JR, Dousset B, Cherqui D, Segol P, Mantion G, Hannoun L, Benhamou G, Launois B, Boillot O, Domergue J, Bismuth H. Results of liver transplantation in the treatment of metastatic neuroendocrine tumors. A 31-case French multicentric report. *Ann Surg* 1997; **225**: 355-364 [PMID: 9114793 DOI: 10.1097/00000658-199704000-00003]

77 **Olausson M**, Friman S, Cahlin C, Nilsson O, Jansson S, Wängberg B, Ahlman H. Indications and results of liver transplantation in patients with neuroendocrine tumors. *World J Surg* 2002; **26**: 998-1004 [PMID: 12016481 DOI: 10.1007/s00268-002-6631-y]

78 **Sawyer AM**, Schade DS. Use of a continuous glucose monitor in the management of inoperable metastatic insulinoma: a case report. *Endocr Pract* 2008; **14**: 880-883 [PMID: 18996817]

79 **Munir A**, Choudhary P, Harrison B, Heller S, Newell-Price J. Continuous glucose monitoring in patients with insulinoma. *Clin Endocrinol* (Oxf) 2008; **68**: 912-918 [PMID: 18088393 DOI: 10.1111/j.1365-2265.2007.03161.x]

80 **Yamashita K**, Okabayashi T, Yokoyama T, Yatabe T, Maeda H, Manabe M, Hanazaki K. The accuracy of a continuous blood glucose monitor during surgery. *Anesth Analg* 2008; **106**: 160-163, table of contents [PMID: 18165572 DOI: 10.1213/01.ane.0000296461.26492.3c]

81 **Yamashita K**, Okabayashi T, Yokoyama T, Yatabe T, Maeda H, Manabe M, Hanazaki K. Accuracy and reliability of continuous blood glucose monitor in post-surgical patients. *Acta Anaesthesiol Scand* 2009; **53**: 66-71 [PMID: 19032568 DOI: 10.1111/j.1399-6576.2008.01799.x]

82 **Hanazaki K**, Nosé Y, Brunicardi FC. Artificial endocrine pancreas. *J Am Coll Surg* 2001; **193**: 310-322 [PMID: 11548802 DOI: 10.1016/S1072-7515(01)01014-6]

83 **Okabayashi T**, Hnazaki K, Nishimori I, Sugimoto T, Maeda H, Yatabe T, Dabanaka K, Kobayashi M, Yamashita K. Continuous post-operative blood glucose monitoring and control using a closed-loop system in patients undergoing hepatic resection. *Dig Dis Sci* 2008; **53**: 1405-1410 [PMID: 17934862 DOI: 10.1007/s10620-007-0010-3]

84 **Okabayashi T**, Nishimori I, Yamashita K, Sugimoto T, Maeda H, Yatabe T, Kohsaki T, Kobayashi M, Hanazaki K. Continuous postoperative blood glucose monitoring and control by artificial pancreas in patients having pancreatic resection: a prospective randomized clinical trial. *Arch Surg* 2009; **144**: 933-937 [PMID: 19841361 DOI: 10.1001/archsurg.2009.176]

85 **Okabayashi T**, Nishimori I, Maeda H, Yamashita K, Yatabe T, Hanazaki K. Effect of intensive insulin therapy using a closed-loop glycemic control system in hepatic resection patients: a prospective randomized clinical trial. *Diabetes Care* 2009; **32**: 1425-1427 [PMID: 19435954 DOI: 10.2337/dc08-2107]

86 **Okabayashi T**, Nishimori I, Yamashita K, Sugimoto T, Yatabe T, Maeda H, Kobayashi M, Hanazaki K. Risk factors and predictors for surgical site infection after hepatic resection. *J Hosp Infect* 2009; **73**: 47-53 [PMID: 19640610 DOI: 10.1016/j.jhin.2009.04.022]

P- Reviewers Matthews V, Iwasaki Y **S- Editor** Gou SX
L- Editor A **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i6.838

World J Gastroenterol 2013 February 14; 19(6): 838-845
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

MINIREVIEWS

Hepatitis B vaccine in celiac disease: Yesterday, today and tomorrow

Giovanna Vitaliti, Andrea Domenico Praticò, Carla Cimino, Giovanna Di Dio, Elena Lionetti, Mario La Rosa, Salvatore Leonardi

Giovanna Vitaliti, Andrea Domenico Praticò, Carla Cimino, Giovanna Di Dio, Elena Lionetti, Mario La Rosa, Salvatore Leonardi, Department of Pediatrics, University of Catania, 95100 Catania, Italy

Author contributions: All authors equally contributed to the building up of the paper, the literature bibliography research and to the writing of the paper.

Correspondence to: Salvatore Leonardi, MD, Department of Pediatrics, University of Catania, 95100 Catania, Italy. leonardi@unict.it

Telephone: +39-953-782764 Fax: +39-953-782385

Received: June 20, 2012 Revised: October 12, 2012
Accepted: October 16, 2012
Published online: February 14, 2013

Abstract

Some studies showed that in celiac patients the immunological response to vaccination is similar to that one found in general population except for vaccine against hepatitis B virus (HBV). The non-responsiveness to HBV vaccine has also been described in healthy people, nevertheless the number of non-responders has been demonstrated to be higher in celiac disease (CD) patients than in healthy controls. Several hypothesis explaining this higher rate of unresponsiveness to HBV vaccine in CD patients have been described, such as the genetic hypothesis, according with CD patients carrying the disease-specific haplotype HLA-B8, DR3, and DQ2, show a lower response to HBV vaccine both in clinical expressed CD patients and in healthy people carrying the same haplotype. On the other hand, it has been demonstrated that the gluten intake during the vaccination seems to influence the response to the same vaccine. Moreover, it has been demonstrated a possible genetic predisposition to hepatitis B vaccine non-responsiveness likely due to the presence of specific human leukocyte antigen haplotypes and specific single nucleotide polymorphism in genes of cytokine/cytokine receptors and toll like receptors, but the pathogenic mechanism responsible for this low responsiveness still remains unclear. The aim of this review is to focus on the possible pathogenic causes of unresponsiveness to HBV vaccine in CD patients and to propose an alternative vaccination schedule in order to improve the responsiveness to HBV vaccine in this at-risk patients.

© 2013 Baishideng. All rights reserved.

Key words: Celiac disease; Non responders; Hepatitis B vaccine; Vaccination schedules

Vitaliti G, Praticò AD, Cimino C, Di Dio G, Lionetti E, La Rosa M, Leonardi S. Hepatitis B vaccine in celiac disease: Yesterday, today and tomorrow. *World J Gastroenterol* 2013; 19(6): 838-845 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i6/838.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i6.838

INTRODUCTION

It is estimated that more than one third of the world's population has been infected with the hepatitis B virus (HBV), causing acute and chronic liver diseases, ranging from fulminant hepatitis to cirrhosis and eventually hepatocellular carcinoma, with an incidence of 620 000 death per year[1-3].

On this regard, in order to prevent this serious health problem, since 1982 a safe and effective hepatitis B vaccine has been available. The first vaccines were plasma-derived, which contained purified hepatitis B surface antigen (HBsAg) obtained from the plasma of people with chronic HBV infection. In the following years, recombinant DNA hepatitis B vaccine has been developed, containing purified HBsAg obtained by culturing genetically engineered yeast or mammalian cells carrying the *HBsAg* gene. Currently recombinant DNA hepatitis B vaccines are predominant-

ly being used, while plasma-derived hepatitis B vaccines are still being used in several low-income countries[4].

At the beginning, the hepatitis B vaccine was considered for use in high risk individuals for acquiring HBV infection. Actually, it has become more widely used and recommendations for hepatitis B vaccination have been extended to all infants in an attempt to achieve protection against HBV infection[5]. Despite these recommendations, 8 countries in Northern Europe (Denmark, Finland, Iceland, Ireland, Netherlands, Norway, Sweden, and the United Kingdom) have yet to implement such a policy, currently adopting an "at-risk" strategy. In 2007, Zuckerman *et al*[6] recommend a reassessment in these country of their hepatitis B prevention strategies considering the difficulty in identifying all at-risk individuals and the lack of effectiveness of the vaccination on at-risk subjects in reducing the overall incidence of hepatitis B.

Italy was one of the first countries to implement an universal strategy of hepatitis B vaccination[7]. Since 1984 HBV vaccination has been recommended and offered free of charge by the National Health Service to high-risk groups-*e.g.*, family members of HBsAg carriers, healthcare workers and in 1991, vaccination became mandatory for all new born and for twelve year-old adolescents[8].

Consequent to this global strategy, a decrease in prevalence of chronic HBV infection among vaccinated children and adolescents has been documented, including people living in high risk area such as China, Hong Kong, Taiwan, the Gambia, Senegal, Alaska and Italy[9].

Usually, a single course of three doses of hepatitis B vaccine administered in a variety of schedules, such as at birth, 1 and 6 mo schedule, the 6, 10 and 14 wk doses and the 2, 4 and 6 mo schedule induces protective levels of antibody to HBsAg in nearly 95% of healthy infants and children[10].

The antibody response to hepatitis B vaccine has been found occurring in more than 90% of the healthy vaccinated subjects[11-13]. However some studies showed that serum anti-HBs levels decreases with the time following vaccination[14,15]. Several factors, such as age, body mass index and smoking at the time of vaccination, have been found to be associated with a lower rate of antibody response to hepatitis B vaccine[16,17], and the decline of HBs antibody titer seemed mainly to be proportional to the antibody titer originally obtained[18].

Long-term follow-up studies of newborn vaccination showed that antibodies become negative in 15%-50% among the vaccine responders within 5 to 10 years[19-21].

The clinical significance of the disappearance of specific antibodies in immunocompetent patients responders to previous vaccination remains object of discussion.

Successfully vaccinated people who have lost antibodies after primary vaccination, usually show a rapid anamnestic response when boosted. This means that the immunological memory for HBsAg can outlast antibody detection, providing long-term protection against the disease. Hence, there is consensus that there is no need to administer booster doses of vaccine to ensure long-term protection in immunocompetent patients[22-25].

On the other hands some authors suggest specific strategies for the vaccination of certain groups of people at high risk such as haemodialysis, celiac and thalassemia patients[26-31].

Vaccinated subjects with an anti-HBs titer less than 10 mIU/mL after completion of primary vaccine series are called "no responders".

Several factors have been associated with a non response to the HBV vaccine. These include inappropriate vaccine storage conditions, vaccination in buttocks, obesity, smoking, drug abuse, infections[32]. There are also chronic conditions, such as chronic alcoholism, chronic kidney disease, human immunodeficiency virus infection, immune-suppression, type I Diabetes Mellitus and Celiac disease that are characterized by a lower response to HBV vaccination[33-45]. Recently, it has been demonstrated a possible genetic predisposition to hepatitis B vaccine non-responsiveness likely due to the presence of specific human leukocyte antigen (HLA) haplotypes and specific single nucleotide polymorphism (SNP) in genes of cytokine/cytokine receptors and toll like receptors (TLR)[42,46], but the pathogenic mechanism responsible for this low responsiveness still remains unclear.

CELIAC DISEASE AND HBV VACCINE: THE HISTORY

Celiac disease (CD) is an autoimmune disorder characterized by a permanent intolerance to ingested gluten, which results in immunologically mediated inflammatory damage to intestinal mucosa[47]. Genetic, environmental and immunological factors seem to be responsible for the disease.

CD is usually characterized by various gastrointestinal (GI) symptoms (*e.g.*, diarrhea, malabsorption, weight loss) associated with consumption of grains containing gluten (wheat, barley, rye). Although some CD patients may have primarily GI symptoms, CD may be detected due to associated extra intestinal disorders, even without GI symptoms, or due to screening for CD based on a positive family history. CD has a strong association with HLA-DQ2 and HLA-DQ8[48]. HLA-DQ2 is present in 90% to 95% of patients with CD[49].

The link between HBV infection and celiac disease seems to be controversial. Relatively little data exist on the relationship between HBV and CD, although one third of the world's population (around 2 billion people) have been infected with HBV. It has been reported that the response rate to HBV vaccination in CD-infected individuals is lower (30%-50%) than in the general population (4%-10%)[42,43] (Table 1). Recently, it has been hypothesized that non intestinal inflammatory diseases may trigger immunologic gluten intolerance in suscepti-

ble individuals, and HBV as far as hepatitis C virus (HCV) were thought to be suitable candidates. However this assumption is still matter of debate. Relatively few data exist on the relationship between HBV and CD. It could be speculated that HBV as well as HCV infections may trigger immunologic gluten intolerance in genetically susceptible people and that chronic HBV segregates a higher percentage of CD patients[50,51]. However this assumption is still a matter of debate.

Possible activation of CD due to the treatment of hepatitis infection is another controversial point. There have been some reports indicating that autoimmune disorders such as insulin-dependent diabetes mellitus and celiac disease can develop during treatment with interferon (IFN)-α for viral hepatitis because of its immune modulatory properties[52,53]. CD activation during interferon α or interferon α plus ribavirin therapy has recently been observed in HCV-positive patients[54], confirming that IFN-α therapy could trigger CD in susceptible subjects during treatment. In the study of Leonardi *et al*[30], although evidence suggested that IFN-α can activate CD4T cells in the lamina propria and cause intestinal tissue damage[55], no patient treated in childhood showed any serological marker of CD at the time of the present study. Due to the small sample size authors could not claim that there was no association between CD and HBV, although a sample size more representative of the prevalence of CD in Italy should help to better examine the relationship between CD and HBV.

Some studies showed that in celiac patients the immunological response to vaccination is similar to that one found in general population except for vaccine against HBV[42,56]. On this regard, it is well known that unresponsiveness to hepatitis B vaccine in healthy people has been attributed to failure of class II major histocompatibility complex molecules in the interaction with processed protein antigen, in the stimulation of T-helper cells, or in both[57,58].

Analysis of previous studies suggested a very high incidence of a particular extended HLA haplotype in no responders to hepatitis B vaccine. In fact, homozygotes for HLA-B8, DR3, and DQ2 were found to have a significantly higher incidence of hepatitis B vaccine non-response[58]. In 1989, Alper *et al*[59] prospectively vaccinated 5 homozygotes and 9 heterozygotes for extended MHC aplotype (HLA-B8, SC01, DR3). Four of the 5 homozygotes produced low levels of HBsAb two months after their third HBV vaccination, whereas all 9 heterozygotes had significantly higher titers of HBsAb. In 1995, Livenson *et al*[60] studied 153 patients with end-stage renal disease immunized with a recombinant HBV vaccine. Homozygotes for HLA-A1, HLA-B8, HLA-DR3 and HLA-DQ2 were found almost exclusively in the non-responder group and a significant higher number of heterozygotes for these alleles was found in the non-responder group compared to the responders[59]. In the same year, Martinetti *et al*[61] performed an HLA study in 9 absolute non-responder (serum titer of anti-HBsAg < 2 mIU/mL) and 8 low-responder (serum antibody level between 2 and 9.9 mIU/mL) infants who underwent, in neonatal period, HBV vaccination. The investigation pointed out that many of these subjects carry HLA haplotypes classically involved in autoimmune diseases: HLA DR7, DQ2, DR4, DQ8 and DR3. Further Godkin *et al*[58] investigated the binding affinities of envelope and core peptides of HBV to particular HLA glycoproteins, and their data supported the direct involvement of HLA-DR3 in HBV vaccine no responsiveness.

Table 1 People who have anti-HBs titre after completion of primary vaccine series less than 10 mIU/mL

	Celiac patients (no responders, %)	Control subjects (no responders, %)
Leonardi *et al*[30]	30/60 (50)	7/60 (11.7)
Ahishali *et al*[42]	8/25 (32)	0/20 (0)
Zingone *et al*[43]	31/51 (60.7)	13/48 (27.08)
Ertekin *et al*[44]	20/52 (38.5)	2/20 (10)
Noh *et al*[56]	13/19 (68.4)	-

Since the HLA-DQ2 haplotype is over-represented in celiac population, it has been postulated that this genetic profile may play a crucial role in predisposing celiac patients to a lower grade of immunization to hepatitis B vaccine[62].

On the other hand some studies argue that in celiac individuals gluten intake at the time of vaccination may influence the vaccine-induced immune response[41,44].

Nevertheless all these hypotheses are still argument of debate and no responsiveness of celiac patients to the HBV vaccine remains a significant public health problem, since the worldwide spread of the disease although there is no convincing evidence that patients with viral hepatitis B carry an increased risk of celiac disease[63].

In 2003, Noh *et al*[62] studied 19 celiac patients and found that 13 did not respond to vaccination. HLA typing was performed on 15 of these subjects (13 no responders and two responders). All tested subjects were apparently either homozygous or heterozygous for DQ2. The authors postulated that the non responsiveness to the hepatitis vaccine in celiac patients is due to the suppression of the Th2 response and to the B cell differentiation due to the high protection performed by the IFN-gamma.

In 2009, Leonardi *et al*[30] retrospectively analysed the response to HBV vaccine in 60 celiac patients and they found that 30 of 60 CD patients (50%) and 7 of 60 control subjects (11.6%) were non-responders to HBV vaccination ($P < 0.0001$).

It is well known that in celiac disease the intestinal damage is caused by interaction between specific deaminated glutamine residues of gliadin and HLA-DQ2 or DQ8 molecules[63].

Both HBsAg protein fragments and gliadin peptides bind to HLA-DQ2 molecules, and their competition

may result in a defective antibody response against the recombinant HBsAg vaccine in active CD[49].

In 2008, Nemes *et al*[41] studied 128 children and adolescent with celiac disease and 113 age-matched control subjects. For all celiac patients diet compliance and CD activity were monitored by measurement of antibodies against transglutaminase and endomysium. The authors demonstrated that the rate of primary non-response to the standard regimen of recombinant HBV vaccination was surprisingly high (74.1%) in undiagnosed and untreated celiac adolescents.

In 2010, Ertem *et al*[64] evaluated anti-HBs titer in celiac patients and healthy children. They found that anti-HBs negativity was significantly higher in celiac patients than in healthy controls. They also demonstrated that response to HBV vaccine in children with CD who were compliant with the gluten free diet (GFD) was not different from that found in the healthy population.

In 2010, even Zingone *et al*[44] hypothesized the possible role of dietary gluten in induction of a suboptimal immune response in celiac patients. They studied 51 celiac patients who were on a GFD and were negative for anti-transglutaminase-IgA antibody at the time of testing. Eleven years after primary vaccination the proportion of vaccinated subjects with anti-HBs antibody $\geqslant$ 10 mIU/mL was significantly lower in celiac than in controls (68.6% *vs* 91.7%; $P < 0.01$). Only three (5.9%) celiac patients were on GFD at the time of primary vaccination. Fourteen out of 16 (87.5%) celiac patients and all controls with anti-HBs < 10 mIU/mL received a booster dose of vaccine. Two weeks after the booster dose, 4 (28.6%) celiac patients and 3 (75%) controls showed an anamnestic response. Three out of 10 celiac patients who did not respond to the booster dose agreed to complete a new vaccine cycle. One month after vaccination completion, all showed anti HBs between 10 and 100 mIU/mL. Thus, the authors concluded that the gluten intake at the time of vaccination could decrease the efficacy of the same vaccination.

To confirm the role of gluten in the unresponsiveness to HBV vaccine in celiac patients, Ertekin *et al*[45] observed 52 children with CD and 20 age healthy children who received HBV vaccination according to the standard immunization schedule. They found that anti-HBs positivity was significantly higher in celiac patients who were compliant to GFD than in those who were noncompliant.

Even in the study leaded by Leonardi *et al*[30], the authors found a significantly higher number of responders in patients younger than 18 mo at diagnosis and a significantly lower number of responders in adolescent patients older than 14 years at diagnosis. This data confirms that in celiac patients a complete cycle of revaccination during a well controlled GFD might be more effective than the primary vaccination performed on a gluten-containing diet.

FUTURE DIRECTION

We have underlined how celiac disease is characterized by a low responsiveness to vaccinations such as HBV vaccine, and both for the widespread of celiac disease, that is high in prevalence of morbidity, and for the wild range of not response to HBV vaccine. The problem of unresponsiveness could represent a matter of world health, because the group of non-responders patients could be considered as a large reservoir of HBV-susceptible people that will persist as healthy carriers, leading to a diffusion of the diseases even in healthy subjects.

This problem drew the literature research towards possible reassessments of immunization strategies to protect this population and to achieve the goal of universal protection.

It would be important, therefore, to assess the possible vaccination strategy in order to reduce this "healthy-reservoir" of infection. On this regard, new vaccination strategies for celiac patients were proposed in literature: the first one the use of booster doses of HBV vaccine by intramuscular route (IM), the second one is the performance of booster doses of HBV vaccine by intradermal route (ID).

In their scientific paper, Zingone *et al*[44] gave an IM dose of HBV vaccine to celiac patients with anti HBs titre < 10 mIU/mL and found that, one month after vaccination completion, all showed anti-HBs titer between 10 and 100 mIU/mL. They concluded that celiac patients may require higher doses of vaccine and/or more injection to achieve full protection. Supporting this hypothesis, in 2008, Ashaili *et al*[43] studied 25 celiac patients and control subjects who were negative for anti-HBs. HBV vaccine was administered to all celiac patients and control subjects in three doses, at month 0, 1, 6 by intramuscular injection into the deltoid muscle. Four weeks after the last dose, anti-HBs was measured quantitatively. Seventeen celiac patients (68%) and all control subjects (100%) were found to respond to HBV vaccination[44].

On the other hands, supporting the hypothesis of a possible role of gluten in the unresponsiveness to HBV vaccine in celiac patients and in severe liver disease[40], Nemes *et al*[41] recommended a revaccination by IM route for celiac patients after treatment with gluten-free diet. They administered a dose of IM vaccine to anti-HBs-negative patients with CD during a controlled gluten-free diet and found that 97.3% of them seroconverted after revaccination. They concluded that the no responder status to primary HBV vaccination is not permanent in CD and may improve after gluten exclusion.

The second strategy addresses on the use of ID booster of HBV vaccine in non-responders patients. The rational of this is that in contrast to IM, which relies on a T-cell mediated response, vaccines introduced directly into the skin activate a dendritic-cell-mediated immune response through a lower doses of antigen[65].

According to these results, in 2010, Leonardi *et al*[66]

revaccinated celiac patients who were non-responders to HBV vaccination with a 2 mg dose of recombinant hepatitis B vaccine administered intradermally. After the first ID dose we found that 40% of patients achieved anti-HBs titer ⩾ 1000 mIU/mL, 20% between 100 and 1000 mIU/mL, and 15% between 10 and 99 mIU/mL.

In order to compare the efficacy and the safety of the two HBV vaccination strategy (IM *vs* ID) in a non-responder population, in 2011, Leonardi *et al*[67] re-vaccinated non-responder celiac patients with ID or IM vaccine. The Authors found a higher percentage of "responders" after the first booster dose (ID = 76.7% *vs* IM = 78.6%) and a deeper increase after the third dose (ID = 90% *vs* IM = 96.4%) of vaccine in both groups. These data seem to suggest that both ID and IM route are effective options to administer a booster dose of HBV vaccine in celiac patients. However the ID route seems to be a better vaccination strategy, as demonstrated by the higher percentage of patients with an anti-HBs title > 1000 IU/L found in ID than in IM group.

In literature there are no other studies comparing the efficacy of HBV vaccination administered by IM or ID route in celiac patients. The two different methods are extensively compared in other pathologic conditions in which we observed a low responsiveness to HBV vaccination. For example in a meta-analysis about the HBV vaccination response in patients with chronic kidney disease, the authors found that pooling of study results demonstrated a decreased risk of failure to respond to HBV vaccine among patients who were vaccinated by intradermal *vs* intramuscular route[27].

There is another topic that should be considered. Actually it is still not clear if non-responder patients are characterized by an immunological anergy since birth, or they lose their immune competence during the time. With this regard, there is general consensus in literature that successfully vaccinated people who have lost antibodies years after primary vaccination usually show a rapid anamnestic response when boosted[68]. This means that the immunological memory for HBsAg can outlast antibody detection, providing long-term protection against the disease and the development of the carrier state. Hence, there should be no need to administer booster doses of vaccine to ensure long-term protection in subjects initially responding to vaccination[22-25]. Thus, it is still not clear if celiac non-responders show an immunity anergy since birth and do not respond since the first dose of vaccination or they loose their antibody protection with the flow of time, as physiologically happens in normal people, even if in a shorter period of time, remaining, thus, protected by an intra-cellular immunity.

For this reason it would be important an early identification of potential "pure" non-responsive patients through the identification of specific markers of unresponsiveness. Recent acquisitions show a possible role of toll-like receptors, cytokines and cytokine receptors polymorphisms associated with no response to hepatitis B vaccine in healthy population. As a matter of fact, Chen *et al*[46] hypothesized that the variations in these structures may act individually or cooperatively in the influence of the duration and intensity of immune response elicited by the hepatitis B vaccine, finding that 4 specific SNPs in the *IL-4*, *IL-4RA*, *IL-13* and *TLR2* genes were closely associated with the serum anti HBsAg response to HBV vaccine. These cytokines and TLR2 seemed to be associated with a status of hepatitis B vaccine-induced protective humoral immune response. If an early identification of responsiveness to HBV vaccine could be speculated in general population by the analysis of specific SNPs, it could be postulated the use of the same analysis in celiac patients, in whom the rate of non-responsiveness is higher, representing a specific marker of the "pure" non-responders. Nevertheless, further studies should clarify the role of these polymorphisms and their possible use as markers of un-responsiveness to HBV vaccine.

In conclusion, we suggest that all patients with CD should be revaccinated to achieve the goal of universal protection. In consideration of the possible relationship between anti-HBs titers and compliance with GFD, they should be revaccinated after the decrease of specific celiac antibodies, that usually occurs after about 1 year of a strict GFD. We also suggest the use of the intradermal route for the revaccination of these patients. The increase of anti-HBs antibodies is in fact satisfactory after ID injection in all patients, a lower dose of the vaccine can be used for immunization and the cellular immune responsiveness to HBsAg can be easily assessed by the development of a skin reaction at the injection site[31]. Thanks to the appearance of this reaction there is no need to test the serum anti-HBs concentration after the booster dose to value the vaccine efficacy, and this could represent a less expensive strategy for the Health Organization to perform HBV booster doses[69]. A recent retrospective cost-benefit analysis of ID hepatitis B vaccination reported a cost reduction exceeding 50% compared with a standard IM vaccine regimen[63]. Moreover the ID route would allow a lower performance of venous withdraws in all patients, reducing the costs linked to serial follow-up withdraws. In literature it is also demonstrated that strong immunological memory persists more than 10 years after immunisation of infants and adolescents with a primary course of vaccination[70]. Furthermore, we suggest to administer a booster dose of vaccine every 10 years to all celiac patients, independently their status of "pure" unresponsiveness, and as they are genetically predisposed to loose their anticorpal memory, these boosters would favour a better immunologic strategy in order to protect celiac non-responders from a possible HBV infection.

REFERENCES

1 **Bozorgi SH**, Ramezani H, Nooranipour M, Ahmadi M, Baghernejad A, Mostajeri A, Kargar-Fard H, Sadri M, Alavian SM. Risk factors of viral hepatitis: yet to explore. *Transfus*

Apher Sci 2012; **47**: 145-149 [PMID: 22858443]
2 **Goldstein ST**, Zhou F, Hadler SC, Bell BP, Mast EE, Margolis HS. A mathematical model to estimate global hepatitis B disease burden and vaccination impact. *Int J Epidemiol* 2005; **34**: 1329-1339 [PMID: 16249217 DOI: 10.1093/ije/dyi206]
3 **Chen DS**. From hepatitis to hepatoma: lessons from type B viral hepatitis. *Science* 1993; **262**: 369-370 [PMID: 8211155 DOI: 10.1126/science.8211155]
4 **Goldstein ST**, Alter MJ, Williams IT, Moyer LA, Judson FN, Mottram K, Fleenor M, Ryder PL, Margolis HS. Incidence and risk factors for acute hepatitis B in the United States, 1982-1998: implications for vaccination programs. *J Infect Dis* 2002; **185**: 713-719 [PMID: 11920288 DOI: 10.1086/339192]
5 **Lemon SM**, Thomas DL. Vaccines to prevent viral hepatitis. *N Engl J Med* 1997; **336**: 196-204 [PMID: 8988900]
6 **Zuckerman J**, van Hattum J, Cafferkey M, Gjørup I, Hoel T, Rummukainen ML, Weiland O. Should hepatitis B vaccination be introduced into childhood immunisation programmes in northern Europe? *Lancet Infect Dis* 2007; **7**: 410-419 [PMID: 17521594 DOI: 10.1016/S1473-3099(07)70136-6]
7 **Romano' L**, Paladini S, Van Damme P, Zanetti AR. The worldwide impact of vaccination on the control and protection of viral hepatitis B. *Dig Liver Dis* 2011; **43** Suppl 1: S2-S7 [PMID: 21195368 DOI: 10.1016/S1590-8658(10)60685-8]
8 **Mele A**, Tosti ME, Mariano A, Pizzuti R, Ferro A, Borrini B, Zotti C, Lopalco P, Curtale F, Balocchini E, Spada E. Acute hepatitis B 14 years after the implementation of universal vaccination in Italy: areas of improvement and emerging challenges. *Clin Infect Dis* 2008; **46**: 868-875 [PMID: 18269332]
9 **Kwon SY**, Lee CH. Epidemiology and prevention of hepatitis B virus infection. *Korean J Hepatol* 2011; **17**: 87-95 [PMID: 21757978 DOI: 10.3350/kjhep.2011.17.2.87]
10 **Da Villa G**, Pelliccia MG, Peluso F, Ricciardi E, Sepe A. Anti-HBs responses in children vaccinated with different schedules of either plasma-derived or HBV DNA recombinant vaccine. *Res Virol* 1997; **148**: 109-114 [PMID: 9108609]
11 **Liao SS**, Li RC, Li H, Yang JY, Zeng XJ, Gong J, Wang SS, Li YP, Zhang KL. Long-term efficacy of plasma-derived hepatitis B vaccine: a 15-year follow-up study among Chinese children. *Vaccine* 1999; **17**: 2661-2666 [PMID: 10418916]
12 **Li H**, Li RC, Liao SS, Yang JY, Zeng XJ, Wang SS. Persistence of hepatitis B vaccine immune protection and response to hepatitis B booster immunization. *World J Gastroenterol* 1998; **4**: 493-496 [PMID: 11819352]
13 **Rendi-Wagner P**, Kundi M, Stemberger H, Wiedermann G, Holzmann H, Hofer M, Wiesinger K, Kollaritsch H. Antibody-response to three recombinant hepatitis B vaccines: comparative evaluation of multicenter travel-clinic based experience. *Vaccine* 2001; **19**: 2055-2060 [PMID: 11228377 DOI: 10.1016/S0264-410X(00)00410-2]
14 **Liu HB**, Meng ZD, Ma JC, Han CQ, Zhang YL, Xing ZC, Zhang YW, Liu YZ, Cao HL. A 12-year cohort study on the efficacy of plasma-derived hepatitis B vaccine in rural newborns. *World J Gastroenterol* 2000; **6**: 381-383 [PMID: 11819603]
15 **Wismans PJ**, van Hattum J, Mudde GC, Endeman HJ, Poel J, de Gast GC. Is booster injection with hepatitis B vaccine necessary in healthy responders? A study of the immune response. *J Hepatol* 1989; **8**: 236-240 [PMID: 2523924]
16 **Wood RC**, MacDonald KL, White KE, Hedberg CW, Hanson M, Osterholm MT. Risk factors for lack of detectable antibody following hepatitis B vaccination of Minnesota health care workers. *JAMA* 1993; **270**: 2935-2939 [PMID: 8254853]
17 **Roome AJ**, Walsh SJ, Cartter ML, Hadler JL. Hepatitis B vaccine responsiveness in Connecticut public safety personnel. *JAMA* 1993; **270**: 2931-2934 [PMID: 8254852]
18 **Banatvala J**, Van Damme P, Oehen S. Lifelong protection against hepatitis B: the role of vaccine immunogenicity in immune memory. *Vaccine* 2000; **19**: 877-885 [PMID: 11115711 DOI: 10.1016/S0264-410X(00)00224-3]
19 **Wu JS**, Hwang LY, Goodman KJ, Beasley RP. Hepatitis B vaccination in high-risk infants: 10-year follow-up. *J Infect Dis* 1999; **179**: 1319-1325 [PMID: 10228050 DOI: 10.1086/314768]
20 **Huang LM**, Chiang BL, Lee CY, Lee PI, Chi WK, Chang MH. Long-term response to hepatitis B vaccination and response to booster in children born to mothers with hepatitis B e antigen. *Hepatology* 1999; **29**: 954-959 [PMID: 10051503 DOI: 10.1002/hep.510290349]
21 **Wainwright RB**, Bulkow LR, Parkinson AJ, Zanis C, McMahon BJ. Protection provided by hepatitis B vaccine in a Yupik Eskimo population--results of a 10-year study. *J Infect Dis* 1997; **175**: 674-677 [PMID: 9041341 DOI: 10.1093/infdis/175.3.674]
22 **Zanetti AR**, Mariano A, Romanò L, D'Amelio R, Chironna M, Coppola RC, Cuccia M, Mangione R, Marrone F, Negrone FS, Parlato A, Zamparo E, Zotti C, Stroffolini T, Mele A. Long-term immunogenicity of hepatitis B vaccination and policy for booster: an Italian multicentre study. *Lancet* 2005; **366**: 1379-1384 [PMID: 16226616 DOI: 10.1016/S0140-6736(05)67568-X]
23 **Gabbuti A**, Romanò L, Blanc P, Meacci F, Amendola A, Mele A, Mazzotta F, Zanetti AR. Long-term immunogenicity of hepatitis B vaccination in a cohort of Italian healthy adolescents. *Vaccine* 2007; **25**: 3129-3132 [PMID: 17291637 DOI: 10.1016/j.vaccine.2007.01.045]
24 **McMahon BJ**, Bruden DL, Petersen KM, Bulkow LR, Parkinson AJ, Nainan O, Khristova M, Zanis C, Peters H, Margolis HS. Antibody levels and protection after hepatitis B vaccination: results of a 15-year follow-up. *Ann Intern Med* 2005; **142**: 333-341 [PMID: 15738452]
25 Are booster immunisations needed for lifelong hepatitis B immunity? European Consensus Group on Hepatitis B Immunity. *Lancet* 2000; **355**: 561-565 [PMID: 10683019 DOI: 10.1016/S0140-6736(99)07239-6]
26 **Fabrizi F**, Andrulli S, Bacchini G, Corti M, Locatelli F. Intradermal versus intramuscular hepatitis b re-vaccination in non-responsive chronic dialysis patients: a prospective randomized study with cost-effectiveness evaluation. *Nephrol Dial Transplant* 1997; **12**: 1204-1211 [PMID: 9198052 DOI: 10.1093/ndt/12.6.1204]
27 **Fabrizi F**, Dixit V, Magnini M, Elli A, Martin P. Meta-analysis: intradermal vs. intramuscular vaccination against hepatitis B virus in patients with chronic kidney disease. *Aliment Pharmacol Ther* 2006; **24**: 497-506 [PMID: 16886915 DOI: 10.1111/j.1365-2036.2006.03002.x]
28 **Barraclough KA**, Wiggins KJ, Hawley CM, van Eps CL, Mudge DW, Johnson DW, Whitby M, Carpenter S, Playford EG. Intradermal versus intramuscular hepatitis B vaccination in hemodialysis patients: a prospective open-label randomized controlled trial in nonresponders to primary vaccination. *Am J Kidney Dis* 2009; **54**: 95-103 [PMID: 19481320 DOI: 10.1053/j.ajkd.2009.03.010]
29 **Poux JM**, Ranger-Rogez S, Lagarde C, Benevent D, Denis F, Leroux-Robert C. [Vaccination against hepatitis B virus. Value of intradermal administration in dialysed patients non responsive to intramuscular approach]. *Presse Med* 1995; **24**: 803-806 [PMID: 7630869]
30 **Leonardi S**, Spina M, Spicuzza L, Rotolo N, La Rosa M. Hepatitis B vaccination failure in celiac disease: is there a need to reassess current immunization strategies? *Vaccine* 2009; **27**: 6030-6033 [PMID: 19682619 DOI: 10.1016/j.vaccine.2009.07.099]
31 **Leonardi S**, Leggio T, Sciacca A, Di Gregorio F, Musumeci S. Intradermal hepatitis B vaccination in thalassaemia. *Arch Dis Child* 1990; **65**: 527-529 [PMID: 2141464]
32 **Yu AS**, Cheung RC, Keeffe EB. Hepatitis B vaccines. *Infect Dis Clin North Am* 2006; **20**: 27-45 [PMID: 16527647 DOI:

10.1016/j.idc.2006.01.004]
33 **Degos F**, Duhamel G, Brechot C, Nalpas B, Courouce AM, Tron F, Berthelot P. Hepatitis B vaccination in chronic alcoholics. *J Hepatol* 1986; **2**: 402-409 [PMID: 2941477]
34 **Zuin G**, Principi N, Tornaghi R, Paccagnini S, Re M, Massironi E, Ragni MC. Impaired response to hepatitis B vaccine in HIV infected children. *Vaccine* 1992; **10**: 857-860 [PMID: 1455911]
35 **Averhoff F**, Mahoney F, Coleman P, Schatz G, Hurwitz E, Margolis H. Immunogenicity of hepatitis B Vaccines. Implications for persons at occupational risk of hepatitis B virus infection. *Am J Prev Med* 1998; **15**: 1-8 [PMID: 9651632]
36 **Peces R**, de la Torre M, Alcázar R, Urra JM. Prospective analysis of the factors influencing the antibody response to hepatitis B vaccine in hemodialysis patients. *Am J Kidney Dis* 1997; **29**: 239-245 [PMID: 9016896]
37 **Li Volti S**, Caruso-Nicoletti M, Biazzo F, Sciacca A, Mandarà G, Mancuso M, Mollica F. Hyporesponsiveness to intradermal administration of hepatitis B vaccine in insulin dependent diabetes mellitus. *Arch Dis Child* 1998; **78**: 54-57 [PMID: 9534677 DOI: 10.1136/adc.78.1.54]
38 **Bouter KP**, Diepersloot RJ, Wismans PJ, Gmelig Meyling FH, Hoekstra JB, Heijtink RA, van Hattum J. Humoral immune response to a yeast-derived hepatitis B vaccine in patients with type 1 diabetes mellitus. *Diabet Med* 1992; **9**: 66-69 [PMID: 1532355]
39 **Leonardi S**, Vitaliti G, Garozzo MT, Miraglia del Giudice M, Marseglia G, La Rosa M. Hepatitis B vaccination failure in children with diabetes mellitus? The debate continues. *Hum Vaccin Immunother* 2012; **8**: 448-452 [PMID: 22370513]
40 **Kaukinen K**, Halme L, Collin P, Färkkilä M, Mäki M, Vehmanen P, Partanen J, Höckerstedt K. Celiac disease in patients with severe liver disease: gluten-free diet may reverse hepatic failure. *Gastroenterology* 2002; **122**: 881-888 [PMID: 11910339]
41 **Nemes E**, Lefler E, Szegedi L, Kapitány A, Kovács JB, Balogh M, Szabados K, Tumpek J, Sipka S, Korponay-Szabó IR. Gluten intake interferes with the humoral immune response to recombinant hepatitis B vaccine in patients with celiac disease. *Pediatrics* 2008; **121**: e1570-e1576 [PMID: 18519462 DOI: 10.1542/peds.2007-2446]
42 **Park SD**, Markowitz J, Pettei M, Weinstein T, Sison CP, Swiss SR, Levine J. Failure to respond to hepatitis B vaccine in children with celiac disease. *J Pediatr Gastroenterol Nutr* 2007; **44**: 431-435 [PMID: 17414139 DOI: 10.1097/MPG.0b013e3180320654]
43 **Ahishali E**, Boztas G, Akyuz F, Ibrisim D, Poturoglu S, Pinarbasi B, Ozdil S, Mungan Z. Response to hepatitis B vaccination in patients with celiac disease. *Dig Dis Sci* 2008; **53**: 2156-2159 [PMID: 18157638 DOI: 10.1007/s10620-007-0128-3]
44 **Zingone F**, Morisco F, Zanetti A, Romanò L, Portella G, Capone P, Andreozzi P, Tortora R, Ciacci C. Long-term antibody persistence and immune memory to hepatitis B virus in adult celiac patients vaccinated as adolescents. *Vaccine* 2011; **29**: 1005-1008 [PMID: 21129395 DOI: 10.1016/j.vaccine.2010.11.060]
45 **Ertekin V**, Tosun MS, Selimoglu MA. Is there need for a new hepatitıs B vaccine schedule for children with celiac disease? *Hepat Mon* 2011; **11**: 634-637 [PMID: 22140387 DOI: 10.5812/kowsar.1735142X.715]
46 **Chen J**, Liang Z, Lu F, Fang X, Liu S, Zeng Y, Zhu F, Chen X, Shen T, Li J, Zhuang H. Toll-like receptors and cytokines/cytokine receptors polymorphisms associate with nonresponse to hepatitis B vaccine. *Vaccine* 2011; **29**: 706-711 [PMID: 21111021 DOI: 10.1016/j.vaccine.2010.11.023]
47 **Murray JA**. The widening spectrum of celiac disease. *Am J Clin Nutr* 1999; **69**: 354-365 [PMID: 10075317]
48 **Freeman HJ**, Chopra A, Clandinin MT, Thomson AB. Recent advances in celiac disease. *World J Gastroenterol* 2011; **17**: 2259-2272 [PMID: 21633592 DOI: 10.3748/wjg.v17.i18.2259]
49 **van Heel DA**, West J. Recent advances in coeliac disease. *Gut* 2006; **55**: 1037-1046 [PMID: 16766754 DOI: 10.1136/gut.2005.075119]
50 **Leonardi S**, La Rosa M. Are hepatitis B virus and celiac disease linked? *Hepat Mon* 2010; **10**: 173-175 [PMID: 22308135]
51 **Rubio-Tapia A**, Murray JA. The liver in celiac disease. *Hepatology* 2007; **46**: 1650-1658 [PMID: 17969053]
52 **Cammarota G**, Cuoco L, Cianci R, Pandolfi F, Gasbarrini G. Onset of coeliac disease during treatment with interferon for chronic hepatitis C. *Lancet* 2000; **356**: 1494-1495 [PMID: 11081540]
53 **Wasmuth HE**, Stolte C, Geier A, Gartung C, Matern S. Induction of multiple autoantibodies to islet cell antigens during treatment with interferon alpha for chronic hepatitis C. *Gut* 2001; **49**: 596-597 [PMID: 11589192]
54 **Adinolfi LE**, Durante Mangoni E, Andreana A. Interferon and ribavirin treatment for chronic hepatitis C may activate celiac disease. *Am J Gastroenterol* 2001; **96**: 607-608 [PMID: 11232725]
55 **Monteleone G**, Pender SL, Alstead E, Hauer AC, Lionetti P, McKenzie C, MacDonald TT. Role of interferon alpha in promoting T helper cell type 1 responses in the small intestine in coeliac disease. *Gut* 2001; **48**: 425-429 [PMID: 11171837]
56 **Leonardi S**, Longo R, Cotugno M, Tardino L, Spina M, Lionetti E, La Rosa M. [Vaccination and celiac disease: results of a retrospective study]. *Minerva Pediatr* 2011; **63**: 363-367 [PMID: 21946447]
57 **Desombere I**, Gijbels Y, Verwulgen A, Leroux-Roels G. Characterization of the T cell recognition of hepatitis B surface antigen (HBsAg) by good and poor responders to hepatitis B vaccines. *Clin Exp Immunol* 2000; **122**: 390-399 [PMID: 11122245 DOI: 10.1046/j.1365-2249.2000.01383.x]
58 **Godkin A**, Davenport M, Hill AV. Molecular analysis of HLA class II associations with hepatitis B virus clearance and vaccine nonresponsiveness. *Hepatology* 2005; **41**: 1383-1390 [PMID: 15915462 DOI: 10.1002/hep.20716]
59 **Alper CA**, Kruskall MS, Marcus-Bagley D, Craven DE, Katz AJ, Brink SJ, Dienstag JL, Awdeh Z, Yunis EJ. Genetic prediction of nonresponse to hepatitis B vaccine. *N Engl J Med* 1989; **321**: 708-712 [PMID: 2528067]
60 **Livenson AR**. [Electrical equipment in medical establishments and electrical safety]. *Med Tekh* 1979; (**1**): 7-12 [PMID: 763114]
61 **Martinetti M**, Cuccia M, Daielli C, Ambroselli F, Gatti C, Pizzochero C, Belloni C, Orsolini P, Salvaneschi L. Anti-HBV neonatal immunization with recombinant vaccine. Part II. Molecular basis of the impaired alloreactivity. *Vaccine* 1995; **13**: 555-560 [PMID: 7483776]
62 **Noh KW**, Poland GA, Murray JA. Hepatitis B vaccine nonresponse and celiac disease. *Am J Gastroenterol* 2003; **98**: 2289-2292 [PMID: 14572581 DOI: 10.1111/j.1572-0241.2003.07701.x]
63 **Gasbarrini G**, Miele L, Malandrino N, Grieco A, Addolorato G, Gasbarrini A, Cammarota G, Bonvicini F. Celiac disease in the 21st century: issues of under- and over-diagnosis. *Int J Immunopathol Pharmacol* 2009; **22**: 1-7 [PMID: 19309546]
64 **Ertem D**, Gonen I, Tanidir C, Ugras M, Yildiz A, Pehlivanoğlu E, Eksioglu-Demiralp E. The response to hepatitis B vaccine: does it differ in celiac disease? *Eur J Gastroenterol Hepatol* 2010; **22**: 787-793 [PMID: 19584738 DOI: 10.1097/MEG.0b013e32832e9d41]
65 **Dubois B**, Bridon JM, Fayette J, Barthélémy C, Banchereau J, Caux C, Brière F. Dendritic cells directly modulate B cell growth and differentiation. *J Leukoc Biol* 1999; **66**: 224-230 [PMID: 10449158]

66 **Leonardi S**, Del Giudice MM, Spicuzza L, Spina M, La Rosa M. Hepatitis B vaccine administered by intradermal route in patients with celiac disease unresponsive to the intramuscular vaccination schedule: a pilot study. *Am J Gastroenterol* 2010; **105**: 2117-2119 [PMID: 20818367 DOI: 10.1038/ajg.2010.195]
67 **Leonardi S**, Praticò AD, Lionetti E, Spina M, Vitaliti G, La Rosa M. Intramuscular vs intradermal route for hepatitis B booster vaccine in celiac children. *World J Gastroenterol* 2012; **18**: 5729-5733 [PMID: 23155313]
68 **WHO Publication**. Hepatitis B vaccines: WHO position paper--recommendations. *Vaccine* 2010; **28**: 589-590 [PMID: 19896455]
69 **Sangfelt P**, Uhnoo I, Reichard O, Weiland O. A low-dose intradermal hepatitis B vaccine programme in health-care workers and students is highly effective and cost saving: a retrospective follow-up survey in the clinical setting. *Scand J Gastroenterol* 2008; **43**: 465-472 [PMID: 18365912 DOI: 10.1080/00365520701733806]
70 **Da Villa G**, Picciottoc L, Elia S, Peluso F, Montanaro F, Maisto T. Hepatitis B vaccination: universal vaccination of newborn babies and children at 12 years of age versus high risk groups. A comparison in the field. *Vaccine* 1995; **13**: 1240-1243 [PMID: 8578810]

P- Reviewers Hwang SG, Yoo BC **S- Editor** Lv S
L- Editor A **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i6.846

World J Gastroenterol 2013 February 14; 19(6): 846-854
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

ORIGINAL ARTICLE

Accommodation and peristalsis are functional responses to obstruction in rat hypertrophic ileum

Simona Bertoni, Francesca Saccani, Rita Gatti, Alberto Rapalli, Lisa Flammini, Vigilio Ballabeni, Elisabetta Barocelli

Simona Bertoni, Francesca Saccani, Alberto Rapalli, Lisa Flammini, Vigilio Ballabeni, Elisabetta Barocelli, Department of Pharmacological, Biological and Applied Chemical Sciences, University of Parma, 43124 Parma, Italy
Rita Gatti, Department of Experimental Medicine, University of Parma, 43125 Parma, Italy
Author contributions: Bertoni S performed the research and wrote the paper; Saccani F and Gatti R performed the immunohistochemistry studies; Rapalli A and Flammini L performed the functional studies; Ballabeni V analyzed the data; Barocelli E designed the research study and wrote the paper.
Correspondence to: Dr. Simona Bertoni, Department of Pharmacological, Biological and Applied Chemical Sciences, University of Parma, Parco Area delle Scienze 27/a, 43124 Parma, Italy. simona.bertoni@unipr.it
Telephone: +39-521-905090 Fax: +39-521-905091
Received: July 4, 2012 Revised: September 13, 2012
Accepted: September 22, 2012
Published online: February 14, 2013

Abstract

AIM: To investigate the effects of chronic obstruction on enteric reflexes evoked by electrical stimulation (EFS) or intraluminal distension of the rat hypertrophic ileum.

METHODS: Motor responses to EFS and to intraluminal distension were studied in the absence and in the presence of various inhibitors of enteric mediators. Ileum segments from operated (chronic ileal obstruction), sham-operated (control) and normal rats were horizontally mounted, connected to a pressure transducer and intraluminally perfused. The effects of selective serotonin receptor (5-HTR) blockers were investigated on distension-induced responses. The cellular localization of 5-HT3Rs was also examined in control and hypertrophic tissues through confocal microscopy.

RESULTS: In non-obstructed segments, EFS elicited tetrodotoxin (TTX)-sensitive responses with high amplitude contraction followed by weak relaxation. In hypertrophic tissues, EFS lowered the baseline pressure and evoked TTX-sensitive contractions significantly larger than normal ($P < 0.01$) or control ($P < 0.05$), and devoid of any relaxation phase ($P < 0.01$ *vs* normal). Incubation with atropine and guanethidine [non-adrenergic non-cholinergic (NANC) conditions] did not modify intestinal tone in normal and control preparations, but reversed the accommodation produced by EFS in hypertrophic tissues, and depressed the amplitude of contractions in all types of tissues. L-NAME and α-chymotrypsin blocked residual NANC motility in all tissues and augmented intraluminal pressure in hypertrophic segments ($P < 0.05$ *vs* NANC conditions). Intraluminal distension of the intestinal wall evoked non-propulsive cycles of contractions and relaxations in non-obstructed tissues. In all hypertrophic segments, strong propulsive strokes, markedly wider ($P < 0.001$), and larger than normal ($P < 0.001$) or control ($P < 0.05$) were elicited. Both motor patterns were blocked under NANC conditions and with simultaneous incubation with L-NAME and α-chymotrypsin. In all types of tissues, incubation with ketanserin or GR125487 did not modify distension-induced motility. In contrast, blockade of 5-HT3Rs by ondansetron concentration-dependently inhibited motor responses in normal and control tissues, but only slightly impaired enteric reflexes in the hypertrophic preparations. Finally, confocal microscopy did not reveal a different cellular distribution of 5-HT3Rs in control and hypertrophic ileum.

CONCLUSION: Accommodation and distension-induced peristalsis of rat hypertrophic ileum are controlled by cholinergic and peptidergic transmission and are negligibly affected by 5-HT3Rs, which modulate distension-induced motility in non-obstructed tissues.

© 2013 Baishideng. All rights reserved.

Key words: 5-HT3 receptor; Intestinal obstruction; Peristalsis; Rat intestinal motility; Serotonin

Bertoni S, Saccani F, Gatti R, Rapalli A, Flammini L, Ballabeni V, Barocelli E. Accommodation and peristalsis are functional responses to obstruction in rat hypertrophic ileum. *World J Gastroenterol* 2013; 19(6): 846-854 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i6/846.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i6.846

INTRODUCTION

Small bowel obstruction is a common clinical problem resulting from a variety of causes: intraluminal (food bolus, gallstones), intramural (neoplasms, strictures) or extramural (adhesions, hernias) factors. It can be partial or complete and, if not properly diagnosed and treated, it may rapidly lead to death[1]. Experimentally, striking morphological and neurochemical changes, affecting all the layers of the gut wall, have been reported to occur orally to the site of a mechanical long-standing partial obstruction. Massive thickening of the muscle coats, neuronal hypertrophy, neoangiogenesis and neoformation of collagen[2], a loss of interstitial cells of Cajal[3,4] and an altered expression of enteric neuropeptides[3,5] are the most prominent alterations detected in the obstructed intestine. Functionally, along with pronounced changes in biomechanical and motility patterns, detected both *in vitro*[6,7] and *in vivo*[8], preservation of the peristaltic reflex in the isolated guinea pig ileum has also been described[9]. Indeed, peristaltic reflex, responsible for the mixing and aboral propulsion of luminal contents *in vivo*, is the result of a highly sophisticated and localized integration between neural and myogenic components; *in vitro*, it can be initiated by local mechanical stimulation of the mucosa or by muscle stretch, as elegantly shown by Trendelenburg's pioneering work[10]. Hypertrophic growth, induced by mechanical obstruction of the rat ileum, affects the functional responses of both smooth muscle layers and of intrinsic innervation[7,11]. Therefore, the present work focused on the consequences of a partial long-standing stenosis on enteric reflexes evoked by electrical field stimulation (EFS) or by intraluminal distension of rat isolated terminal ileum. To this end, the changes in intraluminal pressure elicited by neurogenic stimulation of rat obstructed intestine were investigated in the absence and in the presence of different inhibitors of enteric neurotransmitters which control gut motility, and compared with those triggered in preparations obtained from normal or sham-operated (control) rats. Secondly, given the pivotal role played by endogenous serotonin (5-HT) in the initiation and propagation of enteric reflexes, such as the peristaltic reflex[12,13], special attention was directed at evaluation of its role in specific distension-induced motor patterns exhibited by non-hypertrophic and hypertrophic tissues by investigating the effects of different selective 5-HT receptor (5-HTR) antagonists. The cellular localization of 5-HT_3R was also investigated through confocal microscopy.

MATERIALS AND METHODS

Surgical procedure

Adult female Wistar rats (180-200 g), bred from a local colony, were housed in single cages with a 12 h/12 h light/dark cycle and received food and water *ad libitum*. The rats were randomly assigned to normal group (age-matched animals that did not undergo any type of surgery), a control group (age-matched sham-operated animals subjected to the same intestinal manipulation except for ileal obstruction) and an operated group (25 animals per group). Under general anesthesia (sodium pentobarbital 33 mg/kg *ip*), the rats were operated on according to Gabella's method[14], as previously described[7]. Briefly, a polyethylene ring of diameter 1-2 mm larger than that of the intestine, was applied around the ileum, proximal to the ileo-cecal junction, keeping it initially free for turning around. Postoperatively, the animals were monitored daily with regard to weight, general well-being and distension of the abdomen. Fourteen days after ileal obstruction, when previous experiments had shown that intestinal hypertrophy is fully and homogeneously developed over 10 cm aboral to the stenosis[7], the rats were killed, and functional or immunohistochemical studies were performed on ileal tissue. The experimental protocol complied with the requirements of animal care and was approved by Ministero della Salute, Italy (DL 116/92).

Functional studies

Rats were killed by CO_2 asphyxiation. Full-thickness 4-cm long segments of terminal ileum, immediately proximal to the site of occlusion excised from operated rats, and loops of the same part of the gut excised from normal or control rats, were cleared of their contents by flushing with Krebs-Henseleit solution (composition: NaCl 118.9 mmol/L, KCl 4.6 mmol/L, $CaCl_2$ 2.5 mmol/L, KH_2PO_4 1.2 mmol/L, $NaHCO_3$ 25 mmol/L, $MgSO_4$ 1.2 mmol/L, glucose 11 mmol/L). From each animal, only one preparation was obtained either immediately proximal to the point of obstruction, where hypertrophy was maximal and uniform, or from the corresponding portion of terminal ileum of normal and control rats. According to the method described by Costall *et al*[15], the segments were cannulated at the oral and anal ends, and were mounted in a Mayflower horizontal 20-mL tissue bath (Hugo Sachs Elektronik-Harvard Apparatus GmbH D-79232 March Hugstetten, Germany) filled with Krebs-Henseleit solution gassed with a mixture of 5%CO_2/95%O_2 at 37 ℃, and stretched at their initial length of 4 cm. The inlet cannula was connected *via* tubing to a reservoir filled with nutritive solution, maintained at 37 ℃ and continuously oxygenated, and to a pressure transducer (TSD104A Biopac Systems, 2Biological Instruments, Besozzo, VA, Italy) connected to a MacLab digital data acquisition system to record changes in the intraluminal pressure. The outflow tube at the aboral end had two outlets: one at the level of the bath (A) while the other (B) could be raised above the level of the tissue. A continuous flow of fresh

oxygenated solution was delivered intraluminally at a flow rate of 0.5 mL/min and extraluminally at a speed of 2 mL/min by means of the same peristaltic pump (Gilson Minipuls-3, Gilson Italia SRL, MI, Italy) to remove perfusate and extraluminal solution from the bath. The extraluminal flow was temporarily suspended during the period of incubation of drugs.

Electrical field stimulation: After a period of equilibration of 30 min, during which the fluid level in the outflow tube was equal to that in the bath (drain A open), the intraluminal pressure was augmented by closing drain A and by raising drain B 5 cm above the level of the tissue in the bath (5 cm hydrostatic resistance or low distension, corresponding to a pressure of 3.68 mmHg); 30 V square pulses of 1 ms duration were then delivered to the tissues at 3 Hz frequency through platinum wire electrodes positioned at the two opposite sides of ileum and connected to a generator that provided trains lasting 20 s at 120 s intervals. EFS-evoked changes in intraluminal pressure were registered in the absence (basal conditions) and in the presence of the muscarinic receptor antagonist atropine 1 μmol/L and sympatholytic agent guanethidine 4 μmol/L [non-adrenergic non-cholinergic (NANC) conditions]. The effects produced by the application of nitric oxide synthase (NOS) inhibitor N^G-nitro-*L*-arginine methyl ester (L-NAME) (300 μmol/L) and by the protease α-chymotrypsin (10 IU/mL) on NANC responses evoked by EFS were investigated. Drugs were added to the extraluminal solution.

Distension-induced motility: In a second series of experiments, after a period of equilibration of 30 min, drain A was closed and drain B was elevated 8 cm above the level of the tissue in the bath (8 cm hydrostatic resistance or high distension, corresponding to a pressure of 5.88 mmHg). After another 30 min, once regular motility had been evoked by the increase in the intraluminal pressure, the effects of simultaneous application of atropine 1 μmol/L and guanethidine 4 μmol/L or of single concentrations of the 5-HT_{2A}R antagonist ketanserin (1 μmol/L), the 5-HT_3R antagonist ondansetron (1-10 μmol/L) and the 5-HT_4R antagonist GR125487 (1 μmol/L) were studied over the next 30 min. Each preparation was challenged with a single application of each drug in order to minimize time-dependent variability. About 25% of non-obstructed tissues, which failed to develop a regular motility following distension, were discarded from further investigation in the experiment.

Immunohistochemistry

Tissue preparation and processing. Full-thickness 2-cm long segments of terminal ileum, excised as described above from hypertrophic and control animals, were cleared of their contents by flushing with phosphate buffered saline (PBS) 100 mmol/L at pH 7.4, opened along the mesenteric border, cleaned and pinned flat onto slabs of Sylgard 184 (Dow Corning), with the serosal side exposed. After 2 h fixation with 4% formaldehyde in 100 mmol/L PBS, whole-mount preparations containing the longitudinal muscle layer and the adherent myenteric ganglia were peeled off from the mucosa and the circular muscle under a dissection microscope using fine forceps, and were processed by the immunofluorescence method for double labeling using a free floating technique. After washing in 100 mmol/L PBS, whole mounts were incubated with a mixture of rabbit-anti-rat immunoglobulin G (IgG) primary antibody directed against 5-HT_3R 1:500 (No. 95247, a generous gift from CURE/UCLA Digestive Disease Research Center, Los Angeles, CA, United States) and mouse-anti-rat clathrin IgG 1:100 (Becton Dickinson Italia SpA) for 48 h at 4 ℃. This was followed by a 2-h incubation period at room temperature with a mixture of Alexafluor 488 donkey-anti-rabbit IgG 1:100 (Invitrogen Ltd, United Kingdom) and DyLight 549-conjugated AffiniPure donkey-anti-mouse IgG 1:200 (Jackson ImmunoResearch). Tissues were finally washed with 100 mmol/L PBS, mounted on slides and cover-slipped with ProLong Gold Antifade Reagent (Invitrogen Ltd, United Kingdom). Specificity controls were obtained by omitting primary or secondary antibodies from the incubation solution. Primary and secondary antibodies were diluted in Triton X-100 (0.5% in 100 mmol/L PBS, Sigma). Normal donkey serum (Jackson ImmunoResearch) was added to a final concentration of 10% to reduce the unspecific background staining.

Confocal microscopy specimens were analyzed with a confocal system LSM 510 Meta scan head integrated with the Axiovert 200 mol/L inverted microscope (Carl Zeiss, Jena, Germany). Specimens were observed through a 63 × 1.4 NA oil objective. Alexafluor and DyLight were excited with 488 nm argon and 543 nm He-Ne laser lines, respectively. Image acquisition was carried out in a multitrack mode, with the relevant beamsplitters; barrier filters were 505-530 band pass and 585 long pass for the above signals, respectively. A series of x-y sections was acquired with a z-step of 0.5 μm, to cover the whole height of the samples.

Presentation of results

Data were expressed as mean ± SE of 5 distinct experiments. The following parameters were measured: changes in intestinal tone (baseline intraluminal pressure), index of accommodation capacity; amplitude (area under the pressure-time curve or area under curve for each wave of contraction/relaxation), height and frequency of electrically- or distension-induced contractions/relaxations over a 15 min period immediately prior to and after 15 min incubation with each drug tested. When peristalsis, identifiable by propagating and propulsive waves of contraction (visually confirmed as rings of contraction within 1 cm from the oral end of the loop moving towards the distal end), was induced by distension, a preparatory and an emptying phase could be detected. During the preparatory phase, the intraluminal pressure increased slowly until the threshold pressure was reached and the emptying phase, marked by an abrupt increase in the intraluminal

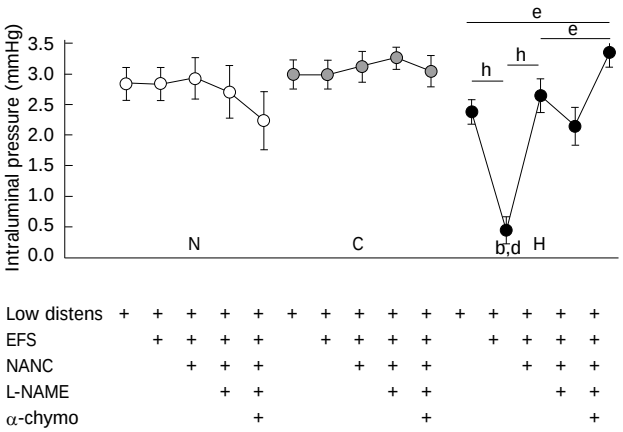


Figure 1 Basal intestinal tone. Changes in basal intestinal tone of normal (N), control (C) and hypertrophic (H) ileal segments, expressed as values of intraluminal pressure (mmHg), exposed to different subsequent conditions: hydrostatic pressure of 5 cm H_2O (Low distens), electrical field stimulation (EFS), NANC conditions (NANC), incubation with N^G-nitro-*L*-arginine methyl ester (L-NAME) 300 μmol/L (L-NAME), incubation with α-chymotrypsin 10 IU/mL (α-chymo). Each value is the mean ± SE of 5 distinct experiments per group. $^bP < 0.001$ *vs* corresponding normal values; $^dP < 0.001$ *vs* corresponding control values; $^eP < 0.05$; $^hP < 0.001$; analysis of variance test followed by Bonferroni's post-test.

pressure, was triggered (peristaltic stroke). The average compliance of the intestinal wall during the preparatory phase (volume infused in the preparatory phase/change in pressure in the preparatory phase) and the average power generated by the intestine during the peristaltic stroke (average pressure in emptying phase × volume expelled/duration of emptying phase) were measured according to Waterman *et al*[16] for 5-8 complete waves of contraction immediately prior to and after 15 min incubation with the drug. In order to calculate the average power, the volume of fluid expelled from the aboral end of the intestine by each peristaltic wave was measured with a measuring cylinder.

Statistical analysis

Data acquisition and analysis were carried out using MacLab digital data acquisition system and applying PowerLab Chart v4.1.1 software (PowerLab/4SP ADI Instruments, Ugo Basile, Comerio, VA, Italy). Statistical analysis was performed by two-way analysis of variance test followed by Bonferroni's post-test unless otherwise indicated. A P value < 0.05 was considered significant, a P value < 0.01 highly significant and a P value < 0.001 extremely significant.

Drugs

The following drugs were used: tetrodotoxin citrate (TTX), atropine sulfate, guanethidine sulfate, L-NAME, α-chymotrypsin, ketanserin tartrate, ondansetron hydrochloride dihydrate, purchased from Sigma Aldrich (St Louis, MO, United States), and GR125487 sulfamate, bought from Tocris Bioscience (Bristol, United Kingdom). All other reagents were of the highest grade commercially available. The stock solutions were prepared by dissolving all drugs in distilled water. The working solutions were prepared fresh on the day of the experiment by diluting the stock solutions with distilled water or nutritive solution. The volume of the drugs added to the organ bath was 1% of the final volume of the bath solution.

RESULTS

Motor responses to electrical field stimulation

During the equilibration period, normal, control and hypertrophic tissues exhibited no or only sporadic spontaneous motility. The raising of the outflow block to 5 cm above the level of the tissue in the bath increased the intraluminal pressure by about 2.5-3.0 mmHg in all the tissues tested (Figure 1), but it did not evoke any wave of contraction or relaxation. In normal and sham-operated segments, EFS did not further modify the intestinal tone but elicited biphasic TTX-sensitive responses comprising a transient high amplitude contraction followed by a weak relaxation (Figure 2A, B and D). In hypertrophic tissues, EFS lowered the intraluminal pressure almost to the same level as in the equilibration phase ($P < 0.001$ *vs* low distension), in contrast to that observed in normal and control tissues ($P < 0.001$) (Figure 1). EFS also evoked TTX-sensitive phasic contractions of significantly larger amplitude in hypertrophic tissue (95.5 ± 17.1 mmHg.s) compared with that in normal tissue (40.3 ± 8.9 mmHg.s, $P < 0.01$) or control tissue (56.0 ± 8.7 mmHg.s, $P < 0.05$), and which were devoid of any relaxation phase ($P < 0.01$ *vs* normal, Figure 2C and D). Incubation with atropine 1 mmol/L and guanethidine 4 mmol/L (NANC conditions) did not modify the intestinal tone in normal and sham-operated preparations but reversed the accommodation produced by EFS in hypertrophic tissues, with intraluminal pressure slowly increasing to the same level reached after raising the outflow block (Figures 1 and 2A-C). Furthermore, the amplitude of contractions in all types of tissues was markedly depressed (Figure 2D). Subsequent exposure to the NOS inhibitor L-NAME 300 mmol/L moderately, although not significantly, increased the contractile responses compared with NANC conditions, while simultaneous incubation with the protease α-chymotrypsin 10 IU/mL blocked the residual NANC motor activity in all the tissues (Figure 2D) and significantly augmented intraluminal pressure in hypertrophic segments ($P < 0.05$ *vs* low distension and *vs* NANC conditions) (Figure 1).

Distension-induced motility

Distension of the intestinal wall, produced by elevating the outflow block to 8 cm above the level of the tissue in the bath, evoked distinct motor patterns in normal and control preparations compared with hypertrophic tissue. In normal and control tissues, intraluminal pressure increased steeply by about 6.5-7.0 mmHg, and non-propulsive, local cycles of contractions and relaxations were exhibited by 75% of the preparations (Figure 3A and B). In contrast, in all hypertrophic segments, intestinal tone first gradually and slowly increased up to a threshold pressure. At this point, rhythmic, anally propagating, strong propulsive strokes, markedly wider than normal and control tissues ($P < 0.001$) (Figure 3D) and markedly

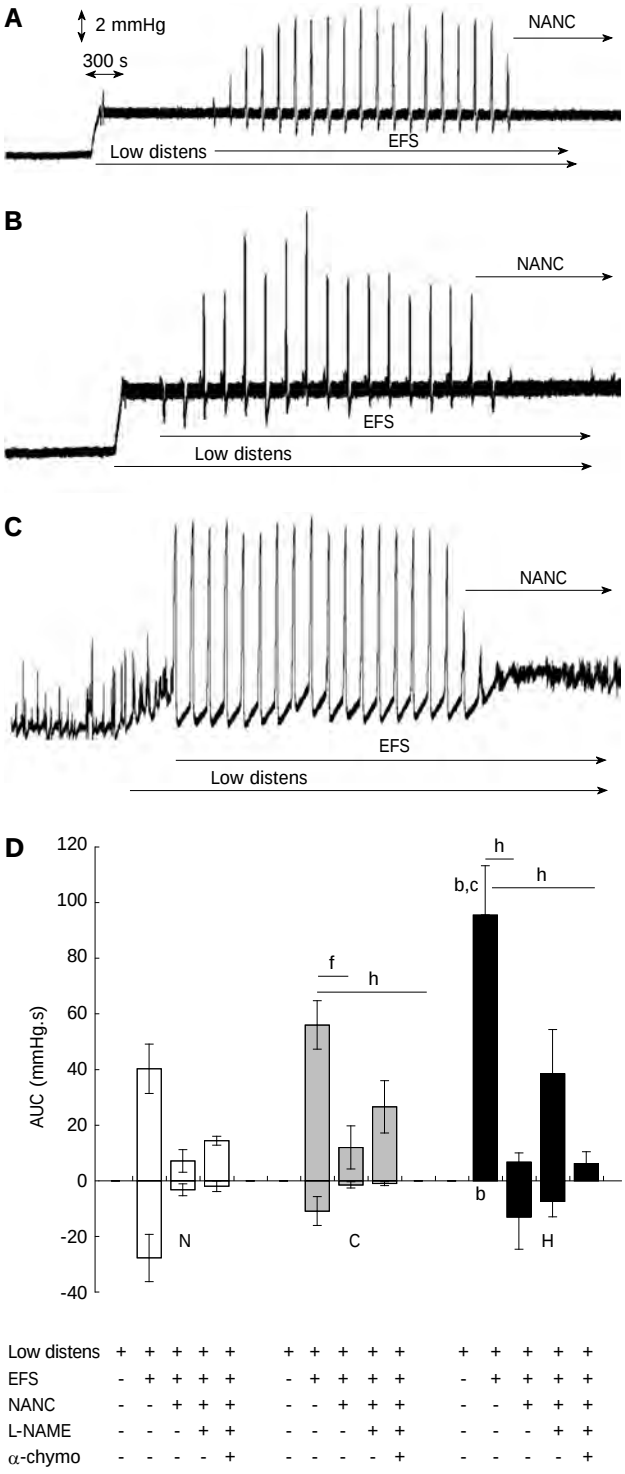


Figure 2 Motor responses to electrical field stimulation. Original tracings representing changes of intraluminal pressure of ileal segments in basal conditions and following subsequent application of a hydrostatic pressure of 5 cm H_2O (Low distens), EFS (30 V, 1 ms, 3 Hz, trains lasting 20 s every 120 s) and NANC conditions (NANC). A: Normal tissues; B: Control tissues; C: Hypertrophic tissues; D: Area under the pressure-time curve of motor responses in normal (N), control (C) and hypertrophic (H) ileal segments following subsequent application of a hydrostatic pressure of 5 cm H_2O (Low distens), EFS (30 V, 1 ms, 3 Hz, trains lasting 20 s every 120 s), NANC conditions (NANC), incubation with L-NAME 300 μmol/L (L-NAME) and incubation with α-chymotrypsin 10 IU/mL (α-chymo). Each value is the mean ± SE of 5 distinct experiments per group. $^{b}P < 0.01$ *vs* corresponding normal values; $^{c}P < 0.05$ *vs* corresponding control values; $^{f}P < 0.01$; $^{h}P < 0.001$; analysis of variance test followed by Bonferroni's post-test.

higher than normal ($P < 0.001$) or control tissue ($P < 0.05$) (Figure 3E) were elicited; simultaneously, basal intraluminal pressure progressively decreased to equilibration values (Figure 3C). Both motor patterns were blocked under NANC conditions and with simultaneous incubation with L-NAME 300 μmol/L and α-chymotrypsin 10 IU/mL (data not shown). In all types of tissues, incubation with the 5-HT_{2A}R antagonist ketanserin 1 μmol/L did not modify any parameter of the distension-induced motility (Figure 3D-F). In contrast, blockade of 5-HT_3R by ondansetron concentration-dependently reduced the amplitude, height and frequency of contractions and relaxations in normal and control tissues; in hypertrophic preparations, only the highest concentration of ondansetron tested (10 μmol/L) had an effect, with a decreased height of the peristaltic strokes without modification of their area or frequency (Figure 3D-F). Regarding antagonism of 5HT_4R by GR125487 1 μmol/L, no significant changes were produced in the distension-induced motility of any tissue (Figure 3D-F).

When the propulsive ability of hypertrophic tissues was taken into consideration, none of the 5-HTR antagonists tested modified the average compliance of the intestinal wall during the preparatory phase (Figure 4), or the efficacy of the propulsion of luminal fluid during the emptying phase, expressed by the average power (data not shown).

Immunohistochemistry

In both sham-operated and hypertrophic specimens, 5-HT_3R immunoreactivity was detected in many neurons and fibers of the ileal myenteric plexus, appearing concentrated primarily near the neuronal plasma membrane; only slight staining was observed also in the cytoplasm (Figure 5A and D). Double-labeling with immunoreactivity for 5-HT_3R and clathrin, a marker for early endosomes, whose distribution seemed mainly cytosolic (Figure 5B and E), did not show any clear significant co-localization, suggesting that in control and hypertrophic tissues the internalization of this ligand-gated ionotropic receptor appeared as an occasional event under basal conditions (Figure 5C and F).

DISCUSSION

The results of the present study indicate that hypertrophy of the intestinal wall induced by a partial chronic obstruction located at the terminal portion of rat ileum is associated with striking changes in the shape and nature of motor patterns elicited by EFS and by intraluminal distension. In particular, an increased accommodation capacity and little involvement of serotonergic neurotransmission in the control of enteric reflexes in hypertrophic tissues compared with normal and control reflexes can be hypothesized. First, when the motor responses evoked by electrical stimulation of intrinsic innervation are taken into consideration, it is worth noting that the typical biphasic profile, comprising a transient contraction fol-

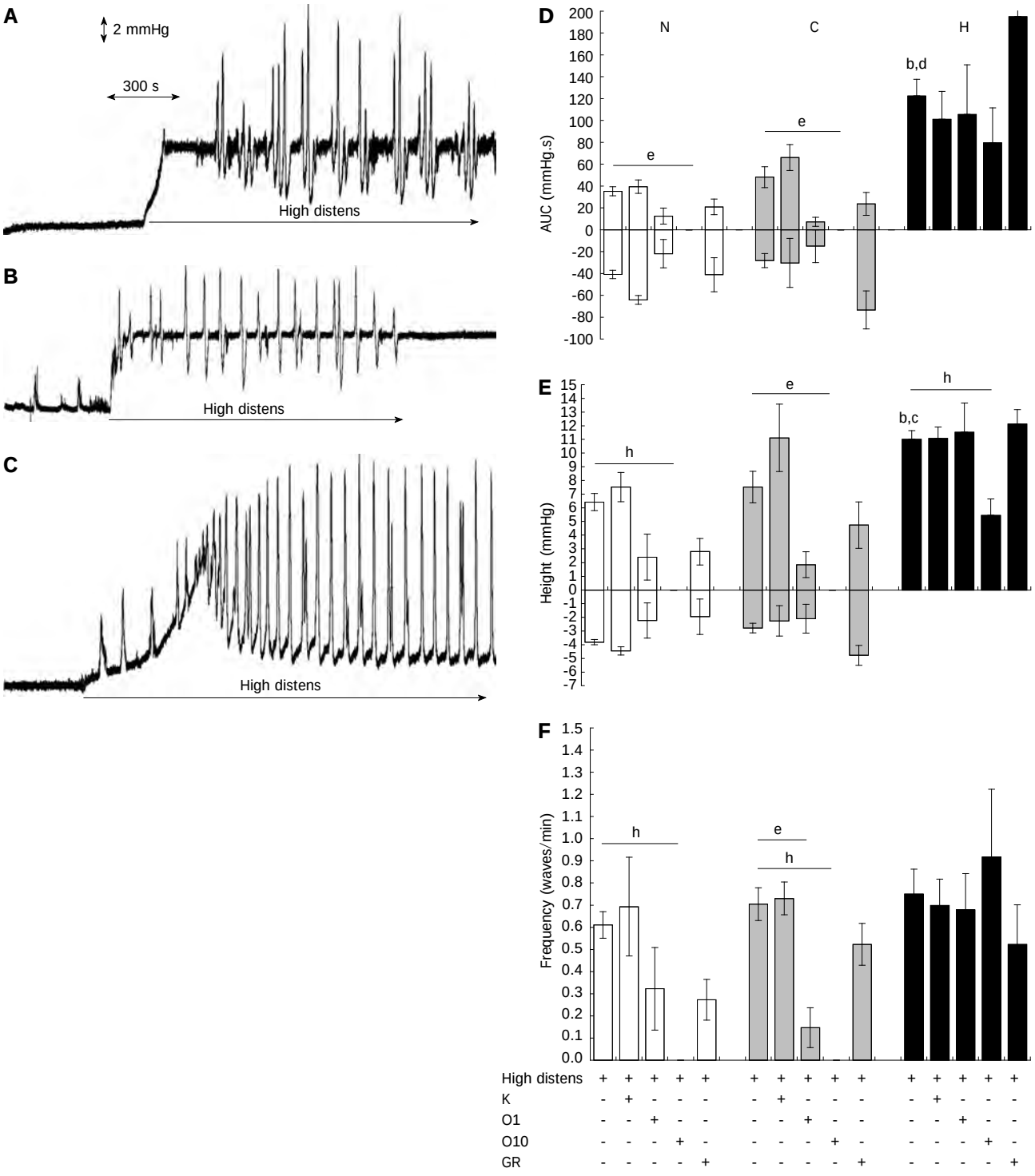


Figure 3 Distension-induced motility. Original tracings representing changes of intraluminal pressure of ileal segments in basal conditions and following application of a hydrostatic pressure of 8 cm H_2O (high distens). A: Normal tissues; B: Control tissues; C: Hypertrophic tissues; D-F: Motor responses in normal (N), control (C) and hypertrophic (H) ileal segments following application of a hydrostatic pressure of 8 cm H_2O (high distens) and incubation with ketanserin 1 μmol/L (K), ondansetron 1 (O1) or 10 (O10) μmol/L or GR125487 1 μmol/L (GR). D: Amplitude (area under the pressure-time curve or AUC); E: Height; F: Frequency. Each value is the mean ± SE of 5 distinct experiments per group. $^{b}P < 0.001$ *vs* corresponding normal values; $^{c}P < 0.05$, $^{d}P < 0.001$ *vs* corresponding control values; $^{e}P < 0.05$; $^{h}P < 0.01$. Analysis of variance test followed by Bonferroni's post-test.

lowed by a weak relaxation in normal and control tissues, was converted into a high-amplitude monophasic contraction in hypertrophic tissue. This modification, similar to that already detected in electrically stimulated hypertrophic ileum when longitudinally mounted[7], can be accounted for by two different factors: a greater force of contraction due to increased muscle mass developed by hypertrophic growth; an increased accommodation capacity as shown by the quick drop in basal intraluminal pressure at the onset of EFS. The subsequent pharmacological investiga-

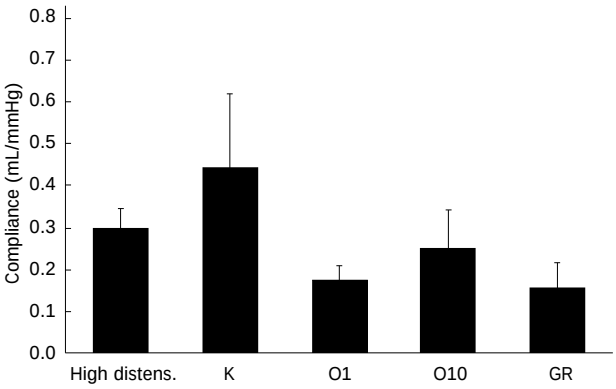


Figure 4 Average compliance of hypertrophic ileum. Changes of the average compliance of hypertrophic intestinal wall following application of a hydrostatic pressure of 8 cm H_2O (high distens.) and incubation with ketanserin 1 μmol/L (K), ondansetron 1 (O1) or 10 (O10) μmol/L or GR125487 1 μmol/L (GR). Each value is the mean ± SE of 5 distinct experiments per group.

tion confirmed the pivotal role played by acetylcholine not only as an excitatory neurotransmitter in normal, control and especially hypertrophic tissues[7], but also as one of the primary transmitters of descending interneurons, corroborating the findings collected in guinea pig small intestine[17]. Indeed, incubation with atropine and guanethidine markedly reduced the contractile phase in all preparations and nearly abolished it in hypertrophic tissues. Furthermore, in obstructed segments, atropine and guanethidine impaired the accommodation reflex elicited by electrical stimulation, by increasing the intraluminal pressure to pre-stimulation values.

Along with acetylcholine, other mediators were involved in the regulation of intestinal motility both in physiological conditions and after chronic obstruction: only simultaneous incubation with the NOS inhibitor L-NAME and the protease α-chymotrypsin completely blocked neurogenic motor responses in all the preparations and further impaired the accommodation capacity in the obstructed tissue. It is worth noting that an increased sensitivity to relaxing mediators, including nitric oxide and vasoactive intestinal peptide (VIP), was exhibited by hypertrophic smooth muscle layers compared with non-obstructed ones[11], possibly concurring with the higher accommodation capacity shown by hypertrophic ileum in these conditions.

In the second part of the research, the increase in hydrostatic pressure on the intestinal wall and the resulting distension triggered atropine- and α-chymotrypsin-sensitive typical motor patterns in normal, control and hypertrophic tissues. In particular, in chronically obstructed segments, distension evoked wide, rhythmic and propulsive peristaltic waves propagating aborally, with shape similar to those elicited by electrical stimulation in the same type of tissues and totally different from the stationary, non-propulsive clusters of cycles arising in normal and control preparations in these experimental conditions. The precise mechanisms underlying this particular motor pattern are not known and the possible contribution given by each morphological and neurochemical alteration, already thoroughly described in the hypertrophic intestine[2-5], requires further investigation. The augmented total collagen content, possibly acting as a force transducer among the components of the contractile system[18], or the higher density of inhibitory neurons expressing VIP or pituitary adenylate cyclase-activating polypeptide[3], may be only two of the reasons for this motor behavior.

The collected results seem however to be consistent with the enhanced responsiveness to contractile agents and relaxing mediators which is seen in isolated hypertrophic smooth muscle layers[11], both aspects likely playing a critical role in the powerful peristaltic reflex. According to the original idea attributed to Bulbring *et al*[13], intraluminal distension initiates the peristaltic reflex through stimulation of intrinsic sensory primary afferent neurons by 5-HT released into the wall of the gut from enterochromaffin cells. Starting from this premise it seemed interesting to investigate the role played by endogenous 5-HT in the modulation of enteric motor reflexes through the application of selective $5HT_{2A}R$, $5\text{-}HT_3R$ or $5\text{-}HT_4R$ antagonists both in non-obstructed and in hypertrophic distended ileum. The findings provided by the present work suggest that in normal and control tissues, distension-induced motility is independent of the activation of smooth muscle $5\text{-}HT_{2A}R$, mediating rat ileum contraction[19], and only slightly affected by blockade of $5HT_4R$. These receptors are chiefly located on terminals of submucosal intrinsic primary afferent neurons, where, by promoting the release of acetylcholine and calcitonin gene-related peptide, they are reported to play a critical role in facilitating the peristaltic reflex along with the putative $5\text{-}HT_{1P}R$[20], towards which no selective antagonist is currently available. Also $5\text{-}HT_3R$ are involved in peristalsis: they are mainly localized on extrinsic sensory nerves and on myenteric neurons, where they initiate giant migrating contractions[20], and may participate in the triggering and propagation of the peristaltic reflex[21,22]. The application of $5\text{-}HT_3R$ antagonist concentration-dependently inhibited distension-induced motor responses in normal and control tissues, while, only slightly at the highest tested concentration did it impair the motor reflexes activated by wall distension in hypertrophic segments, which were refractory to the other 5-HTR antagonists. With regard to this behavior, it is tempting to speculate, and worth further investigation, that the mucosa, hypertrophied following chronic obstruction[23], could contain massive levels of 5-HT, stored in enterochromaffin cells. The mediator, once released by distension of the gut wall, may strongly stimulate $5\text{-}HT_3R$, possibly leading to two effects: the initiation of the peristaltic reflex and the internalization of the ligand-gated ion channel. Indeed, a long-term increase in 5-HT content in the intestinal mucosa has been demonstrated by Freeman *et al*[24] to result in a pronounced $5\text{-}HT_3R$ internalization in myenteric neurons of rat ileum. The results obtained through confocal microscopy in the present study do not seem to support the hypothesis of a different cellular localization of $5\text{-}HT_3$ receptors in control and hypertrophic tissues: on the contrary, in the adopted experimental conditions, internalization of this receptor subtype appears equally unlikely in both kinds of preparations. This

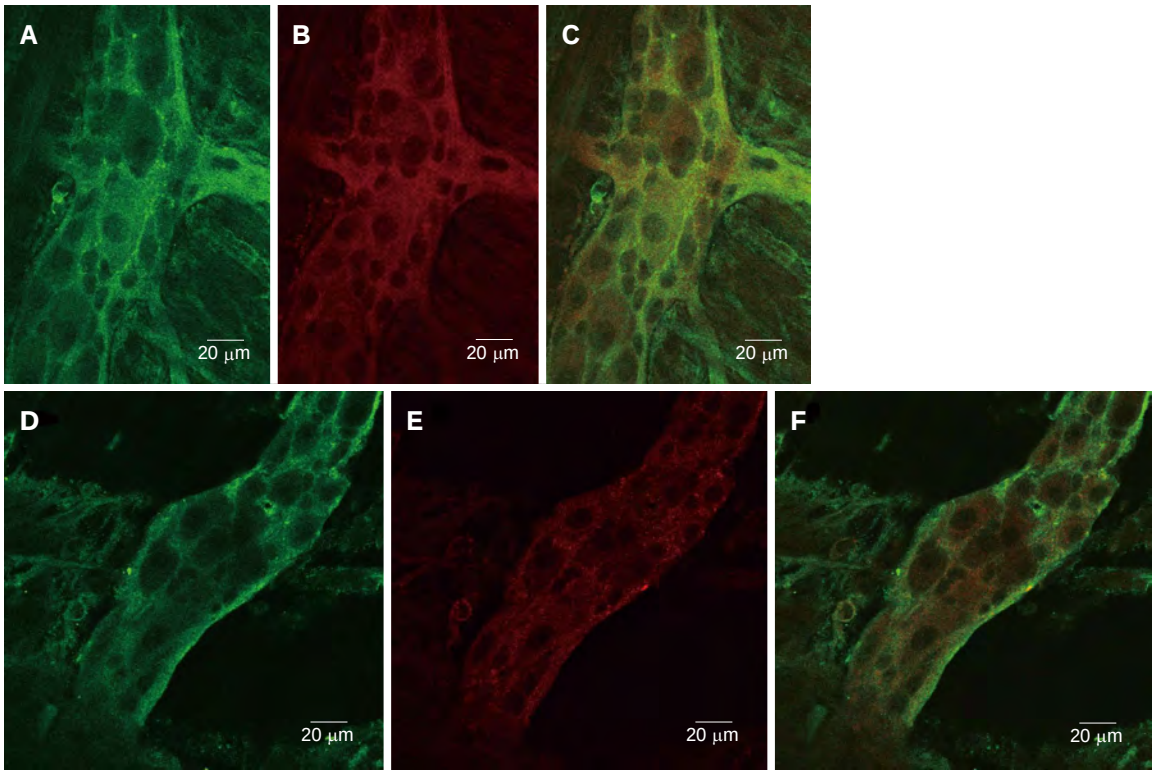


Figure 5 Cellular localization of 5-HT3 receptors in control and hypertrophic ileum. Confocal images (0.5 μm sections; scale bar 20 μm) of myenteric plexus neurons isolated from control and hypertrophic ileal tissues. A: Image showing 5-HT3 receptor immunoreactivity visualized using donkey-anti-rabbit immunoglobulin G (IgG) conjugated with AlexaFluor 488 (green); B: Image showing clathrin immunoreactivity visualized using a donkey-anti-mouse IgG conjugated with Dylight 549 (red); C: Merged images from A and B; D: Image showing 5-HT3 receptor immunoreactivity visualized using donkey-anti-rabbit IgG conjugated with AlexaFluor 488 (green); E: Image showing clathrin immunoreactivity visualized using a donkey-anti-mouse IgG conjugated with Dylight 549 (red); F: Merged images from D and E.

finding, together with the inability of ondansetron even at high concentrations to affect the peristaltic response, casts doubts over the actual involvement of 5-HT$_3$R in the motor reflexes stimulated by mechanical distension in hypertrophic ileum. Given the complexity of factors possibly altering 5-HT availability and metabolism and, therefore, also affecting the pharmacological response to 5-HT$_3$R antagonists, future studies will help to elucidate the role exerted by 5-HT in this experimental model and in intestinal secreto-motor disorders in general, where refractoriness to blockade of 5HT$_3$R has sometimes emerged[25].

In conclusion, on the basis of the findings of this study, it is rational to hypothesize that the ability of hypertrophic intestine to elicit a powerful peristaltic reflex *in vitro* is an expression of the functional plasticity of neural and muscular intestinal tissue, a property aimed at preserving its physiological role in more demanding conditions, and allowing it to maintain chyme propulsion even in the presence of a point of abnormal resistance. This distinctive motor pattern appears to be primarily controlled by cholinergic and peptidergic mediators and only slightly affected by serotonergic transmission, which is critically involved, *via* 5-HT$_3$R, in the modulation of non-propulsive motility triggered by mechanical distension in non-obstructed tissues.

ACKNOWLEDGMENTS

The authors wish to thank Professor Catia Sternini (CURE/UCLA Digestive Disease Research Center, Los Angeles, CA, United States) for kindly providing rabbit-anti-rat IgG primary antibody directed against the 5-HT3R (No. 95247); Dr. Giuseppe Domenichini for his skilful technical assistance and the University of Parma (FIL 2009) for financial support.

COMMENTS

Background

Intestinal obstruction is a frequently encountered clinical problem, resulting from congenital malformations (as in Hirschsprung's disease) or by an acquired obstruction, such as a surgical-induced stenosis or a neoplasm, possibly leading to death if not properly diagnosed and treated. Morphological, biomechanical and neurochemical changes have been thoroughly described orally to the site of an experimental chronic obstruction; however, functional investigations, which would help to gain a deeper insight into the regulatory mechanisms of motility in this pathological condition, are still limited.

Research frontiers

Disruption of digestive motor activity, changes in slow wave activity but also preservation of the peristaltic reflex have been functionally described in different experimental models of intestinal stenosis. In rat ileum, hypertrophic growth, induced by mechanical obstruction, has been demonstrated to affect the responses of both smooth muscle layers and of intrinsic innervation, but no information is available on its effects on a highly sophisticated and integrated process between neural and myogenic components such as intestinal peristalsis.

Innovations and breakthroughs

To the best of knowledge, this is the first report showing that, following obstruction, rat isolated terminal ileum develops a propulsive activity triggered simply by intraluminal distension. Furthermore, the peristaltic activity exhibited by rat hypertrophic ileum appears only slightly affected by serotoninergic transmission, a property quite unexpected on the basis of the pivotal role played, in

general, by endogenous serotonin in the initiation and propagation of enteric reflexes and, in particular, in the non-propulsive motility triggered by mechanical distension in non-obstructed tissues.

Applications

The results of this research improve the understanding of the changes in motility patterns and enteric reflexes triggered by intestinal obstruction, through a pharmacological investigation. Moreover, the observed refractoriness to 5-HT_3 antagonists makes this system an interesting model to study conditions of variable sensitivity to blockade of 5-HT_3 receptors, such as that described for diarrhea-predominant irritable bowel syndrome in female patients treated with alosetron.

Terminology

Peristalsis: A coordinated motor behavior, which allows the intestine to propel its contents in an anal direction; Accommodation: Indicates the ability of the intestine to adapt itself to the distension pressure, and, conversely, is a reflection of the resistance of the intestinal wall to the infused fluid; 5-HT_3 receptor: Ligand-gated ion channel receptor activated by serotonin or 5-HT, localized on extrinsic sensory nerves and on myenteric neurons, where they initiate giant migrating contractions and may participate in the triggering and propagation of the peristaltic reflex.

Peer review

This is a nice study, the findings are interesting and potentially important for clinical medicine. The authors showed that accommodation capacity and distension-induced peristalsis of rat hypertrophic ileum are primarily controlled by cholinergic and peptidergic transmission and negligibly by 5-HT_3 receptors.

REFERENCES

1 **Schuffler MD**, Sinanan MN. Intestinal obstruction and pseudo-obstruction. In: Sleisenger MH, Fordtran JS. Gastrointestinal disease – Pathophysiology/Diagnosis/Management. Philadelphia: WB Saunders editors, 1993: 898-916

2 **Gabella G**. Hypertrophy of visceral smooth muscle. *Anat Embryol* (Berl) 1990; **182**: 409-424 [PMID: 2291488 DOI: 10.1007/BF00178906]

3 **Ekblad E**, Sjuve R, Arner A, Sundler F. Enteric neuronal plasticity and a reduced number of interstitial cells of Cajal in hypertrophic rat ileum. *Gut* 1998; **42**: 836-844 [PMID: 9691923 DOI: 10.1136/gut.42.6.836]

4 **Chang IY**, Glasgow NJ, Takayama I, Horiguchi K, Sanders KM, Ward SM. Loss of interstitial cells of Cajal and development of electrical dysfunction in murine small bowel obstruction. *J Physiol* 2001; **536**: 555-568 [PMID: 11600689 DOI: 10.1111/j.1469-7793.2001.0555c.xd]

5 **Jew JY**, Williams TH, Gabella G, Zhang MQ. The intestine as a model for neuronal plasticity. *Arch Histol Cytol* 1989; **52** Suppl: 167-180 [PMID: 2479400 DOI: 10.1679/aohc.52.Suppl_167]

6 **Storkholm JH**, Zhao J, Villadsen GE, Gregersen H. Spontaneous and bolus-induced motility in the chronically obstructed guinea-pig small intestine in vitro. *Dig Dis Sci* 2008; **53**: 413-420 [PMID: 17562174 DOI: 10.1007/s10620-007-9865-6]

7 **Bertoni S**, Gabella G, Ghizzardi P, Ballabeni V, Impicciatore M, Lagrasta C, Arcari ML, Barocelli E. Motor responses of rat hypertrophic intestine following chronic obstruction. *Neurogastroenterol Motil* 2004; **16**: 365-374 [PMID: 15198659 DOI: 10.1111/j.1365-2982.2004.00510.x]

8 **Coelho JC**, Gouma DJ, Moody FG, Li YF, Senninger N. Gastrointestinal motility following small bowel obstruction in the opossum. *J Surg Res* 1986; **41**: 274-278 [PMID: 3762133 DOI: 10.1016/0022-4804(86)90036-3]

9 **Schulze-Delrieu K**, Brown B, Herman B, Brown CK, Lawrence D, Shirazi S, Palmieri T, Raab J. Preservation of peristaltic reflex in hypertrophied ileum of guinea pig. *Am J Physiol* 1995; **269**: G49-G59 [PMID: 7631801]

10 **Trendelenburg P**. Physiological and pharmacological investigations of small intestinal peristalsis. Translation of the article "Physiologische und pharmakologische Versuche über die Dünndarmperistaltik", Arch. Exp. Pathol. Pharmakol. 81, 55-129, 1917. *Naunyn Schmiedebergs Arch Pharmacol* 2006; **373**: 101-133 [PMID: 17312568]

11 **Bertoni S**, Ballabeni V, Flammini L, Gobbetti T, Impicciatore M, Barocelli E. Intestinal chronic obstruction affects motor responsiveness of rat hypertrophic longitudinal and circular muscles. *Neurogastroenterol Motil* 2008; **20**: 1234-1242 [PMID: 18684211 DOI: 10.1111/j.13652982.2008.01174.x]

12 **Bulbring E**, Crema A. The release of 5-hydroxytryptamine in relation to pressure exerted on the intestinal mucosa. *J Physiol* 1959; **146**: 18-28 [PMID: 13655213]

13 **Bulbring E**, Lin RC. The effect of intraluminal application of 5-hydroxytryptamine and 5-hydroxytryptophan on peristalsis; the local production of 5-HT and its release in relation to intraluminal pressure and propulsive activity. *J Physiol* 1958; **140**: 381-407 [PMID: 13514713]

14 **Gabella G**. Hypertrophy of intestinal smooth muscle. *Cell Tissue Res* 1975; **163**: 199-214 [PMID: 1182787 DOI: 10.1007/BF00221727]

15 **Costall B**, Naylor RJ, Tuladhar BR. 5-HT4 receptor mediated facilitation of the emptying phase of the peristaltic reflex in the guinea-pig isolated ileum. *Br J Pharmacol* 1993; **110**: 1572-1578 [PMID: 8306103]

16 **Waterman SA**, Costa M, Tonini M. Modulation of peristalsis in the guinea-pig isolated small intestine by exogenous and endogenous opioids. *Br J Pharmacol* 1992; **106**: 1004-1010 [PMID: 1356564]

17 **Kunze WA**, Furness JB. The enteric nervous system and regulation of intestinal motility. *Annu Rev Physiol* 1999; **61**: 117-142 [PMID: 10099684 DOI: 10.1146/annurev.physiol.61.1.117]

18 **Storkholm JH**, Zhao J, Villadsen GE, Hager H, Jensen SL, Gregersen H. Biomechanical remodeling of the chronically obstructed Guinea pig small intestine. *Dig Dis Sci* 2007; **52**: 336-346 [PMID: 17219069 DOI: 10.1007/s10620-006-9431-7]

19 **Briejer MR**, Mathis C, Schuurkes JA. 5-HT receptor types in the rat ileum longitudinal muscle: focus on 5-HT2 receptors mediating contraction. *Neurogastroenterol Motil* 1997; **9**: 231-237 [PMID: 9430791 DOI: 10.1046/j.1365-2982.1997.d01-62.x]

20 **Gershon MD**. Review article: serotonin receptors and transporters -- roles in normal and abnormal gastrointestinal motility. *Aliment Pharmacol Ther* 2004; **20** Suppl 7: 3-14 [PMID: 15521849 DOI: 10.1111/j.1365-2036.2004.02180.x]

21 **Monro RL**, Bertrand PP, Bornstein JC. ATP and 5-HT are the principal neurotransmitters in the descending excitatory reflex pathway of the guinea-pig ileum. *Neurogastroenterol Motil* 2002; **14**: 255-264 [PMID: 12061910 DOI: 10.1046/j.1365-2982.2002.00325.x]

22 **Kadowaki M**, Wade PR, Gershon MD. Participation of 5-HT3, 5-HT4, and nicotinic receptors in the peristaltic reflex of guinea pig distal colon. *Am J Physiol* 1996; **271**: G849-G857 [PMID: 8944700]

23 **Bertoni S**, Gabella G. Hypertrophy of mucosa and serosa in the obstructed intestine of rats. *J Anat* 2001; **199**: 725-734 [PMID: 11787826 DOI: 10.1046/j.1469-7580.2001.19960725.x]

24 **Freeman SL**, Glatzle J, Robin CS, Valdellon M, Sternini C, Sharp JW, Raybould HE. Ligand-induced 5-HT3 receptor internalization in enteric neurons in rat ileum. *Gastroenterology* 2006; **131**: 97-107 [PMID: 16831594 DOI: 10.1053/j.gastro.2006.04.013]

25 **Camilleri M**, Atanasova E, Carlson PJ, Ahmad U, Kim HJ, Viramontes BE, McKinzie S, Urrutia R. Serotonin-transporter polymorphism pharmacogenetics in diarrhea-predominant irritable bowel syndrome. *Gastroenterology* 2002; **123**: 425-432 [PMID: 12145795 DOI: 10.1053/gast.2002.34780]

P-Reviewers Bian ZX, Inui A **S- Editor** Gou SX
L- Editor Cant MR **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i6.855

World J Gastroenterol 2013 February 14; 19(6): 855-865
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

ORIGINAL ARTICLE

Role of contrast-enhanced ultrasound in follow-up assessment after ablation for hepatocellular carcinoma

Shu-Guang Zheng, Hui-Xiong Xu, Ming-De Lu, Xiao-Yan Xie, Zuo-Feng Xu, Guang-Jian Liu, Lin-Na Liu

Shu-Guang Zheng, Hui-Xiong Xu, Lin-Na Liu, Department of Medical Ultrasound, Tenth People's Hospital of Tongji University, Shanghai 200072, China
Shu-Guang Zheng, Hui-Xiong Xu, Ming-De Lu, Xiao-Yan Xie, Zuo-Feng Xu, Guang-Jian Liu, Lin-Na Liu, Department of Medical Ultrasonics, the First Affiliated Hospital, Sun Yat-Sen University, Guangzhou 510080, Guangdong Province, China
Author contributions: Zheng SG and Xu HX contributed equally to this work; Xie XY, Xu ZF and Liu GJ performed the majority of experiments; Zheng SG, Xu HX and Lu MD provided analytical tools and were also involved in revising the manuscript; Xu HX and Lu MD collected all the human materials and provided financial support for this work; Zheng SG, Xu HX, Lu MD and Liu LN designed the study and wrote the manuscript.
Supported by Public Welfare Research Special Project from Chinese Ministry of Health, No. 2008-2-10; Key Project from Shanghai Health Bureau, No. 20114003; Shanghai Talent Development Project from Shanghai Human Resource and Social Security Bureau, No. 2012045; National Natural Science Foundation of China, No. 30970837; and Chinese Ministry of Education, NCET-06-0723
Correspondence to: Ming-De Lu, MD, DMSc, Department of Medical Ultrasonics, the First Affiliated Hospital, Sun Yat-Sen University, No. 58, Zhongshan Road 2, Guangzhou 510080, Guangdong Province, China. lumd@live.com
Telephone: +86-20-87765183 Fax: +86-20-87765183
Received: October 23, 2012 Revised: December 17, 2012
Accepted: December 22, 2012
Published online: February 14, 2013

Abstract

AIM: To assess the usefulness of contrast-enhanced ultrasound (CEUS) during follow-up after percutaneous ablation therapy for hepatocellular carcinoma (HCC).

METHODS: A total of 141 patients with HCCs who received percutaneous ablation therapy were assessed by paired follow-up CEUS and contrast-enhanced computed tomography (CECT). The follow-up scheme was designed prospectively and the intervals between CEUS and CECT examinations were less than 14 d. Both images of follow-up CEUS and CECT were reviewed by radiologists. The ablated lesions were evaluated and classified as local tumor progression (LTP) and LTP-free. LTP was defined as regrowth of tumor inside or adjacent to the successfully treated nodule. The detected new intrahepatic recurrences were also evaluated and defined as presence of intrahepatic new foci. On CEUS and CECT, LTP and new intrahepatic recurrence both were displayed as typical enhancement pattern of HCC (*i.e.*, hyper-enhancing during the arterial phase and washout in the late phase). With CECT as the reference standard, the ability of CEUS in detecting LTP or new intrahepatic recurrence during follow-up was evaluated.

RESULTS: During a follow-up period of 1-31 mo (median, 4 mo), 169 paired CEUS and CECT examinations were carried out for the 141 patients. For a total of 221 ablated lesions, 266 comparisons between CEUS and CECT findings were performed. Thirty-three LTPs were detected on CEUS whereas 40 LTPs were detected on CECT, there was significant difference ($P < 0.001$). In comparison with CECT, the numbers of false positive and false negative LTPs detected on CEUS were 6 and 13, respectively; the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and overall accuracy of CEUS in detecting LTPs were 67.5%, 97.4%, 81.8%, 94.4% and 92.3%, respectively. Meanwhile, 131 new intrahepatic recurrent foci were detected on CEUS whereas 183 were detected on CECT, there was also significant difference ($P < 0.05$). In comparison with CECT, the numbers of false positive and false negative intrahepatic recurrences detected on CEUS were 13 and 65, respectively; the sensitivity, specificity, PPV, NPV and overall accuracy of CEUS in detecting new intrahepatic recurrent foci were 77.7%, 92.0%, 92.4%, 76.7% and 84.0%, respectively.

CONCLUSION: The sensitivity of CEUS in detecting LTP and new intrahepatic recurrence after percutaneous ablation therapy is relatively low in comparison

with CECT.

© 2013 Baishideng. All rights reserved.

Key words: Contrast-enhanced ultrasound; Contrast-enhanced computed tomography; Hepatocellular carcinoma; Radiofrequency ablation; Microwave ablation

Zheng SG, Xu HX, Lu MD, Xie XY, Xu ZF, Liu GJ, Liu LN. Role of contrast-enhanced ultrasound in follow-up assessment after ablation for hepatocellular carcinoma. *World J Gastroenterol* 2013; 19(6): 855-865 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i6/855.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i6.855

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common cancer worldwide, the incidence of which is continuously increasing in both western and eastern countries[1]. Percutaneous ablation therapy, such as radiofrequency ablation (RFA) and microwave ablation (MWA), as a minimally invasive and effective treatment modality, has been accepted in the management of hepatic malignance and regarded as one of the best treatment options for patients with early stage HCC who are not suitable for resection or transplantation[2-5]. Several randomized clinical trials have also confirmed that for small HCC, treatment efficacy of thermal ablation is comparable to that of surgical resection[2,6,7].

The evaluation of treatment efficacy after percutaneous ablation therapy for HCC is essential for the determination of subsequent treatment and follow-up strategy[8], which is usually performed by means of imaging modalities such as contrast-enhanced computed tomography (CT) and magnetic resonance imaging (MRI)[2,9,10]. Both of them have been regarded as reliable and accurate imaging tools for post-treatment efficacy evaluation and follow-up[2]. However, contrast-enhanced CT (CECT) and contrast-enhanced MRI (CEMRI) are relatively expensive. In addition, CECT is unsuitable for patients with renal function impairment and allergic reaction to contrast agent. Finally, the radiation exposure in CT examination is always a concern for both clinicians and patients.

The technique of contrast-enhanced ultrasound (CEUS) has the potential to be a substitute for CECT or CEMRI. By using ultrasound contrast agents (UCAs) and contrast specific imaging techniques, CEUS is able to depict the micro- and macro-circulation in the liver and the treated lesion, thus allowing assessment of the treatment efficacy for HCC after percutaneous ablation therapy in a similar fashion with CECT or CEMRI[2,8,11-14]. Besides, UCAs are very safe and the incidence of severe hypersensitivity or allergic reaction is lower than that of current X-ray contrast agents and comparable to MR contrast agents[13,15]. CEUS has been confirmed to be comparable to CECT for characterization of HCC[16-19]. A prospective multicenter study also proved that CEUS is equal to CECT or CEMRI for the assessment of the local treatment response after ablation therapy[11,12]. After local treatment response assessment, the patient is always enrolled into a long-term follow-up scheme for surveillance of local tumor progression (LTP) or new intrahepatic foci[11,12]. In theory, CEUS may be inferior to CECT or CEMRI since the development of new foci may be multiple and may be located in different lobes of the liver, and the arterial enhancement on CEUS only lasts several seconds, thus it is hard to detect all the hypervascular lesions. In addition, the new foci in the blind areas such as liver dome may be invisible on CEUS. To our knowledge, there has been no study evaluating the role of CEUS in the follow-up assessment after percutaneous ablation therapy for HCC[2,12,19,20]. To confirm the hypothesis that CEUS might not be competent to CECT in follow-up assessment after local ablation for HCC, the study was carried out to assess the usefulness of CEUS in detecting LTP and new intrahepatic recurrence in the follow-up after ablation therapy for HCC, with CECT as the reference standard.

MATERIALS AND METHODS

Patients

Between May 2007 and March 2011, 466 consecutive patients with HCCs were referred to the institution for ultrasound (US)-guided percutaneous ablation therapy. These patients met the following enrollment criteria: (1) single HCC not greater than 6 cm in diameter; (2) multiple HCCs up to 5 in number with each tumor measuring 3 cm or smaller; (3) absence of portal venous thrombosis or extrahepatic metastases; (4) liver cirrhosis classified as Child-Pugh class A or B; and (5) prothrombin time ratio greater than 50% and platelet count greater than 60 000/mm^3 (60 × 10^9 /L). Among them, 141 patients (132 men, 9 women; mean age, 53.4 ± 12.1 years; age range, 27-81 years) were enrolled to this follow-up study after ablation. The inclusion criteria were as follows: (1) the patients had no allergic reaction to iodinated contrast agent; (2) CECT confirmed complete ablation of the tumors within 1 mo after ablation therapy; and (3) paired CEUS and CECT were performed during the follow-up and the time interval between CEUS and CECT was less than 14 d. All the data of the 141 patients, including baseline data, clinical data, and imaging data, were collected prospectively and stored in a dedicated database for further analysis. The study was approved by the Institutional Review Board, and written informed consent was obtained from all patients.

Among the 141 patients, 60 patients had recurrent HCCs after partial hepatectomy for primary HCCs and the remaining 81 patients with primary HCCs were treated by US-guided percutaneous ablation therapy as the first therapy. The diagnoses of HCC were confirmed by histopathological examination with specimens obtained from US-guided percutaneous biopsy (n = 35) or clinical data (n = 106). The clinical diagnostic criteria for HCC mainly followed the AASLD and EFSUMBS

practice guideline: the presence of typical CECT and CEUS features (*i.e.*, hyper-enhancement in arterial phase and washout in portal-venous or late phase)[2,15]. Among the clinically confirmed 106 patients, 46 were diagnosed by characteristic imaging findings on CECT and serum α-fetoprotein ≥ 200 ng/mL; 60 patients were diagnosed by history of partial hepatectomy for HCC and typical appearance of HCC recurrence on CECT. The US-guided percutaneous ablation therapies for them included radiofrequency ablation (RFA) (n = 83), percutaneous ethanol ablation (EA) (n = 29), RFA in combination with EA (n = 26), and microwave ablation (MWA) (n = 3).

Ablation techniques

Percutaneous ablation therapy for HCC was performed with local anesthesia and conscious sedation. RFA was carried out with a cooled-tip RFA ablation system (Cool-tip, Radionics, MA, United States), which is a 480 kHz alternative current generator that can produce a maximum power of 200W through a 17 Gmonopolar, cooled-tip needle electrode. The radiofrequency electrode temperature was maintained at less than 18 ℃ by the application of a circulating chilled saline solution to the cannula sheath. A single 3 cm exposed tip RFA electrode was applied[21,22]. MWA was carried out with a microwave delivery system (FORSEA; Qinghai Microwave Electronic Institute, Nanjing, China), which consisted of an MTC-3 microwave generator (FORSEA) with a frequency of 2450 MHz, a power output of 10-150 W, a flexible low-loss cable, and a 14-gauge cooled-shaft antenna. The RFA electrode or MWA antenna was firstly placed at the bottom of the tumor and withdrawn 1.5-2 cm each time to ablate the more superficial portion for large tumors. Multiple insertions were applied to treat tumors larger than 1.5 cm for RFA and 3.0 cm for MWA[21,22]. EA was performed using a Quadra-Fuse™ multi-pronged needle (Rex Medical, Radnor, PA, United States). In general, no greater than 30 mL of 95% ethanol was injected until the hyperechoic cloud covered the whole tumor. For patients with tumor adjacent to critical structures such as hilum or great vessels, RFA was performed in combination with EA. In general, EA was carried out in advance of RFA and RFA was performed 5 min after EA, the aim of which was to increase the coagulation volume whereas limit the damage to adjacent critical structures[23-25]. To prevent possible bleeding or tumor seeding, the needle track was cauterized when the RFA electrode or the MWA antenna was withdrawn. The aim of the procedure was to completely ablate the tumor along with an ablative margin of 0.5-1.0 cm[26,27].

Contrast-enhanced US examination

All the US examinations were performed by one of three skillful radiologists who had more than 7 years experience in CEUS and were unaware of clinical and other imaging information of the patients. Two US machines were used in this study. One was an Acuson Sequoia 512 machine (Siemens Medical Solutions, Mountain View, CA, United States) and the other was an Aplio XV machine (Toshiba Medical Systems, Tokyo, Japan). A 4V1 vector transducer with a frequency range of 1.0-4.0 MHz was applied for Sequoia 512 and a 375 BT convex transducer with a frequency range of 1.9-6.0 MHz was applied for Aplio XV. The installed contrast-specific imaging modes were contrast pulse sequencing (CPS) for Sequoia 512 and contrast harmonic imaging (CHI) for Aplio XV. Both modes work under low acoustic power, and the corresponding mechanical index (MI) ranges were 0.15-0.21 for CPS in Sequoia 512 and 0.05-0.08 for CHI in Aplio XV.

Baseline US (BUS) investigation in B-mode was firstly applied to scan the whole liver, including Doppler technique. Once the treated lesion was found, the lesion size, echogenicity, and location were recorded, and the images that showed best the above-mentioned features were stored digitally in the US machine. Then the transducer was moved to scan other liver to detect if there were suspected new recurrence foci and the above-mentioned features were also recorded if new foci were present. Afterward, the imaging mode was shifted to CEUS mode and the imaging settings were optimized to ensure sufficient tissue cancellation with the maintenance of adequate depth penetration, with the diaphragm remaining barely visible.

The US contrast agent used was SonoVue (Bracco, Milan, Italy), a sulfur hexafluoride-filled microbubble contrast agent. A total of 2.4 mL contrast agent was given intravenously as a 2.4 mL bolus within 2-3 s through the antecubital vein, followed by 5 mL saline flush. Upon start of the SonoVue injection, the stop clock was started and digital cine was recorded simultaneously. During early period of CEUS procedure, the transducer was firstly kept in a stable position to observe the enhancement pattern of the treated lesion and then switched to scan other liver parenchyma. The first 2 min was continuously observed and subsequent intermittent scanning was performed until the disappearance of contrast agent in liver parenchyma. According to the previous studies, the CEUS process was divided into arterial (*i.e.*, 8-30 s from the beginning of contrast agent administration), portal (31-120 s), and late (121-360 s) phases[11,15]. A second or third injection of SonoVue was performed when suspicious new foci were documented on BUS or hypoenhancing new foci were detected in the late phase on CEUS. No patient received more than 3 injections.

Contrast-enhanced CT examination

For the CT examination, the Aquilion 64-slice helical CT machine (Tokyo, Japan) was used. The intervals between CEUS and CECT examinations were less than 14 d and no additional treatment was carried out during this period. The imaging protocol for CT examinations was as follows: 0.5 mm × 64 mm collimation, 120 kV, 150-200 mAs for 64-slice helical CT examination. The standard triphasic scan procedure was used. An unenhanced helical sequence scan through the liver was performed first; thereafter nonionic iodinated contrast material (Ultravist,

Schering, Berlin, Germany) (1.5 mL/kg) was administered via antecubital vein with power injection at a rate of 4 mL/s for 64-slice helical CT. The arterial phase sequence was obtained 25-32 s after contrast material administration, followed by a portal venous phase sequence 70 s after contrast agent administration.

Image interpretation

Two of the three skillful radiologists, who had more than 7-year experience in liver CEUS, evaluated the CEUS images and two experienced radiologists, who had more than 15-year experience in liver CECT, evaluated the treatment response using the CT images. The reviewers were not involved in the US or CT scanning, and were unaware of clinical and other imaging information of the patients. The findings of the treated lesions and new intrahepatic recurrence were observed and the treatment response was evaluated. Complete ablation was defined as nonenhancement in the ablated area; otherwise, ablation was considered incomplete. During the follow-up period, local tumor progression (LTP) was defined as regrowth of tumor inside or adjacent to the successfully treated nodule, which appeared as a hyper-enhancing area during the arterial phase and wash-out during portal-late phases inside the treated lesion on CEUS or CECT[3]. Non-enhancement in the treated area was defined as LTP-free. New intrahepatic recurrence was defined as presence of intrahepatic new foci with typical enhancement pattern of HCC on CEUS or CECT (*i.e.*, hyper-enhancing during the arterial phase and wash-out in the late phase). Development of portal venous tumor thrombosis was also defined as new intrahepatic recurrence. Non-recurrence was defined as no additional HCC lesions found.

Follow-up assessment

In the study design, the local effectiveness of ablation was assessed by CEUS or CECT within one month after ablation. Only the patients with complete ablation were enrolled into the prospectively designed follow-up scheme, and those with incomplete ablation were referred to further treatment, *e.g.*, additional ablation, transcatheter arterial chemoembolization (TACE), Sorafenib, *etc.*, according to the liver function status and tumor staging.

In the follow-up scheme, all patients were evaluated simultaneously with CECT and CEUS every 3 mo for the first 6 mo. If no positive findings were present and the ablation area shrank or disappeared, follow-up at an interval of 6-12 mo was made. At the same time, all patients were also monitored monthly with abdominal color Doppler US, serum AFP, chest radiography and liver function tests for the first 6 mo, and thereafter every 3 to 6 mo. When there were suspicious findings on US (*i.e.*, enlargement of the treated area, changes in US pattern, presence of intralesional Doppler signal, and appearance of new lesion) or elevated AFP, then paired CEUS and CECT were performed to confirm the diagnosis. Once the LTP or new intrahepatic recurrences were detected, the follow-up was over and the patients were referred to further treatment.

Statistical analysis

Continuous data were expressed as mean ± SD. The χ^2 test was used to compare the differences in detecting LTP and new intrahepatic recurrence between CECT and CEUS. Two-tailed $P < 0.05$ was considered statistically significant. With CECT as the reference standard, the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy of CEUS in detecting LTP were computed on the basis of the assessment results of the ablation lesions on each follow-up examination, and those of CEUS in detecting new intrahepatic recurrence were computed on the basis of the patients' screening results on each follow-up examination. Statistical analysis were performed using the SPSS 13.0 software package (SPSS Inc., Chicago, IL, United States).

RESULTS

After percutaneous ablation therapy, the 141 patients with 221 HCCs (the maximum diameter ranged from 0.6 cm to 5.7 cm; mean ± SD, 2.4 ± 1.0 cm) were followed up for 1-31 mo (median, 4 mo; mean ± SD, 6.7 ± 6.4 mo) after complete ablation was confirmed by CECT 1 mo after ablation. The interval between every paired CEUS and CECT examination ranged from 0 to 14 d (median, 1 d; mean ± SD, 3.1 ± 4.3 d).

During the follow-up period, the 141 patients received 169 (once, $n = 118$; twice, $n = 18$; three times, $n = 5$) paired CEUS and CECT examinations. For the 221 ablated lesions (the diameter ranged from 1.2 cm to 7.4 cm; mean ± SD, 4.1 ± 1.1 cm), 266 comparisons between CEUS and CECT findings (185 ablated lesions were examined once; 27 ablated lesions were examined twice; 9 ablated lesions were examined three times) were performed.

During the follow-up, 40 LTPs (the diameter ranged from 0.4 cm to 5.5 cm; mean ± SD, 2.0 ± 1.1 cm) and 183 new intrahepatic recurrences (the diameter ranged from 0.3 cm to 3.8 cm; mean ± SD, 1.5 ± 0.8 cm) were detected on CECT, whereas only 33 and 131 were detected on CEUS. The locations of the LTPs and the new intrahepatic recurrences are summarized in Table 1.

In the 266 comparisons between paired CECT and CEUS for all ablation lesions, CEUS determined that 233 lesions were LTP-free and all showed non-enhancement in the arterial, portal, and late phases (Figure 1A and B). The remaining 33 lesions were determined to be LTP on CEUS and all showed hyper-enhancement in the arterial phase and wash-out ($n = 28$) (Figure 2A and B) or iso-enhancement ($n = 5$) in the portal-late phases. However, on CECT, 226 lesions were determined to be LTP-free and all showed non-enhancement in the arterial, portal, and late phases (Figure 1C and D); and the remaining 40 lesions were determined to be LTP and all showed hyper-enhancement in the arterial phase and wash-out in the portal-late phase (Figure 2C and D).

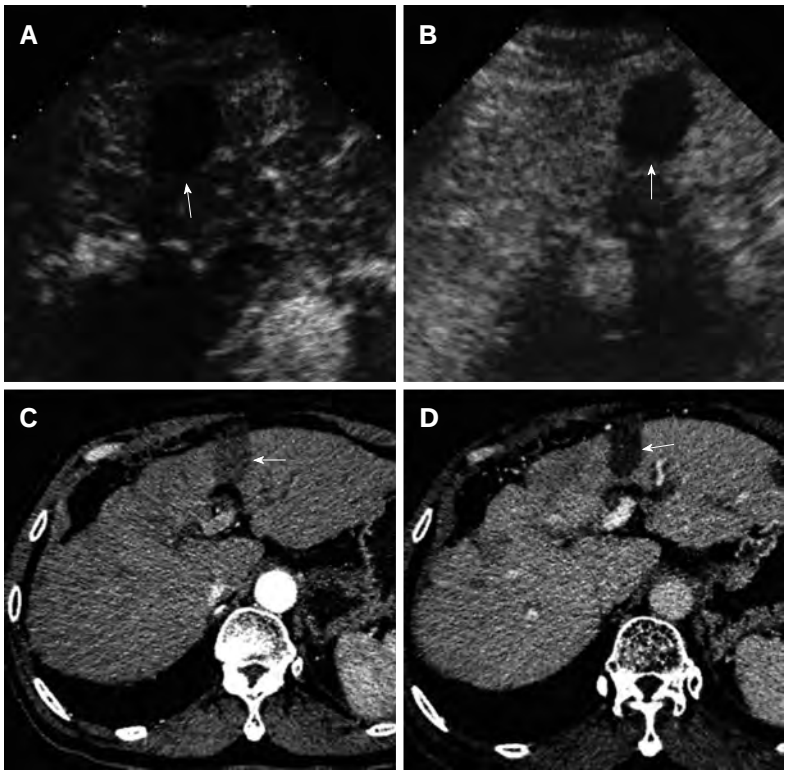


Figure 1 A 57-year-old male patient with hepatocellular carcinoma. Two months after radiofrequency ablation for hepatocellular carcinoma in segment 3 of the liver. On both contrast-enhanced computed tomography (A, B) and contrast-enhanced ultrasound (C, D), the treated lesion (arrow) showed complete necrosis without any enhancement in all vascular phases.

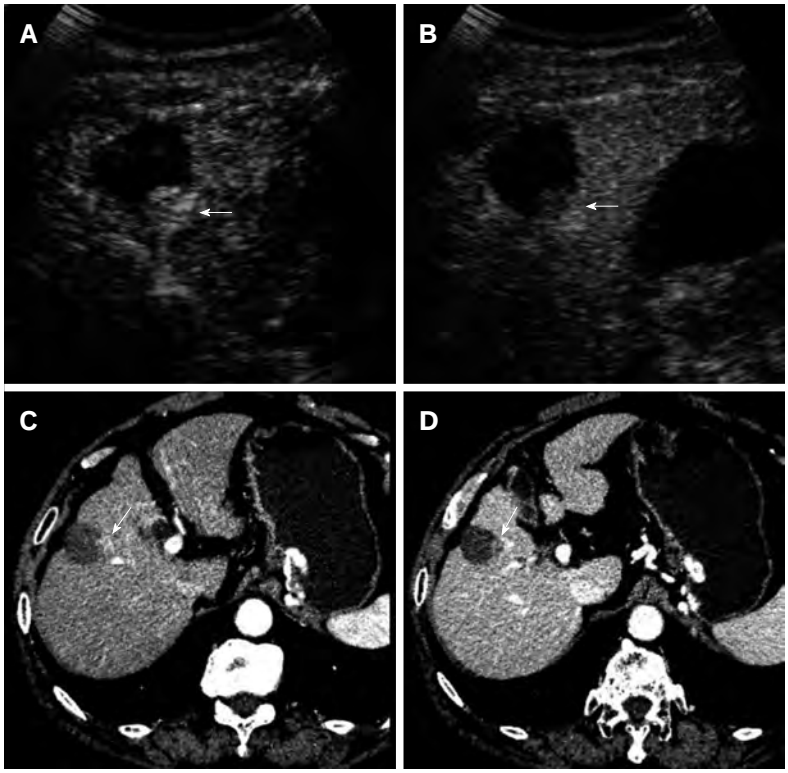


Figure 2 A 70-year-old male patient with hepatocellular carcinoma. Local tumor progression (arrow) was detected 6 mos after radiofrequency ablation in combination with ethanol ablation for hepatocellular carcinoma in segment 5 of the liver. Local tumor progression showed hyper-enhancement in the arterial phase and iso-enhancement in the portal-late phase on contrast-enhanced ultrasound (A, B), whereas hyper-enhancement in the arterial phase and wash-out in the portal-venous phase on contrast-enhanced computed tomography (C, D).

By comparing the number of the ablated lesions, there was significant difference between CECT and CEUS in detecting LTP ($P < 0.001$, Table 2). Differences between CECT and CEUS were also found in the subgroups (< 3 cm *vs* ≥ 3 cm in diameter; single *vs* multiple ablated lesions), (both $P < 0.001$) (Table 2). With CECT as the reference standard, the sensitivity, specificity, PPV, NPV and overall accuracy of CEUS in detecting LTP after percutaneous ablation were 67.5% (27/40), 97.3% (220/226), 81.8% (27/33), 94.4% (220/233), 92.9% (247/266), respectively.

A total of 183 new intrahepatic recurrences were detected on CECT and all showed hyper-enhancement in the arterial phase and wash-out in the portal-venous

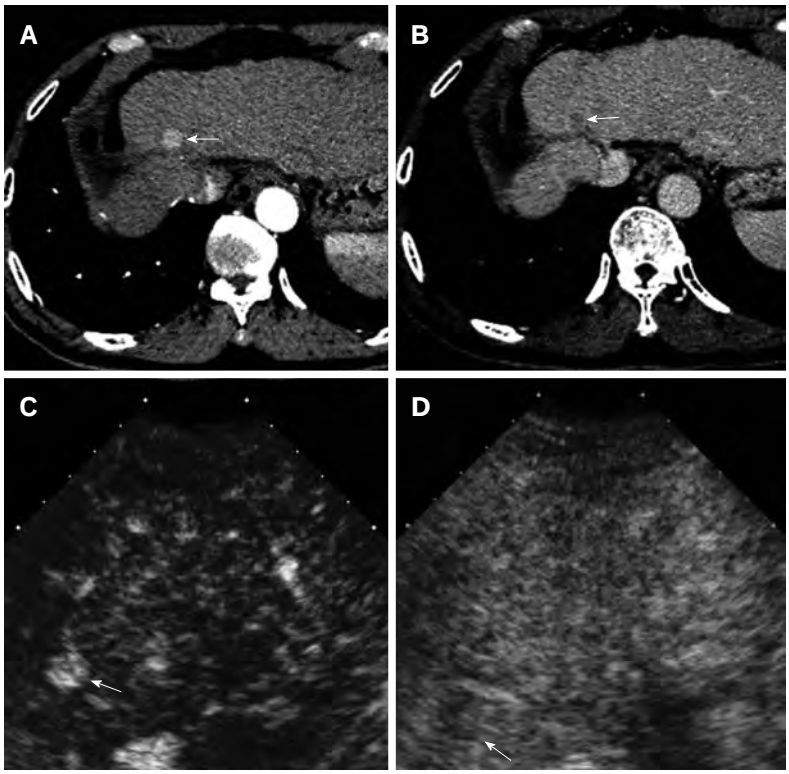


Figure 3 The same patient as shown in Figure 1. A, B: Two months after radiofrequency ablation for hepatocellular carcinoma. A new intrahepatic recurrence (arrow) was detected in segment 4 of the liver, which showed hyper-enhancement in the arterial phase and wash-out in the portal-venous phase on contrast-enhanced computed tomography; C, D: Similar findings were found in the arterial phase and the portal-late phases with contrast-enhanced ultrasound.

Table 1 Location of local tumor progression and new intrahepatic recurrence on contrast-enhanced computed tomography and contrast-enhanced ultrasound

Location	Local tumor progression		New intrahepatic recurrence	
	CEUS	CECT	CEUS	CECT
S1	0	0	1	2
S2	1	2	14	22
S3	2	2	7	13
S4	8	10	27	38
S5	5	7	17	18
S6	7	5	14	23
S7	3	4	17	18
S8	7	10	25	36
PV	0	0	9	11
HV	0	0	0	2
Total	33	40	131	183

Data show the number of the detected lesions. S: Liver segment; PV: Portal vein; HV: Hepatic vein; CEUS: Contrast-enhanced ultrasound; CECT: Contrast-enhanced computed tomography.

phases (Figure 3A and B). Conversely, only 131 recurrent lesions were detected on CEUS (Figure 3C and D). Among them, 107 lesions were in the arterial phase with hyper-enhancement (n = 104) or iso-enhancement (n = 3) and the remaining 24 lesions were missed during the arterial phase. And 124 lesions showed wash-out in the portal-late phases on CEUS and the remaining 7 lesions showed iso-enhancement.

There was significant difference between the follow-up CECT and CEUS in detecting new intrahepatic recurrence when comparing the number of the detected lesions (P = 0.02, Table 3) or the number of the patients with detected lesions (P < 0.01, Table 3). With CECT as the reference standard, the sensitivity, specificity, PPV, NPV and overall accuracy of CEUS in detecting recurrence were 77.7% (73/94), 92.0% (69/75), 92.4% (73/79), 76.7% (69/90), 84.0% (142/169), respectively. The numbers of new intrahepatic recurrence in each patient detected by each follow-up CEUS and CECT are shown in Table 4.

The numbers of false positive and false negative LTPs detected on CEUS were 6 and 13, respectively. Compared with CECT, among the 6 false positive LTPs, 5 misinterpreted hepatic blood vessels (Figure 4) and 1 new intrahepatic recurrence were misdiagnosed as LTPs. The main reasons for false negative LTPs (Figure 5) on CEUS were as follows: near liver dome and obscuration by lung gas (n = 5); deep location (n = 1; depth > 10 cm); obscuration by gastrointestinal tract gas (n = 2); obscuration by enhanced portal vein (n = 1); small lesion (n = 3, all < 0.7 cm in diameter); misdiagnosis as new intrahepatic recurrence (n = 1).

Compared with CECT, the numbers of false positive and false negative intrahepatic recurrences detected on CEUS were 13 and 65, respectively. The causes of false positive recurrences were as follows: 3 regenerative nodules, 9 misinterpreted hepatic blood vessels and 1 LTP were misdiagnosed as new intrahepatic recurrence. Among the 65 false negative recurrences, 4 regenerative nodules and 1 LTP were misdiagnosed as recurrence, and the remaining 60 new intrahepatic recurrences were missed on CEUS. Compared with CECT, the reasons for the missed new intrahepatic recurrences were as follows: multiple lesions (n = 7, > 2 in number), obscuration by gastrointestinal tract gas (n = 10); deep location (n = 4, > 10 cm in depth), near liver dome and obscuration by lung gas (n = 19), small lesion (n = 5, < 1 cm in diameter) and

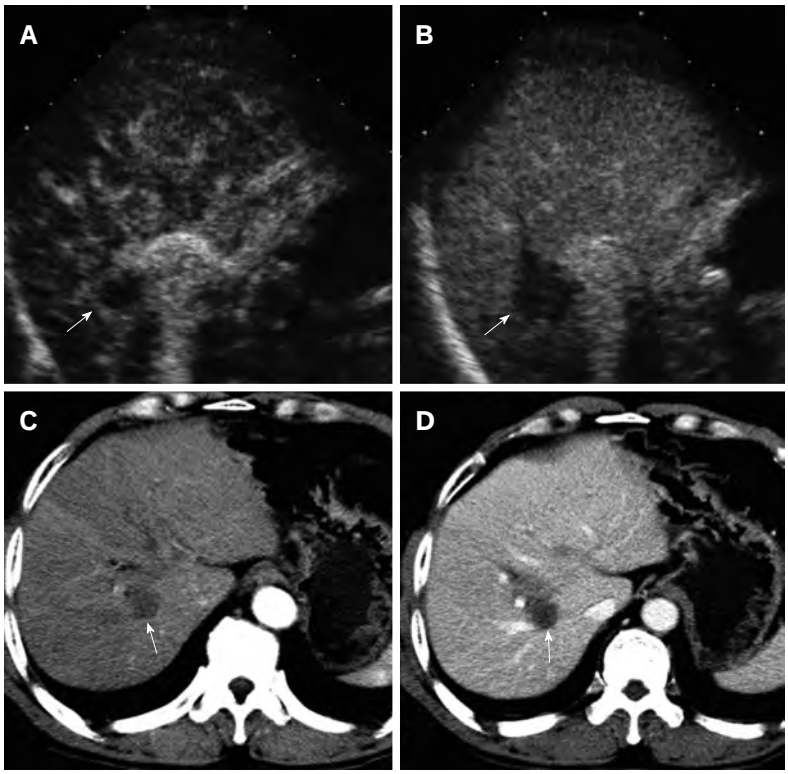


Figure 4 A 62-year-old male patient with hepatocellular carcinoma. Two months after percutaneous ethanol ablation for hepatocellular carcinoma in segment 8 of the liver. A, B: The false positive local tumor progression (arrow) was detected. It showed rim-like hyperenhancement in the arterial phase, wash-out in the portal-late phase on contrast-enhanced ultrasound; C, D: On contrast-enhanced computed tomography, the treated area (arrow) showed complete necrosis without any enhancement in all the vascular phases, but several enhanced hepatic vessels around the treated area.

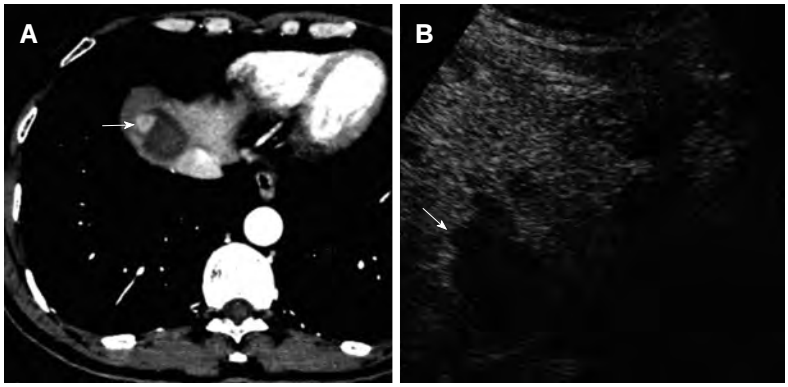


Figure 5 A 54-year-old male patient with hepatocellular carcinoma. Three months after radiofrequency ablation in combination with ethanol ablation for hepatocellular carcinoma in segment 8 of the liver. A: Contrast-enhanced computed tomography showed local tumor progression (arrow) at the periphery of the treated area; B: On contrast-enhanced ultrasound, local tumor progression (arrow) could not be detected, and the treated area was not clearly observed due to unfavorable location near the liver dome.

Table 2 Comparison between contrast-enhanced computed tomography and contrast-enhanced ultrasound in detecting local tumor progression after percutaneous ablation therapy for hepatocellular carcinoma during follow-up

CEUS	CECT														
	All			< 3 cm			≥ 3 cm			Single			Multiple		
	LTP	LTP-free	Total	LTP	LTP-free	Total	LTP	LTP-free	Total	LTP	LTP-free	Total	LTP	LTP-free	Total
LTP	27	6	33	18	4	22	9	2	11	15	5	20	12	1	13
LTP-free	13	220	233	10	165	175	3	55	58	3	74	76	10	146	156
Total	40	226	266	28	169	197	12	57	69	18	79	97	22	147	169
χ^2		125.6			86.7			32.66			48.51			70.79	
P value		< 0.001			< 0.001			< 0.001			< 0.001			< 0.001	

Data show the numbers of the ablated lesions. LTP: Local tumor progression; CEUS: Contrast-enhanced ultrasound; CECT: Contrast-enhanced computed tomography.

unknown causes (n = 15).

DISCUSSION

The treatment efficacy assessment after percutaneous ablation therapy for HCC mainly involves short-term local treatment response evaluation and long-term follow-up assessment. The short-term local treatment response evaluation is difficult as microscopic residual viable HCC is hardly detected by current imaging techniques. There-

Table 3 Comparison in number of detected new intrahepatic recurrence and lesion between contrast-enhanced computed tomography and contrast-enhanced ultrasound

CEUS	CECT		
	Yes	No	Total
New intrahepatic recurrence			
Yes	118	13	131
No	65	0	65
Total	183	13	196
Lesion			
Yes	73	6	79
No	21	69	90
Total	94	75	169

Data show the numbers of detected new intrahepatic recurrence and lesion on CEUS and CECT. The χ^2 test with Yates's correction indicated significant difference in detecting intrahepatic recurrence ($\chi^2 = 5.40$, $P = 0.02$) and lesion ($\chi^2 = 8.33$, $P < 0.01$) between CEUS and CECT. CEUS: Contrast-enhanced ultrasound; CECT: Contrast-enhanced computed tomography.

fore, follow-up scheme after ablation therapies is important. Early detection of LTP or new recurrence during follow-up after percutaneous ablation for HCC is critical and will facilitate retreatment at an early stage[3]. The short-term local treatment response evaluation is usually carried out within 1 mo after ablation therapy[2,8,28]. Contrast-enhanced imaging studies are the most widely accepted modalities to assess the local treatment response[10,12,28]. In contrast to the follow-up assessment, local treatment response focused on the specified known lesion, whereas not the whole liver. Many studies have shown that in local treatment response evaluation, CEUS is comparable with CECT or CEMRI[11,13,20].

However, up till now, no studies have been performed to evaluate the efficacy of CEUS in the follow-up assessment. This issue is very important since some centers may only use CEUS for follow-up because of the convenience of CEUS and the unwareness of the limitations of CEUS. In this study, the efficacy of CEUS in follow-up was firstly evaluated, in comparison with the widely accepted modality of CECT. The long-term follow-up assessment (up to 31 mo; mean ± SD, 6.7 ± 6.4 mo) provided a sufficient surveillance for HCC progression after ablation and the short interval (3.1 ± 4.3 d) between the paired CEUS and CECT examination was able to guarantee that the lesions were observed under the same status of vascularity for comparison between CEUS and CECT.

Many studies have confirmed the accuracy of CEUS in local treatment response evaluation, with the CECT or CEMRI as the reference standard. Among these studies, a prospective multicenter study showed that the sensitivity and the accuracy were as high as 97.0% and 94.2%, respectively[11,20]. The accuracy (92.9%) of follow-up CEUS in detecting LTP in this study was comparable to that in local treatment response evaluation, so were the specificity (97.3%), PPV (81.8%) and NPV (94.4%). However, the relatively low sensitivity (67.5%) showed that CEUS was not comparable to CECT ($P < 0.001$). The low sensitivity was partly due to short arterial phase duration of CEUS and the intrinsic shortcomings of US technique such as inability to detect the lesions in the dome or small lesions, and obscuration by gas from gastrointestinal tract or lung.

It is unknown whether CEUS is competent for the detection of new intrahepatic recurrence after HCC ablation as compared with CECT. According to the published literatures, although CEUS is comparable with CECT/MRI in the detection of liver metastasis, CEUS is incompetent to CECT for HCC surveillance, owing to the insufficient access to the lesion near the liver dome, short duration in arterial phase and the variable appearances in late phase[15,29-31]. Correas *et al*[32] found that CEUS had a sensitivity of 78% and an accuracy of 70% for detection of liver metastases by scanning entire liver parenchyma, similar to the 77.7% and 84% for detection of new intrahepatic recurrences in our study. In this study, CECT was significantly superior to CEUS for the detection of new intrahepatic recurrent foci and CEUS was unable to detect 65 (35.5%) of 183 lesions. The possible reasons might be as follows: small lesion, unfavorable location (i.e., deep location, near liver dome, near gastrointestinal tract or large hepatic blood vessel), atypical enhancement pattern, and background of fatty or cirrhotic liver[19,28,33,34].

During the routine CEUS procedure, the hepatic arterial phase starts from 10-20 s after injection of UCAs, and lasts approximately 10-15 s. Furthermore, the arterial phase presents the optimal contrast enhancement for detecting LTPs and recurrence[15]. However, the short duration of the arterial phase makes it hard to scan the whole liver and screen all suspected lesions, while CECT can scan the entire liver in a few seconds[35]. In this study, a total of 24 (18.3%, 24/131) new intrahepatic recurrences were missed in the arterial phase.

In the portal-late phases, though CEUS can guarantee sufficient duration for whole liver scan, some LTPs and new intrahepatic recurrences usually show iso-enhancement [5 (15.2%, 5/33) and 7 (5.3%, 7/131) in this study, respectively], making them indistinguishable from the surrounding liver parenchyma[28,33,36].

Besides the above-mentioned limitations of CEUS, there were some factors related to the false negative results on CEUS, *e.g.*, near liver dome and obscuration by lung gas (5 LTPs and 19 recurrences) and obscuration by gastrointestinal tract gas (2 LTPs and 10 recurrences), which usually were displayed on CECT whereas not on CEUS. On the other hand, deep location (depth > 10 cm; 1 LTP and 4 recurrences) and small size (< 1.0 cm in diameter; 1 LTP and 5 recurrences) were not easy to be detected on CEUS due to acoustic attenuation and inconspicuous enhancement. Additionally, in this study 15 recurrences were missed on CEUS and it was unable to figure out definite reason in comparison with CECT.

It was notable that the misinterpreted abnormal hepatic blood vessels due to the presence of hepatic blood vessel enhancement in the vicinity of suspicious lesion (Figure 4) was the main reason of the false positive results

Table 4 Number of new intrahepatic recurrence detected by follow-up contrast-enhanced ultrasound and contrast-enhanced computed tomography

CEUS	CECT						
N	0	1	2	3	4	5	Total
0	69 (0/0)	13 (0/13)	7 (0/14)	1 (0/3)	0 (0/0)	0 (0/0)	90 (0/30)
1	4 (4/0)	25 (25/25)	9 (9/18)	8 (8/24)	1 (1/4)	0 (0/0)	47 (47/71)
2	2 (4/0)	5 (10/5)	8 (16/16)	3 (6/9)	0 (0/0)	0 (0/0)	18 (36/30)
3	0 (0/0)	0 (0/0)	0 (0/0)	7 (21/21)	1 (3/4)	1 (3/5)	9 (27/30)
4	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	3 (12/12)	1 (4/5)	4 (16/17)
5	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	0 (0/0)	1 (5/5)	1 (5/5)
Total	75 (8/0)	43 (35/43)	24 (25/48)	19 (35/57)	5 (16/20)	3 (12/15)	169 (131/183)

N represents the number of the detected recurrence in each patient. Data are the numbers of follow-up examinations and data in parenthesis are the numbers of new intrahepatic recurrences (detected on CEUS/CECT). CEUS: Contrast-enhanced ultrasound; CECT: Contrast-enhanced computed tomography.

on CEUS, which mainly involved arteriovenous shunting or marginally enhanced artifact of large vessel and may be caused by the ablation or severe liver cirrhosis. The misinterpreted hepatic blood vessels usually showed hyperenhancement in the arterial phase and iso-enhancement in the portal-late phase on CEUS[13,34]. In this study, a total of 14 misinterpreted hepatic blood vessels were misdiagnosed as false positive LTPs (83.3%, 5/6) or new intrahepatic recurrence (69.2%, 9/13) by CEUS.

There are some limitations in this study: firstly, although the study was designed prospectively, not all the HCC patients who underwent ablation therapy received follow-up assessment by this paired CEUS and CECT examinations due to the low compliance of the patients, which may lead to bias of patient selection. Secondly, CECT was used as the reference standard in this study and not all the detected LTPs and new intrahepatic recurrences were confirmed by pathology. However, it was hard to obtain pathological results for all the lesions found in the follow-up due to the ethical concern and under current situation CECT is still acceptable to be used as the standard. Thirdly, we did not evaluate the role of CEUS using different ablation techniques. There might be some differences of the incidence of LTP or new intrahepatic recurrence between different ablation techniques such as RFA and EA, *etc*., because they have different efficacies in treating HCC. However, the role of the study was not to evaluate the treatment efficacy of different ablation therapies, but to evaluate the ability of CEUS in treatment response assessment. In theory, the viable tumor tissue will show arterial hypervascularity on CEUS, whether it is residual tumor tissue or the recurrent tumor, and whether it is after RFA or after EA. In addition, the number of patients undergoing EA was small. Finally, further prospective study with a large number of cases is necessary to confirm the results of the present study and to evaluate the real value of CEUS in the follow-up.

In conclusion, the sensitivity of CEUS in detecting LTP and new intrahepatic recurrence after percutaneous ablation therapy is relatively low in comparison with CECT. CEUS cannot replace CECT in the follow-up assessment after percutaneous ablation for HCC.

COMMENTS

Background

For the patients with hepatocellular carcinoma (HCC) after percutaneous ablation therapy, the regular follow-up after ablation can detect local tumor progression (LTP) and new recurrence as early as possible, so as to facilitate further treatment in time, and therefore can benefit the survival. Thus the follow-up assessment, similar to surveillance and diagnosis, plays a key role in the management of HCC. However, at present, only contrast-enhanced computed tomography (CECT) and contrast-enhanced magnetic resonance imaging (CEMRI), are recommended as accurate and reliable imaging tools and applied to the follow-up assessment. Unfortunately, some factors, such as high cost, radiation and side effect of agents, limit its application.

Research frontiers

Contrast-enhanced ultrasound (CEUS) is a new imaging technique developed in recent decade. A lot of previous studies have demonstrated that CEUS is comparable to CECT and CEMRI in the area of characterization and treatment response assessment of HCC. Regarding the role of CEUS in follow-up assessment for HCC after ablation, most studies just focus on targeted lesion assessment and seldom investigate its capability of detecting LTP and new intrahepatic recurrence by scanning whole liver. Whether CEUS can be competent to this follow-up assessment is still controversial.

Innovations and breakthroughs

In most of the previous studies, CEUS has a good performance for treatment response assessment after HCC ablation, while just for the specific lesions. In this study, the authors aimed to investigate the ability of CEUS by scanning whole liver for detecting the LTPs and new intrahepatic recurrences, most of which are unknown in number, size and location, *etc*. It is concluded that CEUS is inferior to CECT in the follow-up assessment of HCC after ablation, which is mainly due to the innate defect of CEUS, such as limited acoustic penetration, display scope and relatively short duration of artery phase. Additionally, the incompetence of CEUS in the follow-up assessment might result from some traits of LTPs and new intrahepatic recurrences after HCC ablation, such as its small size, deep location, and atypical enhancement patterns.

Applications

The study results suggest that in follow-up assessment after HCC ablation CEUS can not take place of CECT and CEMRI for whole liver scanning, but can act as an adjuvant imaging tool for assessing the specific lesions.

Terminology

Treatment response assessment is performed in a month after HCC ablation by using CECT or CEMRI to assess the efficacy of ablation. Follow-up assessment: after complete ablation of HCC is confirmed by treatment response assessment, the patients will be follow-up regularly for monitoring the progression and recurrence.

Peer review

In this prospective study, the authors investigated extensively the role of CEUS in the follow-up of HCC patients undergoing radiofrequency ablations with CECT as the reference standard. The conclusion drawn by the authors is that the ability of CEUS in detecting LTP and new intrahepatic recurrence after percutaneous abla-

tion therapy is inferior to CECT. This is the first study to evaluate the role of CEUS in the follow-up assessment after percutaneous ablation therapy for HCC and the results are relevant and objective. The conclusions are very important, which indicate that people should not overestimate the role of CEUS in the follow-up even though it is meaningful in local treatment evaluation.

REFERENCES

1 **Parkin DM**, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108 [PMID: 15761078 DOI: 10.3322/caac.20107]

2 **Bruix J**, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666 DOI: 10.1002/hep.24199]

3 **Kim SK**, Lim HK, Kim YH, Lee WJ, Lee SJ, Kim SH, Lim JH, Kim SA. Hepatocellular carcinoma treated with radiofrequency ablation: spectrum of imaging findings. *Radiographics* 2003; **23**: 107-121 [PMID: 12533646 DOI: 10.1148/rg.231025055]

4 **Xu HX**, Wang Y, Lu MD, Liu LN. Percutaneous ultrasound-guided thermal ablation for intrahepatic cholangiocarcinoma. *Br J Radiol* 2012; **85**: 1078-1084 [PMID: 22374282 DOI: 10.1259/bjr/24563774]

5 **Llovet JM**, Bruix J. Novel advancements in the management of hepatocellular carcinoma in 2008. *J Hepatol* 2008; **48** Suppl 1: S20-S37 [PMID: 18304676 DOI: 10.1016/j.jhep.2008.01.022]

6 **Livraghi T**, Meloni F, Di Stasi M, Rolle E, Solbiati L, Tinelli C, Rossi S. Sustained complete response and complications rates after radiofrequency ablation of very early hepatocellular carcinoma in cirrhosis: Is resection still the treatment of choice? *Hepatology* 2008; **47**: 82-89 [PMID: 18008357 DOI: 10.1002/hep.21933]

7 **Tiong L**, Maddern GJ. Systematic review and meta-analysis of survival and disease recurrence after radiofrequency ablation for hepatocellular carcinoma. *Br J Surg* 2011; **98**: 1210-1224 [PMID: 21766289 DOI: 10.1002/bjs.7669]

8 **Salvaggio G**, Campisi A, Lo Greco V, Cannella I, Meloni MF, Caruso G. Evaluation of posttreatment response of hepatocellular carcinoma: comparison of ultrasonography with second-generation ultrasound contrast agent and multidetector CT. *Abdom Imaging* 2010; **35**: 447-453 [PMID: 19562414 DOI: 10.1007/s00261-009-9551-6]

9 **Solbiati L**, Ierace T, Tonolini M, Cova L. Guidance and monitoring of radiofrequency liver tumor ablation with contrast-enhanced ultrasound. *Eur J Radiol* 2004; **51** Suppl: S19-S23 [PMID: 15311434]

10 **Sparchez Z**, Radu P, Anton O, Socaciu M, Badea R. Contrast enhanced ultrasound in assessing therapeutic response in ablative treatments of hepatocellular carcinoma. *J Gastrointestin Liver Dis* 2009; **18**: 243-248 [PMID: 19565061]

11 **Lu MD**, Yu XL, Li AH, Jiang TA, Chen MH, Zhao BZ, Zhou XD, Wang JR. Comparison of contrast enhanced ultrasound and contrast enhanced CT or MRI in monitoring percutaneous thermal ablation procedure in patients with hepatocellular carcinoma: a multi-center study in China. *Ultrasound Med Biol* 2007; **33**: 1736-1749 [PMID: 17629608 DOI: 10.1016/j.ultrasmedbio.2007.05.004]

12 **Ricci P**, Cantisani V, Drudi F, Pagliara E, Bezzi M, Meloni F, Calliada F, Erturk SM, D'Andrea V, D'Ambrosio U, Passariello R. Is contrast-enhanced US alternative to spiral CT in the assessment of treatment outcome of radiofrequency ablation in hepatocellular carcinoma? *Ultraschall Med* 2009; **30**: 252-258 [PMID: 19280552 DOI: 10.1055/s-2008-1027727]

13 **Vilana R**, Bianchi L, Varela M, Nicolau C, Sánchez M, Ayuso C, García M, Sala M, Llovet JM, Bruix J, Bru C. Is microbubble-enhanced ultrasonography sufficient for assessment of response to percutaneous treatment in patients with early hepatocellular carcinoma? *Eur Radiol* 2006; **16**: 2454-2462 [PMID: 16710666 DOI: 10.1007/s00330-006-0264-8]

14 **Xu HX**, Lu MD, Liu LN, Zhang YF, Guo LH, Xu JM, Liu C. Discrimination between neoplastic and non-neoplastic lesions in cirrhotic liver using contrast-enhanced ultrasound. *Br J Radiol* 2012; **85**: 1376-1384 [PMID: 22553290 DOI: 10.1259/bjr/19932596]

15 **Claudon M**, Cosgrove D, Albrecht T, Bolondi L, Bosio M, Calliada F, Correas JM, Darge K, Dietrich C, D'Onofrio M, Evans DH, Filice C, Greiner L, Jäger K, Jong Nd, Leen E, Lencioni R, Lindsell D, Martegani A, Meairs S, Nolsøe C, Piscaglia F, Ricci P, Seidel G, Skjoldbye B, Solbiati L, Thorelius L, Tranquart F, Weskott HP, Whittingham T. Guidelines and good clinical practice recommendations for contrast enhanced ultrasound (CEUS) - update 2008. *Ultraschall Med* 2008; **29**: 28-44 [PMID: 18270887 DOI: 10.1055/s-2007-963785]

16 **Xu HX**, Chen LD, Xie XY, Xie XH, Xu ZF, Liu GJ, Lin MX, Wang Z, Lu MD. Enhancement pattern of hilar cholangiocarcinoma: contrast-enhanced ultrasound versus contrast-enhanced computed tomography. *Eur J Radiol* 2010; **75**: 197-202 [PMID: 19464836 DOI: 10.1016/j.ejrad.2009.04.060]

17 **Xu HX**, Xie XY, Lu MD, Liu GJ, Xu ZF, Zheng YL, Liang JY, Chen LD. Contrast-enhanced sonography in the diagnosis of small hepatocellular carcinoma < or = 2 cm. *J Clin Ultrasound* 2008; **36**: 257-266 [PMID: 18088056 DOI: 10.1002/jcu.20433]

18 **Liu GJ**, Xu HX, Lu MD, Xie XY, Xu ZF, Zheng YL, Liang JY. Correlation between enhancement pattern of hepatocellular carcinoma on real-time contrast-enhanced ultrasound and tumour cellular differentiation on histopathology. *Br J Radiol* 2007; **80**: 321-330 [PMID: 17005515 DOI: 10.1259/bjr/54805002]

19 **Liu GJ**, Xu HX, Lu MD, Xie XY, Xu ZF, Zheng YL, Liang JY. Enhancement pattern of hepatocellular carcinoma: comparison of real-time contrast-enhanced ultrasound and contrast-enhanced computed tomography. *Clin Imaging* 2006; **30**: 315-321 [PMID: 16919551 DOI: 10.1016/j.clinimag.2006.03.031]

20 **Kim CK**, Choi D, Lim HK, Kim SH, Lee WJ, Kim MJ, Lee JY, Jeon YH, Lee J, Lee SJ, Lim JH. Therapeutic response assessment of percutaneous radiofrequency ablation for hepatocellular carcinoma: utility of contrast-enhanced agent detection imaging. *Eur J Radiol* 2005; **56**: 66-73 [PMID: 15913940 DOI: 10.1016/j.ejrad.2005.03.023]

21 **Xu HX**, Lu MD. Comparison between radiofrequency ablation and percutaneous microwave coagulation therapy for small hepatocellular carcinomas — a reply. *Clin Radiol* 2006; **61**: 801-802 [DOI: 10.1016/j.crad.2006.05.005]

22 **Lu MD**, Yin XY, Xie XY, Xu HX, Xu ZF, Liu GJ, Kuang M, Zheng YL. Percutaneous thermal ablation for recurrent hepatocellular carcinoma after hepatectomy. *Br J Surg* 2005; **92**: 1393-1398 [PMID: 16044409 DOI: 10.1002/bjs.5102]

23 **Wong SN**, Lin CJ, Lin CC, Chen WT, Cua IH, Lin SM. Combined percutaneous radiofrequency ablation and ethanol injection for hepatocellular carcinoma in high-risk locations. *AJR Am J Roentgenol* 2008; **190**: W187-W195 [PMID: 18287411 DOI: 10.2214/AJR.07.2537]

24 **Luo BM**, Wen YL, Yang HY, Zhi H, Xiao XY, Ou B, Pan JS, Ma JH. Percutaneous ethanol injection, radiofrequency and their combination in treatment of hepatocellular carcinoma. *World J Gastroenterol* 2005; **11**: 6277-6280 [PMID: 16419155]

25 **Mahnken AH**, Bruners P, Günther RW. Local ablative therapies in HCC: percutaneous ethanol injection and radiofrequency ablation. *Dig Dis* 2009; **27**: 148-156 [PMID: 19546553 DOI: 10.1159/000218347]

26 **Kuang M**, Lu MD, Xie XY, Xu HX, Mo LQ, Liu GJ, Xu ZF, Zheng YL, Liang JY. Liver cancer: increased microwave delivery to ablation zone with cooled-shaft antenna--experimental and clinical studies. *Radiology* 2007; **242**: 914-924 [PMID: 17229876 DOI: 10.1148/radiol.2423052028]

27 **Kuang M**, Lu MD, Xie XY, Xu HX, Xu ZF, Liu GJ, Yin XY, Huang JF, Lencioni R. Ethanol ablation of hepatocellular carcinoma Up to 5.0 cm by using a multipronged injection needle with high-dose strategy. *Radiology* 2009; **253**: 552-561

[PMID: 19709992 DOI: 10.1148/radiol.2532082021]
28 **Bartolotta TV**, Taibbi A, Midiri M, De Maria M. Hepatocellular cancer response to radiofrequency tumor ablation: contrast-enhanced ultrasound. *Abdom Imaging* 2008; **33**: 501-511 [PMID: 17786507 DOI: 10.1007/s00261-007-9294-1]
29 **Jang HJ**, Yu H, Kim TK. Contrast-enhanced ultrasound in the detection and characterization of liver tumors. *Cancer Imaging* 2009; **9**: 96-103 [PMID: 19933022 DOI: 10.1102/1470-7330.2009.0015]
30 **Hoeffel C**, Job L, Ladam-Marcus V, Vitry F, Cadiot G, Marcus C. Detection of hepatic metastases from carcinoid tumor: prospective evaluation of contrast-enhanced ultrasonography. *Dig Dis Sci* 2009; **54**: 2040-2046 [PMID: 19034651 DOI: 10.1007/s10620-008-0570-x]
31 **Larsen LP**, Rosenkilde M, Christensen H, Bang N, Bolvig L, Christiansen T, Laurberg S. Can contrast-enhanced ultrasonography replace multidetector-computed tomography in the detection of liver metastases from colorectal cancer? *Eur J Radiol* 2009; **69**: 308-313 [PMID: 18068925 DOI: 10.1016/j.ejrad.2007.10.023]
32 **Correas JM**, Low G, Needleman L, Robbin ML, Cosgrove D, Sidhu PS, Harvey CJ, Albrecht T, Jakobsen JA, Brabrand K, Jenett M, Bates J, Claudon M, Leen E. Contrast enhanced ultrasound in the detection of liver metastases: a prospective multi-centre dose testing study using a perfluorobutane microbubble contrast agent (NC100100). *Eur Radiol* 2011; **21**: 1739-1746 [PMID: 21479856 DOI: 10.1007/s00330-011-2114-6]
33 **Nicolau C**, Catalá V, Vilana R, Gilabert R, Bianchi L, Solé M, Pagés M, Brú C. Evaluation of hepatocellular carcinoma using SonoVue, a second generation ultrasound contrast agent: correlation with cellular differentiation. *Eur Radiol* 2004; **14**: 1092-1099 [PMID: 15007620 DOI: 10.1007/s00330-004-2298-0]
34 **Pompili M**, Riccardi L, Covino M, Barbaro B, Di Stasi C, Orefice R, Gasbarrini G, Rapaccini GL. Contrast-enhanced gray-scale harmonic ultrasound in the efficacy assessment of ablation treatments for hepatocellular carcinoma. *Liver Int* 2005; **25**: 954-961 [PMID: 16162152 DOI: 10.1111/j.1478-3231.2005.01135.x]
35 **Lencioni R**, Piscaglia F, Bolondi L. Contrast-enhanced ultrasound in the diagnosis of hepatocellular carcinoma. *J Hepatol* 2008; **48**: 848-857 [PMID: 18328590 DOI: 10.1016/j.jhep.2008.02.005]
36 **Bolondi L**, Gaiani S, Celli N, Golfieri R, Grigioni WF, Leoni S, Venturi AM, Piscaglia F. Characterization of small nodules in cirrhosis by assessment of vascularity: the problem of hypovascular hepatocellular carcinoma. *Hepatology* 2005; **42**: 27-34 [PMID: 15954118 DOI: 10.1002/hep.20728]

P- Reviewers Chen F, Xu HX **S- Editor** Song XX
L- Editor Ma JY **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i6.866

World J Gastroenterol 2013 February 14; 19(6): 866-873
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

ORIGINAL ARTICLE

Interference of suppressor of cytokine signaling 3 promotes epithelial-mesenchymal transition in MHCC97H cells

Yuan-Yuan Ji, Zhi-Dong Wang, Zong-Fang Li, Ke Li

Yuan-Yuan Ji, Ke Li, Scientific Research Center, the Second Affiliated Hospital, Xi'an Jiaotong University School of Medicine, Xi'an 710004, Shaanxi Province, China
Zhi-Dong Wang, Zong-Fang Li, Department of General Surgery and Engineering Research Center of Biotherapy and Translation Medicine, Shaanxi Province, the Second Affiliated Hospital, Xi'an Jiaotong University School of Medicine, Xi'an 710004, Shaanxi Province, China
Author contributions: Ji YY and Wang ZD designed the research and contributed equally to this work; Ji YY and Wang ZD performed the research; Ji YY, Wang ZD and Li K contributed to materials and methods; Ji YY and Li K analyzed the data; Ji YY, Wang ZD and Li ZF wrote the manuscript.
Supported by Program for Changjiang Scholars and Innovative Research Team in Universities, PCSIRT No. 1171; National Natural Science Foundation of China, No. 81201925 and No. 81001588; Specialized Research Fund of the Second Affiliated Hospital of Xi'an Jiaotong University School of Medicine of China, No. RC(XM)201108; and the Fundamental Research Funds for the Central Universities, No. 08143048
Correspondence to: Zhi-Dong Wang, MD, PhD, Department of General Surgery and Engineering Research Center of Biotherapy and Translation Medicine, Shaanxi Province, the Second Affiliated Hospital, Xi'an Jiaotong University School of Medicine, No. 157, West 5th Road, Xi'an 710004, Shaanxi Province, China. xawzd@163.com
Telephone: +86-29-87679386 Fax: +86-29-87679386
Received: September 18, 2012 Revised: December 12, 2012
Accepted: December 22, 2012
Published online: February 14, 2013

Abstract

AIM: To investigate the role of suppressor of cytokine signaling 3 (SOCS3) silencing in epithelial-mesenchymal transition (EMT) involved in a human hepatocellular carcinoma MHCC97H cell line.

METHODS: MHCC97H cells were transiently transfected with SOCS3 small-interfering RNA (siRNA). Morphological changes of the transfected cells were observed under microscope. Expressions of E-cadherin, Vimentin and α-smooth muscle actin (α-SMA) were identified with immunofluorescence. Furthermore, protein expressions and mRNA levels of characteristic markers of EMT (E-cadherin, Vimentin, α-SMA and Snail) were detected by Western blotting, quantitative real-time polymerase chain reaction. Transforming growth factor-β1 (TGF-β1) levels in the supernatant were measured with enzyme-linked immunosorbent assay.

RESULTS: The transfected cells with SOCS3 siRNA showed a morphological alteration from a typical cobblestone morphology to mesenchymal spindle-shaped and fusiform features. SOCS3 siRNA lessened immunofluorescent expression of E-cadherin, but elicited immunofluorescent expressions of Vimentin and α-SMA in MHCC97H cells. More importantly, compared with the negative control, depletion of SOCS3 resulted in the decrease of the epithelial marker E-cadherin ($P < 0.05$), and the increase of the mesenchymal markers Vimentin and α-SMA and the transcription factor Snail in MHCC97H cells ($P < 0.05$). Moreover, compared with the negative control, SOCS3 siRNA evidently enhanced TGF-β1 secretion in MHCC97H cells (200.20 ± 29.02 pg/mL *vs* 490.20 ± 92.43 pg/mL, $P < 0.05$).

CONCLUSION: SOCS3 silencing is able to promote EMT in MHCC97H cells *via* changing the phenotypic characteristics and modulating the characteristic markers.

© 2013 Baishideng. All rights reserved.

Key words: Hepatocellular carcinoma; Epithelial-mesenchymal transition; Suppressor of cytokine signaling; E-cadherin; Snail

Ji YY, Wang ZD, Li ZF, Li K. Interference of suppressor of cytokine signaling 3 promotes epithelial-mesenchymal transition in MHCC97H cells. *World J Gastroenterol* 2013; 19(6): 866-873 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i6/866.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i6.866

INTRODUCTION

Hepatocellular carcinoma (HCC), the primary malignancy of the liver, ranks the sixth in incidence and the third in cancer-related deaths worldwide[1]. Poor prognosis of HCC is associated with a high potential of vascular invasion, metastasis, and recurrence even after curative surgical resection[2]. The main cause of death in HCC patients is intrahepatic metastasis, but the underlying mechanism is still not fully understood. Epithelial-mesenchymal transition (EMT) is the morphological and molecular change that occurs when epithelial cells lose their characteristics, gain mesenchymal properties and become motile, which is a key event in tumor invasion and metastasis[3-5]. The most common character of EMT is that the cells turn to spindle-like morphology from compact and well-arranged epithelial structure[6]. The epithelial marker E-cadherin plays a central role in cell-cell adhesion junctions in maintenance of cell polarity[7-9]. Loss of E-cadherin expression is commonly related to tumor invasiveness, metastasis and poor prognosis, including HCC[8]. Furthermore, the expression of mesenchymal markers such as vimentin and α-smooth muscle actin (α-SMA), along with the expression of transcription factors such as Snail is an essential molecular marker of EMT[10]. Snail is able to repress E-cadherin and overexpression of Snail has been reported to be correlated with HCC metastasis through the induction of EMT[11,12]. Moreover, the levels of transforming growth factor-β1 (TGF-β1) are very high in many cancer cells, which are related to EMT and a high incidence of metastasis[4]. In particular, TGF-β1 induced-EMT has been found to be associated with HCC invasion and metastasis[13]. EMT is being increasingly recognized as a crucial step that promotes tumor invasiveness and metastasis, thus understanding the influential factor of EMT could allow the development of novel therapies targeting at HCC invasion and metastasis.

Suppressor of cytokine signaling (SOCS) family proteins have been implicated in the negative regulation of various cytokines[14,15]. Recently, emerging evidence suggests that SOCS may be tumor suppressors. It has been postulated that SOCS can decelerate or inhibit the progression of cirrhosis and HCC[16,17]. Intriguingly, SOCS3 silencing is a significant predictor or poor survival indicating that SOCS3 might play a special role in limiting late-stage HCC progression[18]. Although SOCS3 may be involved in the suppression of tumor growth and metastasis of HCC[19,20], its role in hepatoma carcinoma cells has not been completely established. Thus, it will be of interest to learn more about whether SOCS3 depletion is able to promote EMT of HCC. The objectives of the present study were to primarily investigate the role of SOCS3 silencing in EMT involved in MHCC97H cells.

MATERIALS AND METHODS

Reagents

Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were purchased from Gibco BRL (Carlsbad, CA, United States). Polyclonal anti-human E-cadherin, Vimentin, Snail, SOCS3, anti-α-SMA and anti-β-SMA antibodies were products of Santa Cruz Biotechnology (Santa Cruz, CA, United States). Human TGF-β1 enzyme-linked immunosorbent assay (ELISA) kit was obtained from Invitrogen (Carlsbad, CA, United States). siRNA specific for SOCS3 (siGENOME SMARTpool, M-004299-08-0005) and negative control siRNA (siGENOME Non-Targeting siRNA Pool, D-001206-13-05), siGLO Green (6-FAM) Transfection Indicator (D-001630-01-05), DharmaFECT 4 transfection reagent (T-2002-04), and siGLO Green (6-FAM) Transfection Indicator (D-001630-01-05) were purchased from Dharmacon (Lafayette, CO, United States)

Cell culture

Human MHCC97H cell, a typical HCC cell line with a high metastatic potential[21], was obtained from Liver Cancer Institute of Fudan University (Shanghai, China). MHCC97H cells were cultured with DMEM supplemented with 10% FBS in a humidified incubator at 5% CO_2 and 37 ℃.

Small-interfering RNA transfection

MHCC97H cells (1×10^5) were seeded into 6-well plates and were grown until 60%-80% confluent. The cells were transiently transfected with 25 nmol/L of SOCS3 small-interfering RNA (siRNA) or negative control siRNA (NC siRNA) using Dharma FECT 4 transfection reagents according to the manufacturer's instructions. After 24 h, fluorescent images of transfected cells were observed under fluorescence microscope (Nikon, Japan). After 48 h, protein expression and mRNA levels of SOCS3 were detected by Western blotting, quantitative real-time polymerase chain reaction (PCR) and reverse transcription (RT)-PCR. Transfection rates of 60%-70% of the cells were accepted for all the experiments.

Examination of morphological changes

After application of NC siRNA or SOCS3 siRNA for 48 h, morphological changes of the transfected cells were observed under an inverted phase-contrast microscope (Nikon, Japan). The photographs were taken at 100× and 200× magnifications by a digital camera.

Immunofluorescence

MHCC97H cells were plated on cover slips and grown to confluence, and were transiently transfected with 25 nmol/L of SOCS3 siRNA or NC siRNA for 48 h. After the treatment, the cells were fixed with 4% formaldehyde-PBS for 15 min. The cell membranes were fenestrated with 0.3% Triton-100-PBS, and nonspecific binding sites were blocked with 10% goat serum. The cells were incubated with anti-human antibody including E-cadherin (1:100) or Vimentin (1:200) or α-SMA (1:100) and then incubated with the secondary antibody conjugated to fluorescein isothiocyanate. The immunolabeled cells were observed under fluorescence microscope (Nikon, Japan).

Table 1 Primers used for real-time polymerase chain reaction analysis

Gene	Primer sequence	Accession number	Expected size (bp)
E-cadherin	5′-CCCGGGACAACGTTTATTAC-3′ 5′-GCTGGCTCAAGTCAAAGTCC-3′	NM_004360.3	190
Vimentin	5′-AAAGTGTGGCTGCCAAGAAC-3′ 5′-AGCCTCAGAGAGGTCAGCAA-3′	NM_003380.2	200
α-SMA	5′-GCGCAAATACTCGGTGTGGA-3′ 5′-CCCCCCCATTGAGAAGATTC-3′	NM_001141945.1	170
Snail	5′-TGGTTGCTTCAAGGACACAT-3′ 5′-GTTGCAGTGAGGGCAAGAA-3′	NM_003068.3	141
SOCS3	5′-CAGGAATGTAGCAGCGATGGAA-3′ 5′-CCTGTCCAGCCCAATACCTGA-3′	NM_003955.3	125
β-actin	5′-ATCGTGCGTGACATTAAGGAGAAG-3′ 5′-AGGAAGGAAGGCTGGAAGAGTG -3′	NM_001101	179

α-SMA: α-smooth muscle actin; SOCS3: Suppressor of cytokine signaling 3.

ELISA

After application of NC siRNA or SOCS3 siRNA for 48 h, concentration of TGF-β1 in the supernatant of the cells was measured by ELISA kits according to the manufacturer's instructions.

Western blotting analysis

As described previously[22], protein samples (25 μg) were separated on sodium dodecyl sulfate polyacrylamide gel electrophoresis gels and transferred onto a polyvinylidene difluoride membrane (Bio-Rad Laboratories, Hercules, CA, United States). The membranes were blocked with 5% nonfat dry milk in Tris-buffered saline containing 0.1% Tween 20, and incubated with specific antibodies against E-cadherin (1:200), Vimentin (1:200), Snail (1:100), SOCS3 (1:200), α-SMA (1:400) and β-actin (1:400). The expression of β-actin was used as a loading control. Reagents (Pierce Corp., Rockford, IL, United States) for the enhanced chemiluminescence were applied to the blots, and the light signals were detected by X-ray film. Optical densities of the bands were scanned and quantified with the Syngene Gene Tools (Syngene Corp., Cambridge, United Kingdom). Three independent experiments were carried out to study protein expression.

Quantitative real-time PCR and RT-PCR

mRNA levels were determined by our previous method[22]. Total RNA was isolated using a TRIzol Kit (Invitrogen Corp., Carlsbad, CA, United States). cDNA was synthesized from 1 μg samples of total RNA using Revert Aid™ First Strand cDNA Synthesis Kit (Fermentas, St. Leon-Rot, Germany) following the manufacturer's instructions. Real-time PCR was performed with the SYBR Premix Ex Taq™ Ⅱ Perfect Real Time kit (Takara, Japan) on an ABI QPCR System (Applied Biosystems, CA, United States) following the manufacturer's instructions. The samples were run in triplicate. Primers for human E-cadherin, Vimentin, Snail, SOCS3, α-actin and β-actin were designed with Beacon designer v 4.0 (Premier Biosoft, United States) (Table 1). Traditional PCR was performed according to the manufacturer's instructions. The RT-PCR products were analyzed by electrophoresis through 2% agarose gels containing ethidium bromide. A melting point dissociation curve generated by the instrument was used to confirm that only a single product was present. Quantization of relative gene expression was calculated by the comparative Ct method ($2^{-\Delta\Delta CT}$) as described by the manufacturer. Data were normalized to human β-actin mRNA levels. Three independent experiments were carried out to study mRNA levels.

Statistical analysis

All data were expressed as the mean ± SD from three different experiments. Statistical analysis were carried out with Student's *t* test for independent samples. In all cases, a value of $P < 0.05$ was considered statistically significant.

RESULTS

Fluorescent detection of transfected cells

After MHCC97H cells were transiently transfected with SOCS3 siRNA (25 nmol/L) and siGLO Green Transfection Indicator (50 nmol/L) for 24 h, fluorescent expression was observed under fluorescence microscope. Compared with the control, the transfected cells with SOCS3 siRNA showed a green fluorescence (Figure 1). The results illustrated that SOCS3 siRNA had been successfully transfected into MHCC97H cells.

Effect of SOCS3 siRNA on morphological changes of MHCC97H cells

To investigate the role of SOCS3 in morphological changes of MHCC97H cells, the cells were transiently transfected with NC siRNA or SOCS3 siRNA for 48 h. As shown in Figure 2, knockdown of SOCS3 resulted in a significant change in cell morphology, as demonstrated by phase-contrast microscopy, with transition from a typical cobblestone morphology to mesenchymal spindle-shaped and fusiform features. The acquisition of a fibroblastic morphology suggested that MHCC97H cells could undergo the mesenchymal change after treated with SOCS3 siRNA.

Effect of SOCS3 siRNA on immunofluorescent expressions of E-cadherin, Vimentin and α-SMA in MHCC97H cells

To examine the effect of SOCS3 on immunofluorescent expressions of E-cadherin, Vimentin and α-SMA in MHCC97H cells, the cells were transiently transfected with NC siRNA or SOCS3 siRNA for 48 h, and expressions of E-cadherin, Vimentin and α-SMA were identified with immunocytofluorescence. The results showed that SOCS3 siRNA might lessen immunofluorescent expression of E-cadherin, but elicit immunofluorescent expressions of Vimentin and α-SMA in MHCC97H cells (Figure 3).

Effect of SOCS3 siRNA on characteristic markers of EMT in MHCC97H cells

To further explore the impact of SOCS3 on characteristic markers of EMT in MHCC97H cells, the cells were

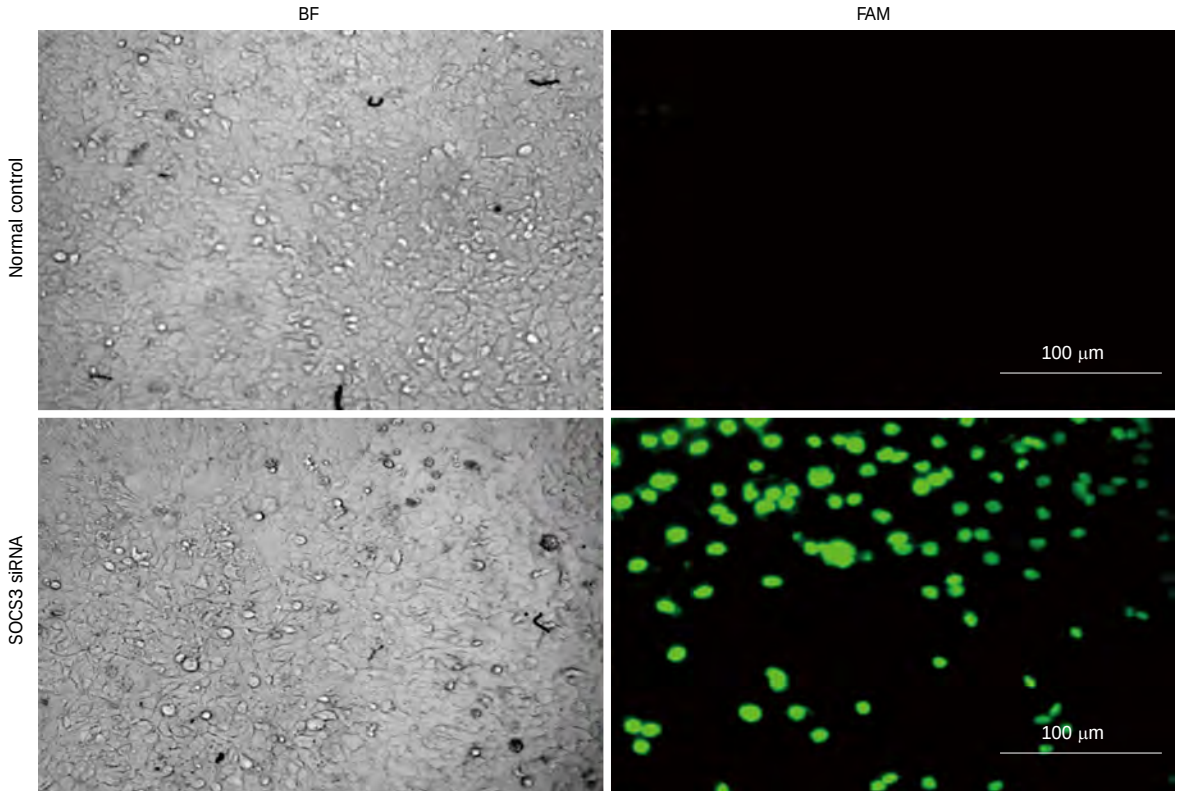


Figure 1 Fluorescent detection of transfected cells. MHCC97H cells were transiently transfected with suppressor of cytokine signaling 3 (SOCS3) small-interfering RNA (siRNA) for 24 h, green fluorescence was observed under fluorescence microscope. BF: Bright field; FAM: Fluorescence from fluorescein-labeled scramble-siRNA.

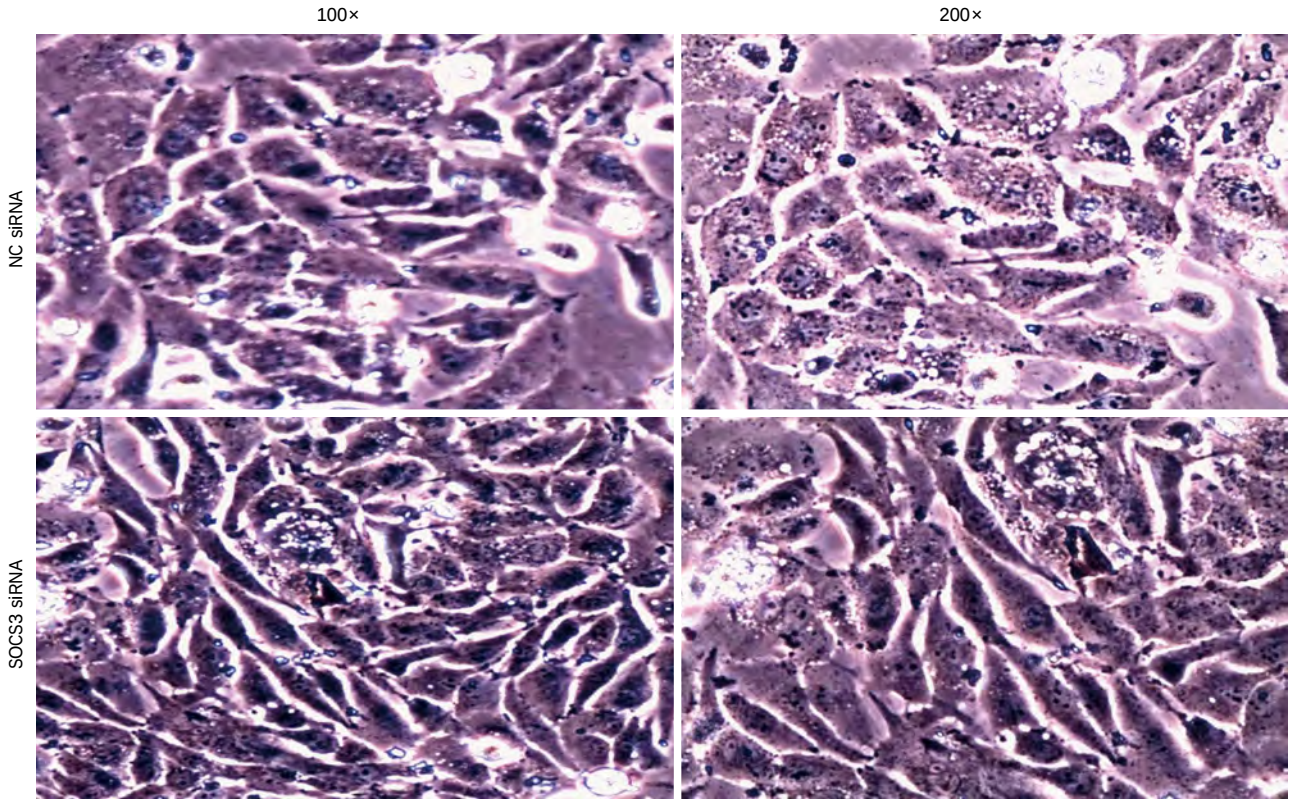


Figure 2 Effect of suppressor of cytokine signaling 3 small-interfering RNA on morphological changes of MHCC97H cells. After application of negative control (NC) small-interfering RNA (siRNA) or suppressor of cytokine signaling 3 (SOCS3) siRNA for 48 h, morphological changes of cells were recorded with an inverted phase-contrast microscope.

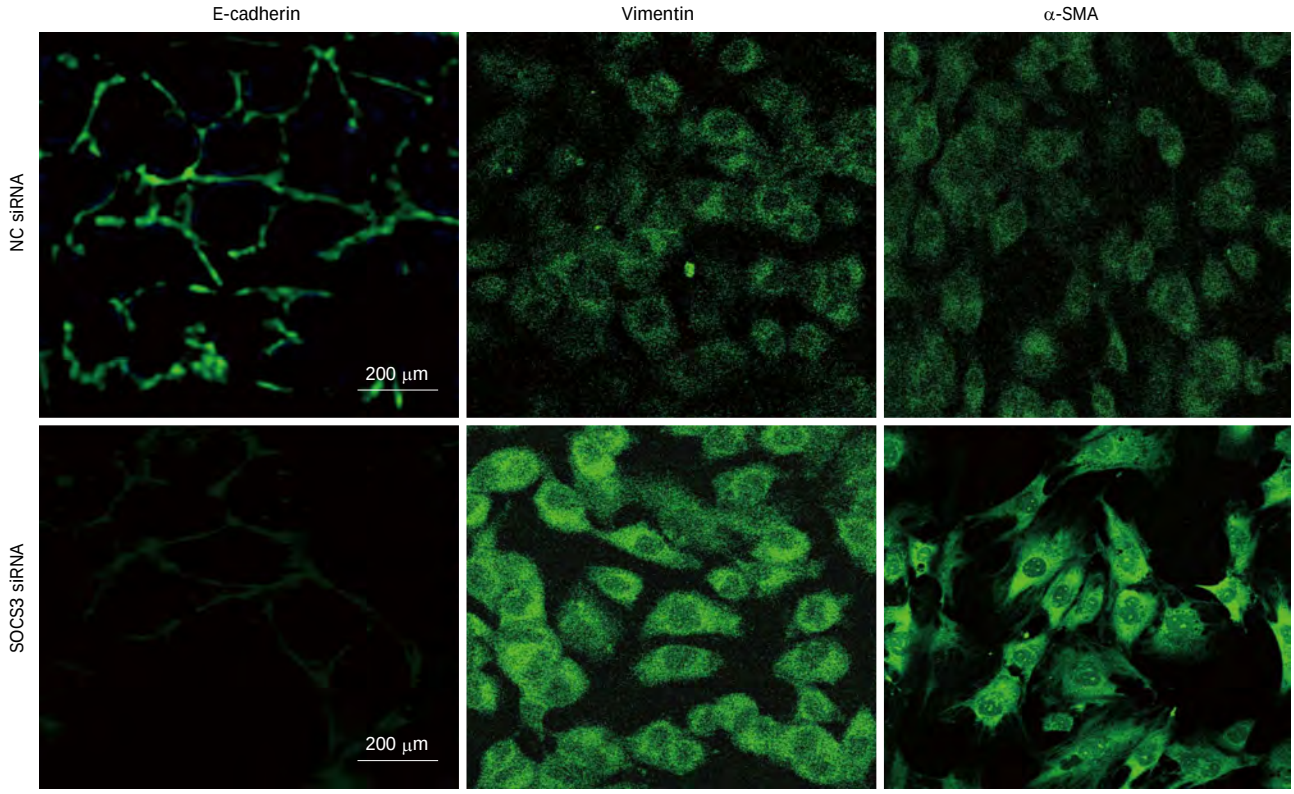


Figure 3 Effect of suppressor of cytokine signaling 3 small-interfering RNA on immunofluorescent expressions of E-cadherin, Vimentin and α-smooth muscle actin in MHCC97H cells. The cells were transiently transfected with negative control (NC) small-interfering RNA (siRNA) or suppressor of cytokine signaling 3 (SOCS3) siRNA for 48 h, expressions of E-cadherin, Vimentin and α-smooth muscle actin (SMA) were identified with immunofluorescence.

transiently transfected with NC siRNA or SOCS3 siRNA for 48 h, and protein expression and mRNA levels of SOCS3 were detected. Knock down efficiency of SOCS3 was 69.2% or 63.5% as determined by Western blot or quantitative real-time PCR ($P < 0.05$) (Figure 4A and B). These results showed that SOCS3 siRNA could efficiently reduce protein expression and mRNA levels of SOCS3. Meanwhile, depletion of SOCS3 resulted in the decrease of the epithelial marker E-cadherin, and the increase of the mesenchymal markers Vimentin, α-SMA and Snail in MHCC97H cells ($P < 0.05$) (Figure 4C and D).

Effect of SOCS3 siRNA on TGF-β1 levels in MHCC97H cells

The above characteristic markers of EMT (E-cadherin, Vimentin, α-SMA and Snail) modulate the process of EMT, and TGF-β1 is believed to play a major role in this process. We then observed TGF-β1 levels in MHCC97H cells after SOCS3 silencing. As described in Figure 5, compared with the negative control, depletion of SOCS3 remarkably aggravated TGF-β1 secretion in MHCC97H cells (200.20 ± 29.02 pg/mL *vs* 490.20 ± 92.43 pg/mL, $P < 0.05$), suggesting that SOCS3 is able to decrease TGF-β1 generation of MHCC97H cells.

DISCUSSION

The present study demonstrated that the transfected cells with SOCS3 siRNA showed a morphological alteration from a typical cobblestone morphology to mesenchymal spindle-shaped and fusiform features, suggesting that MHCC97H cells might adopt the mesenchymal cell phenotype. More importantly, we found that lack of SOCS3 resulted in the decrease of the epithelial marker E-cadherin, and the increase of the mesenchymal markers Vimentin and α-SMA and the expression of transcription factor Snail in MHCC97H cells. In addition, SOCS3 siRNA evidently enhanced TGF-β1 secretion in MHCC97H cells. These results reveal that interference of SOCS3 is able to facilitate EMT involved in MHCC97H cells.

SOCS3 functions as a negative regulator of the Jak/Stat signal transduction pathway and has been described as a tumor suppressor in human cancers[23,24]. It has also been reported that SOCS3 silencing plays a role in human HCC and SOCS3 has a tumor suppressor role in mouse models of HCC[25]. So far, the relationship between SOCS3 and EMT in HCC is still elusive. As a result, our present study has focused on the effect of SOCS3 silencing on EMT in MHCC97H cells. We found for the first time that SOCS silencing was associated with EMT process in MHCC97H cells because of changes of the phenotypic characteristics.

EMT is a key and dynamic process that facilitates invasion of tumor cells. In addition to changes of the phenotypic characteristics, tumor cells are reported to gain the molecular characteristics of EMT including down-regulation of epithelial marker (E-cadherin) and up-regulation of mesenchymal markers (Vimentin and α-SMA)[26].

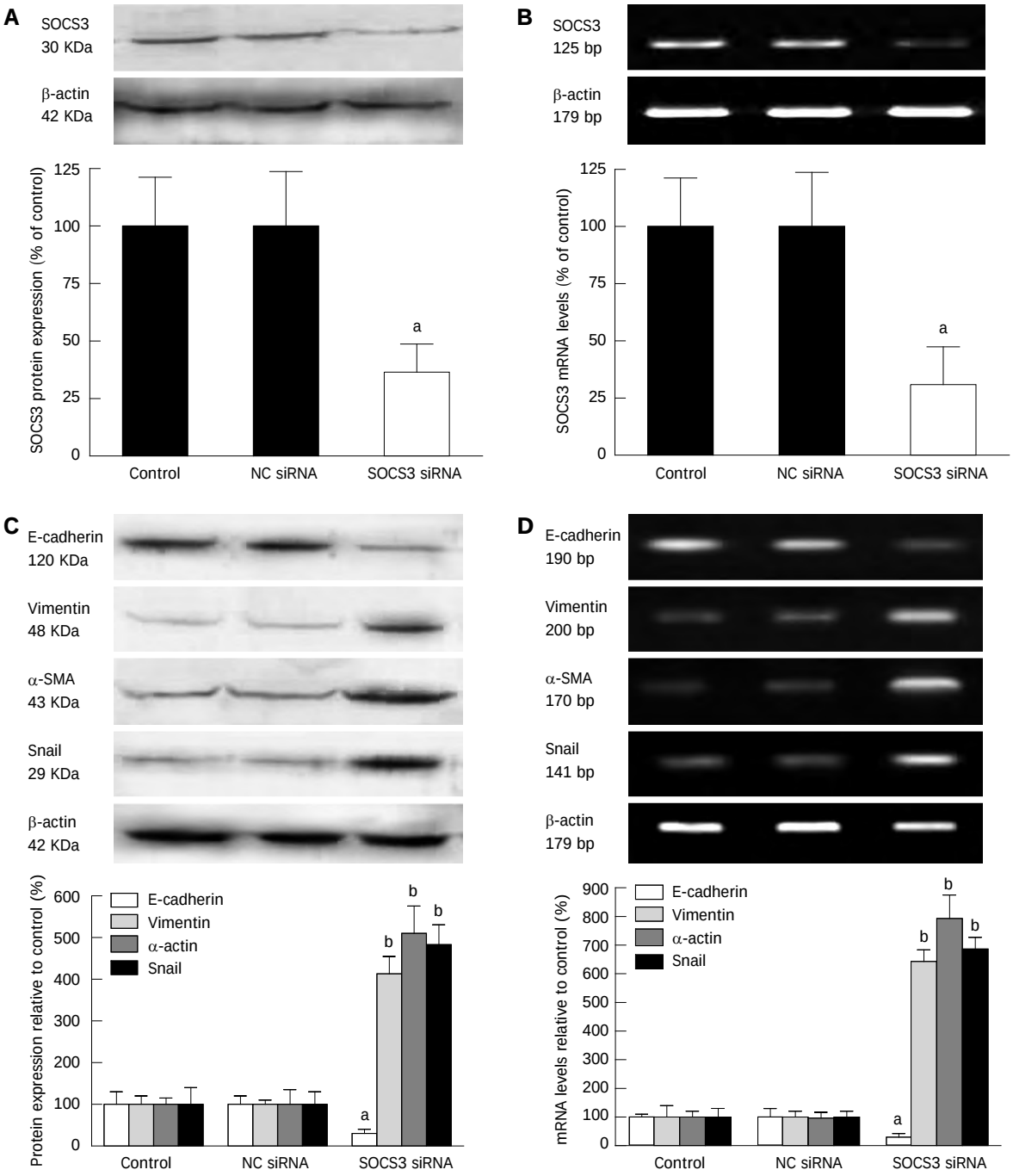


Figure 4 Effect of suppressor of cytokine signaling 3 small-interfering RNA on expression of epithelial-mesenchymal transition-associated molecules in MHCC97H cells. The cells were transiently transfected with negative control (NC) small-interfering RNA (siRNA) or suppressor of cytokine signaling 3 (SOCS3) siRNA for 48 h. A: Protein expression and mRNA levels of SOCS3 in MHCC97H cells were detected by Western blotting; B: Reverse transcription-polymerase chain reaction (RT-PCR) and quantitative real-time PCR; C: Protein expression and mRNA levels of epithelial-mesenchymal transition-associated molecules in MHCC97H cells were analyzed by Western blotting; D: RT-PCR and quantitative real-time PCR after normalization to β-actin mRNA. Data are presented as the mean ± SD from three independent experiments. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* NC siRNA.

Loss of E-cadherin and gain of Vimentin or α-SMA are the most important consequence of EMT that facilitate invasion and metastasis of tumor cells[27-29]. In the present study, transfection of MHCC97H cells with SOCS3 siRNA resulted in down-regulation of epithelial marker E-cadherin and up-regulation of mesenchymal markers Vimentin and α-SMA, further confirming the promotion of EMT by SOCS3 silencing in MHCC97H cells.

Snail has been shown to be a strong repressor of transcription of the E-cadherin gene and evokes tumorigenic and invasive properties in epithelial cells upon overexpression[30]. It has been reported that the high expression of Snail leads to EMT and enhances motility and invasiveness of tumor cells[31]. Inverse correlation of Snail and E-cadherin was detected in HCC-derived cell lines[32]. In the present study, examination of Snail expression in

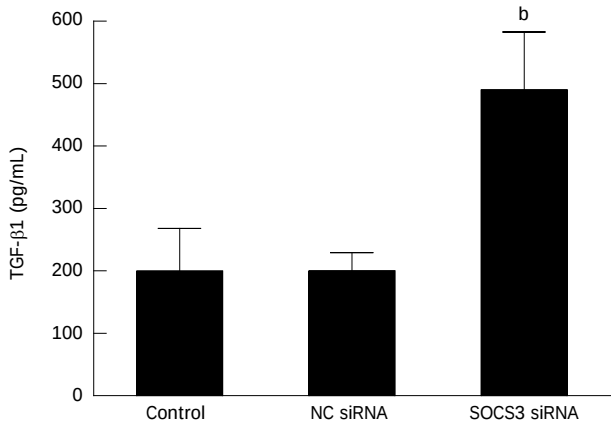


Figure 5 Effect of suppressor of cytokine signaling 3 small-interfering RNA on transforming growth factor-β1 levels in MHCC97H cells. After application of negative control (NC) small-interfering RNA (siRNA) or suppressor of cytokine signaling 3 (SOCS3) siRNA for 48 h, concentration of transforming growth factor-β1 in the supernatant of the cells was measured by enzyme-linked immunosorbent assay. Data are presented as the mean ± SD from three independent experiments. $^{b}P < 0.01$ *vs* NC siRNA. TGF: Transforming growth factor.

MHCC97H cells with SOCS3 siRNA transfection indicated a significant increase in protein expression and mRNA levels of Snail, possibly leading to loss of E-cadherin resulting in the induction of EMT. These results are consistent with the mechanism of induction of EMT[11,12].

TGF-β1 is known to be the most potent inducer of EMT, and it initiates morphological transition of the cells from an epithelial to a fibroblastic appearance, accompanied by loss of epithelial cell marker such as E-cadherin and a gain of mesenchymal cell marker such as Vimentin[33,34]. During the late stages of HCC tumorgenesis, TGF-β1 stimulates cellular invasion through the EMT program[35]. A previous study also demonstrates that TGF-β1 expression is enhanced by SOCS3 gene deletion in HCC[25]. In this work, we further detected TGF-β1 levels in MHCC97H cells transfected with SOCS3 siRNA. The results displayed that depletion of SOCS3 remarkably aggravated TGF-β1 secretion in MHCC97H cells, further illustrating the induction of EMT by SOCS3 silencing.

In conclusion, the present study provides direct evidence that SOCS3 silencing is able to promote EMT in MHCC97H cells *via* changing the phenotypic characteristics and modulating the characteristic markers, suggesting that SOCS3 could play a potential role in EMT in MHCC97H cells. We also identified a novel relationship between SOCS3 silencing and EMT in MHCC97H cells, however, further *in vitro* and *in vivo* studies are necessary to find out the exact molecular mechanism associated with the effect of SOCS3 in EMT of HCC.

COMMENTS

Background

The main cause of death of patients with hepatocellular carcinoma (HCC) is intrahepatic metastasis, but the underlying mechanism is still not fully understood. Epithelial-mesenchymal transition (EMT) is being increasingly recognized as a crucial step that promotes HCC invasiveness and metastasis. However, the role of suppressor of cytokine signaling (SOCS) in EMT involved in HCC is not well documented.

Research frontiers

This study demonstrated that depletion of SOCS3 resulted in the decrease of the epithelial marker E-cadherin, and the increase of the mesenchymal markers Vimentin and α-smooth muscle actin and the expression of transcription factor Snail in MHCC97H cells. In addition, SOCS3 small-interfering RNA evidently enhanced transforming growth factor-β1 secretion in MHCC97H cells. These results reveal that interference of SOCS3 is able to facilitate EMT involved in MHCC97H cells.

Innovations and breakthroughs

The present study provides direct evidence that SOCS3 silencing has the ability to promote EMT in MHCC97H cells *via* changing the phenotypic characteristics and modulating the characteristic markers, suggesting that SOCS3 could play a potential role in EMT in MHCC97H cells.

Applications

The authors found a novel relationship between SOCS3 silencing and EMT in HCC, thus understanding the influential factor of EMT could allow the development of novel therapies targeting at HCC invasion and metastasis.

Terminology

EMT is the morphological and molecular changes that occur when epithelial cells lose their characteristics, gain mesenchymal properties and become motile, which is a key event in tumor invasion and metastasis; The SOCS proteins are a family of negative regulatory proteins related to wide cytokine and growth correlation factor, especially by the signal molecule in the pathway of Janus kinase/signal transducer and transcription activator.

Peer review

This study presented results suggesting that SOCS3 silencing has the ability to promote EMT in MHCC97H cells. It will help to define the role of SOCS3 as a tumor suppressor in HCC. The manuscript is well presented and of interest and it can contribute to increase the knowledge of SOCS3 silencing on EMT involved in MHCC97H cell line.

REFERENCES

1 **Farazi PA**, DePinho RA. Hepatocellular carcinoma pathogenesis: from genes to environment. *Nat Rev Cancer* 2006; **6**: 674-687 [PMID: 16929323 DOI: 10.1038/nrc1934]
2 **Tung-Ping Poon R**, Fan ST, Wong J. Risk factors, prevention, and management of postoperative recurrence after resection of hepatocellular carcinoma. *Ann Surg* 2000; **232**: 10-24 [PMID: 10862190 DOI: 10.1097/00000658-200007000-00003]
3 **Lee JM**, Dedhar S, Kalluri R, Thompson EW. The epithelial-mesenchymal transition: new insights in signaling, development, and disease. *J Cell Biol* 2006; **172**: 973-981 [PMID: 16567498 DOI: 10.1083/jcb.200601018]
4 **Thiery JP**, Acloque H, Huang RY, Nieto MA. Epithelial-mesenchymal transitions in development and disease. *Cell* 2009; **139**: 871-890 [PMID: 19945376 DOI: 10.1016/j.cell.2009.11.007]
5 **Thiery JP**. Epithelial-mesenchymal transitions in tumour progression. *Nat Rev Cancer* 2002; **2**: 442-454 [PMID: 12189386 DOI: 10.1038/nrc822]
6 **Kang Y**, Massagué J. Epithelial-mesenchymal transitions: twist in development and metastasis. *Cell* 2004; **118**: 277-279 [PMID: 15294153 DOI: 10.1016/j.cell.2004.07.011]
7 **Yang J**, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C, Savagner P, Gitelman I, Richardson A, Weinberg RA. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell* 2004; **117**: 927-939 [PMID: 15210113 DOI: 10.1016/j.cell.2004.06.006]
8 **Hirohashi S**. Inactivation of the E-cadherin-mediated cell adhesion system in human cancers. *Am J Pathol* 1998; **153**: 333-339 [PMID: 9708792 DOI: 10.1016/S0002-9440(10)65575-7]
9 **Sugimachi K**, Taguchi K, Aishima S, Tanaka S, Shimada M, Kajiyama K, Sugimachi K, Tsuneyoshi M. Altered expression of beta-catenin without genetic mutation in intrahepatic cholangiocarcinoma. *Mod Pathol* 2001; **14**: 900-905 [PMID: 11557787 DOI: 10.1038/modpathol.3880409]
10 **Gavert N**, Ben-Ze'ev A. Epithelial-mesenchymal transition

and the invasive potential of tumors. *Trends Mol Med* 2008; **14**: 199-209 [PMID: 18406208 DOI: 10.1016/j.molmed.2008.03.004]
11 **Cano A**, Pérez-Moreno MA, Rodrigo I, Locascio A, Blanco MJ, del Barrio MG, Portillo F, Nieto MA. The transcription factor snail controls epithelial-mesenchymal transitions by repressing E-cadherin expression. *Nat Cell Biol* 2000; **2**: 76-83 [PMID: 10655586 DOI: 10.1038/35000025]
12 **Yang MH**, Chen CL, Chau GY, Chiou SH, Su CW, Chou TY, Peng WL, Wu JC. Comprehensive analysis of the independent effect of twist and snail in promoting metastasis of hepatocellular carcinoma. *Hepatology* 2009; **50**: 1464-1474 [PMID: 19821482 DOI: 10.1002/hep.23221]
13 **Fransvea E**, Angelotti U, Antonaci S, Giannelli G. Blocking transforming growth factor-beta up-regulates E-cadherin and reduces migration and invasion of hepatocellular carcinoma cells. *Hepatology* 2008; **47**: 1557-1566 [PMID: 18318443 DOI: 10.1002/hep.22201]
14 **Kubo M**, Hanada T, Yoshimura A. Suppressors of cytokine signaling and immunity. *Nat Immunol* 2003; **4**: 1169-1176 [PMID: 14639467 DOI: 10.1038/ni1012]
15 **Stoiber D**, Kovarik P, Cohney S, Johnston JA, Steinlein P, Decker T. Lipopolysaccharide induces in macrophages the synthesis of the suppressor of cytokine signaling 3 and suppresses signal transduction in response to the activating factor IFN-gamma. *J Immunol* 1999; **163**: 2640-2647 [PMID: 10453004]
16 **Chu PY**, Yeh CM, Hsu NC, Chang YS, Chang JG, Yeh KT. Epigenetic alteration of the SOCS1 gene in hepatocellular carcinoma. *Swiss Med Wkly* 2010; **140**: w13065 [PMID: 20648401 DOI: 10.4414/smw.2010.13065]
17 **Ogata H**, Kobayashi T, Chinen T, Takaki H, Sanada T, Minoda Y, Koga K, Takaesu G, Maehara Y, Iida M, Yoshimura A. Deletion of the SOCS3 gene in liver parenchymal cells promotes hepatitis-induced hepatocarcinogenesis. *Gastroenterology* 2006; **131**: 179-193 [PMID: 16831601 DOI: 10.1053/j.gastro.2006.04.025]
18 **Calvisi DF**, Ladu S, Gorden A, Farina M, Conner EA, Lee JS, Factor VM, Thorgeirsson SS. Ubiquitous activation of Ras and Jak/Stat pathways in human HCC. *Gastroenterology* 2006; **130**: 1117-1128 [PMID: 16618406 DOI: 10.1053/j.gastro.2006.01.006]
19 **Riehle KJ**, Campbell JS, McMahan RS, Johnson MM, Beyer RP, Bammler TK, Fausto N. Regulation of liver regeneration and hepatocarcinogenesis by suppressor of cytokine signaling 3. *J Exp Med* 2008; **205**: 91-103 [PMID: 18158318 DOI: 10.1084/jem.20070820]
20 **Elliott J**. SOCS3 in liver regeneration and hepatocarcinoma. *Mol Interv* 2008; **8**: 19-21 [PMID: 18332480 DOI: 10.1124/mi.8.1.5]
21 **Wang Z**, Zhou J, Fan J, Tan CJ, Qiu SJ, Yu Y, Huang XW, Tang ZY. Sirolimus inhibits the growth and metastatic progression of hepatocellular carcinoma. *J Cancer Res Clin Oncol* 2009; **135**: 715-722 [PMID: 19002496 DOI: 10.1007/s00432-008-0506-z]
22 **Ji Y**, Wang Z, Li Z, Li K, Le X, Zhang T. Angiotensin II induces angiogenic factors production partly via AT1/JAK2/STAT3/SOCS3 signaling pathway in MHCC97H cells. *Cell Physiol Biochem* 2012; **29**: 863-874 [PMID: 22613986 DOI: 10.1159/000171034]
23 **Baltayiannis G**, Baltayiannis N, Tsianos EV. Suppressors of cytokine signaling as tumor repressors. Silencing of SOCS3 facilitates tumor formation and growth in lung and liver. *J BUON* 2008; **13**: 263-265 [PMID: 18555475]
24 **Lindemann C**, Hackmann O, Delic S, Schmidt N, Reifenberger G, Riemenschneider MJ. SOCS3 promoter methylation is mutually exclusive to EGFR amplification in gliomas and promotes glioma cell invasion through STAT3 and FAK activation. *Acta Neuropathol* 2011; **122**: 241-251 [PMID: 21590492 DOI: 10.1007/s00401-011-0832-0]
25 **Ogata H**, Chinen T, Yoshida T, Kinjyo I, Takaesu G, Shiraishi H, Iida M, Kobayashi T, Yoshimura A. Loss of SOCS3 in the liver promotes fibrosis by enhancing STAT3-mediated TGF-beta1 production. *Oncogene* 2006; **25**: 2520-2530 [PMID: 16474852 DOI: 10.1038/sj.onc.1209281]
26 **Bonnomet A**, Brysse A, Tachsidis A, Waltham M, Thompson EW, Polette M, Gilles C. Epithelial-to-mesenchymal transitions and circulating tumor cells. *J Mammary Gland Biol Neoplasia* 2010; **15**: 261-273 [PMID: 20449641 DOI: 10.1007/s10911-010-9174-0]
27 **Schmalhofer O**, Brabletz S, Brabletz T. E-cadherin, beta-catenin, and ZEB1 in malignant progression of cancer. *Cancer Metastasis Rev* 2009; **28**: 151-166 [PMID: 19153669 DOI: 10.1007/s10555-008-9179-y]
28 **Guarino M**, Rubino B, Ballabio G. The role of epithelial-mesenchymal transition in cancer pathology. *Pathology* 2007; **39**: 305-318 [PMID: 17558857 DOI: 10.1080/00313020701329914]
29 **Huber MA**, Kraut N, Beug H. Molecular requirements for epithelial-mesenchymal transition during tumor progression. *Curr Opin Cell Biol* 2005; **17**: 548-558 [PMID: 16098727 DOI: 10.1016/j.ceb.2005.08.001]
30 **Moreno-Bueno G**, Cubillo E, Sarrió D, Peinado H, Rodríguez-Pinilla SM, Villa S, Bolós V, Jordá M, Fabra A, Portillo F, Palacios J, Cano A. Genetic profiling of epithelial cells expressing E-cadherin repressors reveals a distinct role for Snail, Slug, and E47 factors in epithelial-mesenchymal transition. *Cancer Res* 2006; **66**: 9543-9556 [PMID: 17018611 DOI: 10.1158/0008-5472.CAN-06-0479]
31 **Kurrey NK**, K A, Bapat SA. Snail and Slug are major determinants of ovarian cancer invasiveness at the transcription level. *Gynecol Oncol* 2005; **97**: 155-165 [PMID: 15790452 DOI: 10.1016/j.ygyno.2004.12.043]
32 **Jiao W**, Miyazaki K, Kitajima Y. Inverse correlation between E-cadherin and Snail expression in hepatocellular carcinoma cell lines in vitro and in vivo. *Br J Cancer* 2002; **86**: 98-101 [PMID: 11857019 DOI: 10.1038/sj.bjc.6600017]
33 **Zavadil J**, Böttinger EP. TGF-beta and epithelial-to-mesenchymal transitions. *Oncogene* 2005; **24**: 5764-5774 [PMID: 16123809 DOI: 10.1038/sj.onc.1208927]
34 **Liu Y**. Epithelial to mesenchymal transition in renal fibrogenesis: pathologic significance, molecular mechanism, and therapeutic intervention. *J Am Soc Nephrol* 2004; **15**: 1-12 [PMID: 14694152 DOI: 10.1097/01.ASN.0000106015.29070.E7]
35 **Xu J**, Lamouille S, Derynck R. TGF-beta-induced epithelial to mesenchymal transition. *Cell Res* 2009; **19**: 156-172 [PMID: 19153598 DOI: 10.1038/cr.2009.5]

P- Reviewers Grassi G, Zhou YM, Aghakhani A
S- Editor Huang XZ **L- Editor** Ma JY **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i6.874

World J Gastroenterol 2013 February 14; 19(6): 874-881
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Intraductal ultrasound substantiates diagnostics of bile duct strictures of uncertain etiology

Tobias Meister, Hauke S Heinzow, Carina Woestmeyer, Philipp Lenz, Josef Menzel, Torsten Kucharzik, Wolfram Domschke, Dirk Domagk

Tobias Meister, Hauke S Heinzow, Carina Woestmeyer, Philipp Lenz, Josef Menzel, Torsten Kucharzik, Wolfram Domschke, Dirk Domagk, Department of Medicine B, University of Münster, D-48149 Münster, Germany
Tobias Meister, Department of Medicine Ⅱ, HELIOS Albert-Schweitzer University Teaching Hospital, D-37154 Northeim, Germany
Josef Menzel, Department of Medicine Ⅱ, Klinikum Ingolstadt, D-85049 Ingolstadt, Germany
Torsten Kucharzik, Department of Medicine, University Teaching Hospital Lüneburg, D-21339 Lüneburg, Germany
Author contributions: Meister T and Heinzow HS contributed equally to this work; Meister T contributed to design of the study, analysis and interpretation of data, drafting of the manuscript; Heinzow HS contributed to analysis and interpretation of data, drafting of the manuscript, technical and material support; Woestmeyer C contributed to acquisition of data; Lenz P contributed to critical revision of the manuscript for important intellectual content, technical support; Menzel J contributed to performance of the intraductal ultrasound (IDUS) procedures, revision of the manuscript for important intellectual content; Kucharzik T contributed to performance of the IDUS procedures, critical revision of the manuscript for important intellectual content, study concept, analysis and interpretation of data; Domschke W contributed to critical revision of the manuscript for important intellectual content; Domagk D contributed to senior author, study concept and design, performance of the IDUS procedures, analysis and interpretation of data, drafting of the manuscript, statistical analysis, study supervision and final approval of the version to be published.
Supported by A research fellowship from the Faculty of Medicine, Westfälische Wilhelms-Universität Münster
Correspondence to: Tobias Meister, MD, Department of Medicine Ⅱ, HELIOS Albert-Schweitzer Teaching Hospital, Sturmbäume 8-10, D-37154 Northeim, Germany. tobiasmeister@gmx.de
Telephone: +49-5551-971240 Fax: +49-5551-971420
Received: September 6, 2012 Revised: October 3, 2012
Accepted: October 19, 2012
Published online: February 14, 2013

Abstract

AIM: To report the largest patient cohort study investigating the diagnostic yield of intraductal ultrasound (IDUS) in indeterminate strictures of the common bile duct.

METHODS: A patient cohort with bile duct strictures of unknown etiology was examined by IDUS. Sensitivity, specificity and accuracy rates of IDUS were calculated relating to the definite diagnoses proved by histopathology or long-term follow-up in those patients who did not undergo surgery. Analysis of the endosonographic report allowed drawing conclusions with respect to the T and N staging in 147 patients. IDUS staging was compared to the postoperative histopathological staging data allowing calculation of sensitivity, specificity and accuracy rates for T and N stages. The endoscopic retrograde cholangio-pancreatography and IDUS procedures were performed under fluoroscopic guidance using a side-viewing duodenoscope (Olympus TJF 160, Olympus, Ltd., Tokyo, Japan). All procedures were performed under conscious sedation (propofol combined with pethidine) according to the German guidelines. For IDUS, a 6 F or 8 F ultrasound miniprobe was employed with a radial scanner of 15-20 MHz at the tip of the probe (Aloka Co., Tokyo, Japan).

RESULTS: A total of 397 patients (210 males, 187 females, mean age 61.43 ± 13 years) with indeterminate bile duct strictures were included. Two hundred and sixty-four patients were referred to the department of surgery for operative exploration, thus surgical histopathological correlation was available for those patients. Out of 264 patients, 174 had malignant disease proven by surgery, in 90 patients benign disease was found. In these patients decision for surgical exploration was made due to suspicion for malignant disease in multimodal diagnostics (computed tomography scan, endoscopic ultrasound or magnetic resonance imaging). Twenty benign bile duct strictures were misclassified by IDUS as malignant while 14 patients with malignant strictures were initially misdiagnosed by IDUS as benign resulting in sensitivity, specificity and accuracy rates

of 93.2%, 89.5% and 91.4%, respectively. In the subgroup analysis of malignancy prediction, IDUS showed best performance in cholangiocellular carcinoma as underlying disease (sensitivity rate, 97.6%) followed by pancreatic carcinoma (93.8%), gallbladder cancer (88.9%) and ampullary cancer (80.8%). A total of 133 patients were not surgically explored. 32 patients had palliative therapy due to extended tumor disease in IDUS and other imaging modalities. Ninety-five patients had benign diagnosis by IDUS, forceps biopsy and radiographic imaging and were followed by a surveillance protocol with a follow-up of at least 12 mo; the mean follow-up was 39.7 mo. Tumor localization within the common bile duct did not have a significant influence on prediction of malignancy by IDUS. The accuracy rate for discriminating early T stage tumors (T1) was 84% while for T2 and T3 malignancies the accuracy rates were 73% and 71%, respectively. Relating to N0 and N1 staging, IDUS procedure achieved accuracy rates of 69% for N0 and N1, respectively. Limitations: Pretest likelihood of 52% may not rule out bias and overinterpretation due to the clinical scenario or other prior performed imaging tests.

CONCLUSION: IDUS shows good results for accurate diagnostics of bile duct strictures of uncertain etiology thus allowing for adequate further clinical management.

© 2013 Baishideng. All rights reserved.

Key words: Intraductal ultrasound; Bile duct strictures; Accuracy

Meister T, Heinzow HS, Woestmeyer C, Lenz P, Menzel J, Kucharzik T, Domschke W, Domagk D. Intraductal ultrasound substantiates diagnostics of bile duct strictures of uncertain etiology. *World J Gastroenterol* 2013; 19(6): 874-881 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i6/874.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i6.874

INTRODUCTION

There is still ongoing debate about adequate diagnostics in bile duct strictures of unknown etiology. The application of endoscopic retrograde cholangio-pancreatography (ERCP) is considered to be an essential tool in bile duct strictures in which additional need for intervention is given[1]. The main advantage of ERCP over other imaging modalities is the ability to achieve biliary decompression and to take transpapillary specimens for histological or cytological analysis in the very same session. A large trial on biliary brush cytology, however, described poor results thus indicating the need for further studies in the analysis of appropriate tissue sampling[2]. The use of intraductal ultrasound (IDUS) performed during ERCP enables the investigator to obtain additional information concerning the bile duct wall and the periductal tissue[3]. Specifically, IDUS gives clinically important data by visualizing the wall layers in biliary strictures and estimating the extent of potentially cancerous infiltration, also enabling the investigator to perform targeted biopsies. Thus, IDUS might be instrumental in choosing the appropriate therapeutic approach and may improve our potential to differentiate benign and malignant strictures. In this matter, however, previous studies, although partly of a prospective design, have only investigated limited numbers of patients[4-6]. On the other hand, cholangioscopy is at present time a promising diagnostic technique in diagnosing bile duct strictures of unknown origin. However, so far only limited data evaluating the diagnostic impact of cholangioscopy is available. In a prospective study with 35 patients included, the sensitivity of SpyGlass-directed biopsy of intraductal lesions was 71%[7,8].

In the present large cohort of patients, we aimed to evaluate the diagnostic yield of IDUS in patients scheduled for ERCP due to indeterminate strictures or filling defects of the common bile duct.

MATERIALS AND METHODS

Data collection

At the tertiary referral center of Münster University Hospital, we retrospectively analyzed the data of our patient cohort undergoing ERCP in combination with IDUS for diagnostics of indeterminate strictures of the bile duct during 2002-2009. All patients with bile duct stenosis who had undergone ERCP and IDUS during the study period could be identified by looking for codes K83.1 and procedures 3.055 and 1440.6 according to the International Classification of Diseases. A total of 397 patients were found in our analysis who were referred to the Department of Medicine B at Münster University Hospital. Clinical records of patients were collected and carefully analyzed. Baseline characteristics, diagnostic techniques employed and histopathology were retrieved as shown in Table 1.

Exclusions

Patients not having been histopathologically controlled by surgery, forceps biopsy or with an endoscopic follow-up < 1 year in suspected benign biliary stenosis were excluded from this study.

Procedures

All 397 patients enrolled in the present study underwent ERCP with the additional application of intraductal ultrasound. In our cohort, there was no case in which a positive tissue diagnosis was already known at the time of IDUS investigation. The individual procedure was performed after written informed consent had been obtained from the patients or related persons for the endoscopic procedures. All endoscopic maneuvers were executed by highly experienced investigators according to the generally accepted guidelines with an ERCP case volume above 200/year[9]. The ERCP and IDUS procedures were performed under fluoroscopic guidance using a side-viewing duodenoscope (Olympus TJF 160, Olympus, Ltd., Tokyo, Japan). All procedures were performed

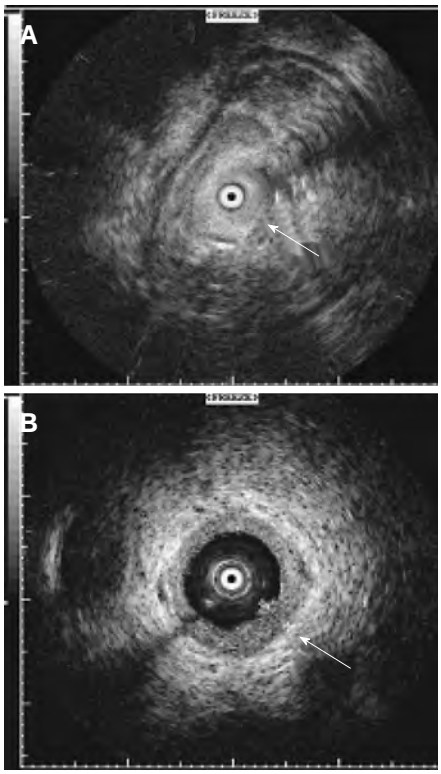


Figure 1 An exemplary benign and malignant bile duct stenosis. A: Benign bile duct stricture showing a homogeneous hyperechoic lesion with smooth margins (arrow); B: Malignant stricture showing bile duct wall thickening and irregular margins (arrow).

under conscious sedation (propofol combined with pethidine) according to the German guidelines[10]. For IDUS, a 6 F or 8 F ultrasound miniprobe was employed with a radial scanner of 15-20 MHz at the tip of the probe (Aloka Co., Tokyo, Japan). Thus a radial real-time image of 360° view was possible for optimum investigation of the area surrounding the probe. The visible depth was about 20 mm with a resolution of up to 0.1 mm (Figure 1). Three hundred and twelve IDUS procedures included additional forceps biopsies. Endoscopic transpapillary biopsies (n = 4-8 specimens) were taken out of the biliary strictures by straight or angled endoscopic forceps (MTW Endoscopy, Wesel, Germany). If insertion of the forceps into the stricture was not feasible, biopsies were retrieved from the distal margins of the bile duct stenosis[11]. Patients with eligibility for surgery were transferred to the Department of General Surgery, Münster University Hospital.

IDUS T and N staging

Two hundred and sixty-four patients were surgically explored (66% of patients). In 174 cases malignancy was proven, of those 14 patients were initially misdiagnosed by IDUS as benign, thus in these cases uT stages were not available. Thirteen of the 174 patients had surgical exploration but due to extended disease with inoperability T and N stage assessment was not done. Thus, retrospective analysis of the endosonographic report allowed drawing conclusions with respect to the T and N staging in 147 patients. IDUS staging was based on the latest TNM classification system[12]. IDUS classification of N stages was as follows: N0-no regional lymph node metastasis; N1-regional lymph node metastasis. Lymph nodes were considered positive, if at least one of the following criteria could be assessed: lymph node larger than 10 mm, delineated borders, hypoechoic structure resembling the primary tumor, roundish shape.

IDUS staging was compared to the postoperative histopathological staging data allowing calculation of sensitivity, specificity and accuracy rates for T and N stages. Due to the limited penetration depth of IDUS, M staging was not performed.

Follow-up

All patients with suspected benign strictures had routine follow-up the day following intervention as well as every three months the first year, every 6 mo the second year and annually up to the third year after intervention at our department. The follow-up procedure was performed according to a surveillance protocol and included laboratory testing and abdominal ultrasound. In cases of biliary plastic stent insertion, the follow-up procedures included ERCP and where appropriate biliary plastic stent changing.

Statistical analysis

Data were analyzed using SPSS 17.0 (Chicago, IL, United States). Results were expressed as medians and ranges. For each of the diagnostic measures, sensitivity, specificity and accuracy rates were calculated for each individual T and N stage. In all cases, gold standard was the histopathologic staging of specimens. Comparison of accuracy rates between groups (localization of stricture) was performed by using the Mann-Whitney U test and the χ^2 test as appropriate. Differences were considered statistically significant if $P < 0.05$. For statistical analysis, sensitivity, specificity, pre-test likelihood and accuracy rates were calculated as follows: sensitivity = true positives/(true positives + false negatives); specificity = true negatives/(true negatives + false positives); pre-test likelihood = truly malignant cases/total cases; accuracy = (true positives + true negatives)/total cases.

RESULTS

Patient characteristics

The study cohort included 397 patients (210 men and 187 women; median age 61.4 ± 13 years). Two hundred and sixty-four patients were referred to the department of surgery for operative exploration, thus surgical histopathological correlation was available for those patients. Out of 264 patients, 174 had malignant disease proven by surgery, in 90 patients benign disease was found. In these patients decision for surgical exploration was made due to suspicion for malignant disease in multimodal diagnostics [computed tomography (CT) scan, endoscopic ultrasound (EUS) or magnetic resonance imaging (MRI)]. A total of 133 patients were not surgically explored.

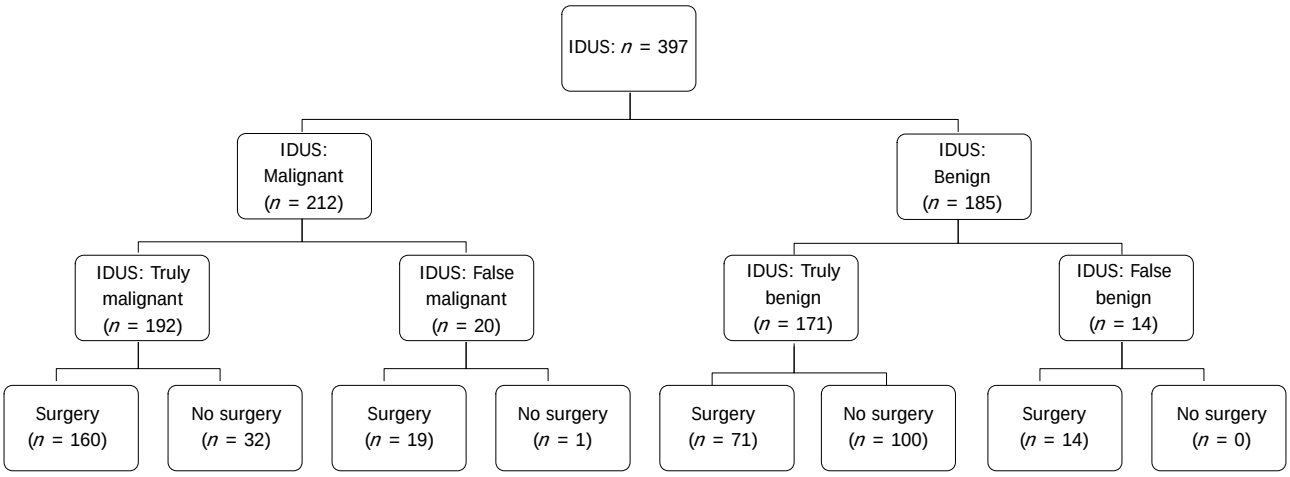


Figure 2 Flow chart showing the enrollment of study patients and distribution based on intraductal ultrasound diagnosis. IDUS: Intraductal ultrasound.

Table 1 Baseline characteristics of the patient cohort

Baseline characteristics	
Patients (*n*)	397
Age (yr), mean ± SE	61.43 ± 13
Sex (M/F)	210/187
IDUS performed (*n*)	397
Follow-up (mo), mean ± SE	39.7 ± 23.1
Follow-up range (mo)	12-100
Procedures (*n*)	
Clinical follow-up	95
Surgery	264
Palliative therapy	32
Calculus extraction	6
Localization of stricture (CBD)	
Proximal third	59
Middle third	46
Distal third	292
Final diagnosis (*n*)	
Normal bile duct	25
Benign disease	
Papillitis	30
Ampullary adenoma	18
Cholangitis	17
PSC	8
Mirizzi syndrome	7
Choledocholithiasis	14
Pancreatitis	59
Pseudocyst	4
Portal vein thrombosis	1
Papilloma of pancreatic duct	3
Choledochal cyst	1
Postoperative stenosis	1
Pancreatic cystadenoma	2
Caroli's syndrome	1
Malignant disease	
Cholangiocarcinoma	85
Pancreatic carcinoma	80
Ampullary carcinoma	26
Gallbladder carcinoma	9
Hepatocellular carcinoma	6

CBD: Common bile duct; PSC: Primary sclerosing cholangitis; M: Man; F: Female; IDUS: Intraductal ultrasound.

Thirty-two patients had palliative therapy due to extended tumor disease in IDUS and other imaging modalities. In 6 patients calculus extraction was performed due to choledocholithiasis. Ninety-five patients had benign diagnosis by IDUS, forceps biopsy and radiographic imaging and were followed by a surveillance protocol with a follow-up of at least 12 mo; the mean follow-up was 39.7 mo (Table 1). A flow chart showing the enrollment of the study patients with distribution based on IDUS diagnosis is presented in Figure 2.

IDUS

IDUS was performed in all patients enrolled. Pre-test likelihood for malignant stenosis in our patient cohort of 52% (95%CI: 47%-57%) was calculated. Twenty benign bile duct strictures were initially misclassified by IDUS as malignant while 14 malignant bile duct strictures were initially interpreted by IDUS as benign resulting in sensitivity, specificity and accuracy rates of 93.2%, 89.5% and 91.4%, respectively (Tables 2 and 3). In the subgroup analysis of malignancy prediction, IDUS showed best performance in cholangiocellular carcinoma as underlying disease (sensitivity rate, 97.6%) followed by pancreatic carcinoma (93.8%), gallbladder cancer (88.9%) and ampullary cancer (80.8%) (Tables 2 and 3).

Accuracy rates of IDUS for malignant stenoses in the proximal, middle or distal third of the common bile duct did not differ statistically in terms of localization (Table 4).

Accuracy of IDUS in T and N staging

Overall T and N staging results with the intraductal ultrasound miniprobe are given in Tables 5 and 6. The accuracy rate for discriminating early T stage tumors (T1) was 84% while for T2 and T3 malignancies the accuracy rates were 73% and 71%, respectively (Table 5). Relating to N0 and N1 staging, IDUS achieved accuracy rates of 69% each (Table 6).

DISCUSSION

Endoscopic ultrasound has proved to be an accurate imaging device in the diagnostics and staging of malig-

Table 2 Staging results of intraductal ultrasound (*n* = 397)

Method, classification	Final diagnosis according to histopathology or long-term follow-up						
	Benign lesion	Carcinoma	CCC	Pancreatic CA	Ampullar CA	GB CA	HCC
IDUS, benign	171	14	2	5	5	1	1
IDUS, malignant	20	192	83	75	21	8	5

CA: Carcinoma; GB CA: Gallbladder cancer; CCC: Cholangiocellular carcinoma; HCC: Hepatocellular carcinoma; IDUS: Intraductal ultrasound.

Table 3 Sensitivity, specificity and accuracy rates of intraductal ultrasound (*n* = 397)

Tumor	Sensitivity (95%CI)	Specificity (95%CI)	Accuracy (95%CI)
All tumors	0.93 (0.90-0.97)	0.89 (0.85-0.94)	0.91 (0.89-0.94)
CCC	0.98 (0.94-1.0)	0.98 (0.94-1.0)	0.92 (0.89-0.95)
Pancreatic CA	0.94 (0.88-0.99)	0.90 (0.85-0.94)	0.91 (0.87-0.94)
Ampullary CA	0.81 (0.66-0.96)	0.90 (0.85-0.94)	0.89 (0.84-0.93)
GB CA	0.89 (0.68-1.0)	0.90 (0.85-0.94)	0.90 (0.85-0.94)
HCC	0.83 (0.54-1.0)	0.90 (0.85-0.94)	0.89 (0.85-0.94)

CA: Carcinoma; GB CA: Gallbladder cancer; CCC: Cholangiocellular carcinoma; HCC: Hepatocellular carcinoma; IDUS: Intraductal ultrasound.

Table 4 Statistical analysis of intraductal ultrasound accuracy rates relating to localization of bile duct strictures (*n* = 397)

Localization of stenosis	*N*	Accuracy (95%CI)	Test for significance	*P* value
Proximal third	55/59	0.93 (0.87-1.0)	Proximal *vs* middle third	0.958
Middle third	43/46	0.93 (0.87-1.0)	Proximal *vs* distal third	0.280
Distal third	260/292	0.89 (0.85-0.93)	Middle *vs* distal third	0.308

Table 5 Histopathological *vs* intraductal ultrasound T staging

IDUS	Histopathology			
	pT1	pT2	pT3/4	∑
uT1	12	9	13	34
uT2	1	26	24	51
uT3	1	5	56	62
∑	14	40	93	147
Sensitivity (95%CI)	0.86 (0.67-1.0)	0.65 (0.50-0.80)	0.60 (0.50-0.70)	
Specificity (95%CI)	0.83 (0.77-0.90)	0.77 (0.69-0.85)	0.89 (0.81-0.97)	
Accuracy (95%CI)	0.84 (0.78-0.89)	0.73 (0.66-0.81)	0.71 (0.63-0.78)	

IDUS: Intraductal ultrasound.

Table 6 N staging accuracy-histopathology *vs* intraductal ultrasound

IDUS	Histopathology		
	pN0	pN1	∑
uN0	44	23	67
uN1	23	57	80
∑	67	80	147
Sensitivity (95%CI)	0.66 (0.61-0.81)	0.61 (0.57-0.79)	
Specificity (95%CI)	0.61 (0.54-0.77)	0.66 (0.54-0.77)	
Accuracy (95%CI)	0.69 (0.61-0.76)	0.69 (0.61-0.76)	

IDUS: Intraductal ultrasound.

nant bile duct strictures[13]. However, in previous studies, although partly of a prospective design, only limited numbers of patients were investigated[4-6]. Therefore, in the present to our knowledge largest patient cohort, we aimed to substantiate the diagnostic yield of IDUS.

IDUS has also proved superior to other imaging modalities such as CT and MRI[14-16]. Its limitation, however, is the minor ultrasonic penetration depth. Consequently, intraductal ultrasonography tends to understage tumors of the pancreaticobiliary tract. Therefore, IDUS does not seem useful in extensive lymph node staging[17].

It is consistently accepted that in the bilio-pancreatic tract depiction of tumors by means of ultrasound and CT is difficult, but these tumors can appropriately be represented through endoscopic ultrasound[18] or IDUS[19,20]. Although IDUS is superior to EUS regarding diagnostics of tumor extension in the pancreatic and biliary duct, the use of IDUS has not been taken up widely yet.

A promising diagnostic tool in the differentiation of bile duct strictures is single- operator peroral cholangioscopy (SOC) as it provides direct visualization of the bile duct and facilitates diagnostic procedures and therapeutic intervention. The diagnostic utility of SOC for indeterminate biliary lesions has been the topic of a few recently published studies[21,22].

However, only limited data with smaller patient cohorts exist. Promising results with an accuracy of SOC for diagnosing malignant lesions has been reported to range between 64% and 89%. SOC-guided biopsies have been shown to be adequate in 72% to 82%[21-23]. Therefore, its therapeutic and diagnostic yield in patients with bile duct strictures remains uncertain. A prospective randomized trial comparing various endoscopic imaging and radiographic imaging modalities with each other would be desirable, especially in a multi-center study design.

In our patient cohort of 397 patients with bile duct strictures of initially unknown etiology, using IDUS we observed sensitivity, specificity and accuracy rates of 93%, 89% and 91%, respectively, for discriminating malignant from benign lesions. In previous studies with a limited number of patients, IDUS accuracy rates between 84% and 95% are described as presented in Table 7. IDUS also showed excellent results in regard to localization of biliary stenoses with an accuracy rate of 93% (proximal part of bile duct), 93% (middle) and 89% (distal), respectively (*P* values not significant) (Table 4). In our study, endoscopic transpapillary biopsies (ETP) were additionally obtained in 312 cases. Out of 226 bile duct strictures initially diagnosed to be benign by ETP, 101 cases eventually turned out as malignancies. There were no false-positive results in forceps biopsy histopathology leading to sensitivity, specificity and accuracy rates of 46%, 100% and 67.6%, respectively[11]. Thus the authors conclude that ETPs are of limited value for detecting additional malignant bile

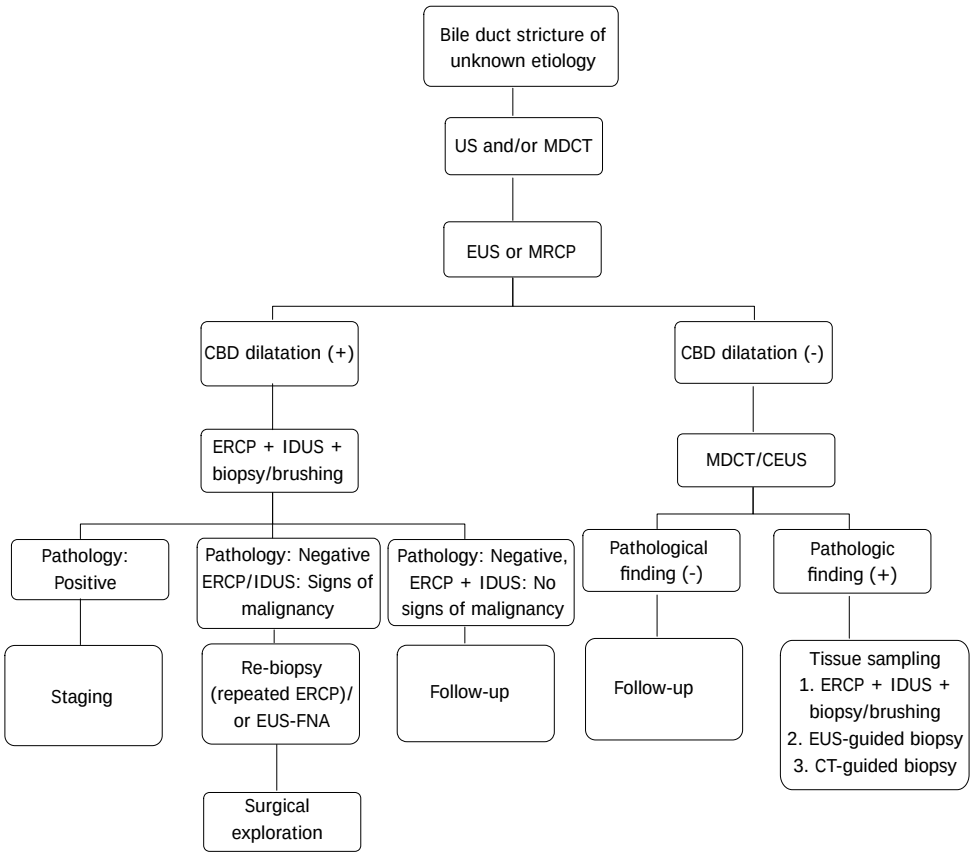


Figure 3 Suggested algorithm for the evaluation of bile duct strictures of uncertain etiology. US: Ultrasound; MDCT: Multidetector computed tomography; EUS: Endoscopic ultrasound; MRCP: Magnetic resonance cholangio-pancreatography; CBD: Common bile duct; IDUS: Intraductal ultrasound; CEUS: Contrast enhanced ultrasound; EUS-FNA: Endoscopic ultrasound with fine needle aspiration; CT: Computed tomography.

Table 7 Meta-analysis of intraductal ultrasound accuracy in detecting bile duct malignancy

Study	No. of patients	Accuracy of IDUS
Menzel *et al*[15]	57	89%
Tamada *et al*[30]	19	84%
Domagk *et al*[14]	60	88%
Domagk *et al*[31]	33	88%
Domagk *et al*[29]	30	95%

IDUS: Intraductal ultrasound.

duct strictures.

In matters of tumor entity, IDUS demonstrated best results in cholangiocellular carcinoma (CCC) with accuracy and sensitivity rates of 92% and 98%, respectively. As far as T staging is concerned, upon IDUS the normal bile duct wall appears as either two or three layers. However, in some patients differentiation of the fibromuscular layer from the perimuscular connective tissue can be difficult thus limiting the ability to distinguish CCC stages 1 and 2, although this distinction is usually not clinically relevant with respect to treatment options[24]. According to a study conducted earlier by our group[25], the various layers of the extrahepatic bile duct wall as described by the Union for International Cancer Control 7th tumor-node-metastasis (TNM) classification[12] are not consistently demonstrable histomorphologically, immunohistochemically or by endosonographic imaging. Domagk *et al*[25] suggested that difficulties in distinguishing tumor invasion encroaching beyond the bile duct (T2) and into the pancreas (T3) pose problems for the present TNM-classification. A better metric may be to combine T2- and T3-staged tumors into one single class.

T staging of other tumor entities such as pancreatic, ampullary or gallbladder cancer may be limited in mini-probe intraductal ultrasound due to its maximum penetration depth of 20 mm. The T stage, *e.g.*, of pancreatic tumors among other factors relies on tumor size. Further, the assessment of vascular tumor infiltration is restricted due to the technical limitation of penetration depth and absence of potential duplex sonography. In a study by Tamada *et al*[26,27], vascular infiltration of CCC was assessed: Depiction of the right hepatic artery was possible in 100% of the cases while in less than 20% of the cases depiction of the common hepatic artery or the left hepatic artery succeeded. On that account, the assessment of tumor infiltration into the above mentioned vessels was inaccurate.

In summary, IDUS tends to understage tumors of the pancreaticobiliary tract. Specific analysis of our patient cohort revealed overall T staging accuracy rates as follows: T1 84%, T2 73% and T3/T4 71% as displayed in Table 5. N staging accuracy was calculated as 69% (Table 6).

In recent years, other imaging techniques like magnetic resonance cholangio-pancreatography (MRCP) and multi-detector computed tomography have also been evaluated for their diagnostic sensitivity and specificity in biliary duct tumors. In a first prospective comparison of the diagnostic accuracy of ERCP, MRCP, CT and EUS in biliary duct strictures by Rösch *et al*[28], 50 patients were

analyzed. In their patient cohort, relating to diagnosis of malignancy sensitivity and specificity rates of 85%/75% for ERCP/PTC, 85%/71% for MRCP, 77%/63% for CT, and 79%/62% for EUS were observed. Those authors concluded that although MRCP provides the same imaging information as direct cholangiography, it has only limited specificity for the diagnosis of malignant strictures[28]. A prospective study by our group confirmed superiority of ERCP supplemented by IDUS in differentiating benign from malignant strictures as compared with MRI[29]. Classification of T stages by endolumenal ultrasound was also the topic of an investigation conducted by Menzel *et al*[15] demonstrating similar accuracy rates (77.7%) for T staging as shown in the present study.

Admittedly, as limitations of the present study the retrospective design and certain bias have to be mentioned. In our cohort, there was no case in which a positive tissue diagnosis was already known at the time of IDUS investigation but our pre-test likelihood of 52% may not rule out bias and over-interpretation due to the clinical scenario or other prior performed imaging tests. Our patient cohort is certainly a highly selected one making the probability of malignant bile duct stricture more obvious. On the one hand, the prevalence of malignancy in our patient cohort was 52% as expressed by the pre-test likelihood. On the other hand, the accuracy of IDUS for detecting malignant bile duct strictures exceeds 91%. Therefore, our analysis of a very large patient cohort shows good results of IDUS with regard to accurate diagnostics of bile duct strictures of uncertain etiology and, thus, allows for adequate further clinical management. In particular, IDUS is suitable for early T stage prediction.

Summing up, we suggest the following algorithm for the evaluation of bile duct strictures of uncertain etiology as displayed in Figure 3.

ACKNOWLEDGMENTS

Thanks to Professor Gary L Powell for helpful and valuable suggestions regarding the manuscript and true friendship for over 25 years.

COMMENTS

Background

Adequate diagnostics in bile duct strictures of unknown etiology is still a challenging task. Many different imaging techniques compete with each other. Intraductal ultrasound (IDUS) might be instrumental in choosing the appropriate therapeutic approach and may improve our potential to differentiate benign and malignant strictures.

Research frontiers

The authors undertook the largest European retrospective study to evaluate the diagnostic yield of IDUS in patients scheduled for endoscopic retrograde cholangio-pancreatography due to indeterminate strictures of the common bile duct.

Innovations and breakthroughs

IDUS shows good results for accurate diagnostics of bile duct strictures of uncertain etiology thus allowing for adequate further clinical management. In particular, IDUS is suitable for early T stage prediction.

Applications

By analyzing the accuracy of IDUS in the diagnostics of bile duct strictures of uncertain etiology, the authors contribute to a better diagnostic strategy of this difficult issue. This may lead to an improved patient care with an optimal diagnostic approach to patients with bile duct strictures of uncertain etiology.

Terminology

IDUS enables the investigation of the common bile duct wall and the periductal tissue with high resolution.

Peer review

Authors evaluated the role of IDUS in the differential diagnosis of indeterminate biliary strictures. This retrospective study involved such a large number of patients with surgical standards. It is a well-written paper, in which the authors have studied an important issue in hepatobiliary medicine.

REFERENCES

1 **Choudari CP**, Fogel E, Gottlieb K, Sherman S, Lehman GA. Therapeutic biliary endoscopy. *Endoscopy* 1998; **30**: 163-173 [PMID: 9592658]
2 **Glasbrenner B**, Ardan M, Boeck W, Preclik G, Möller P, Adler G. Prospective evaluation of brush cytology of biliary strictures during endoscopic retrograde cholangiopancreatography. *Endoscopy* 1999; **31**: 712-717 [PMID: 10604612]
3 **Menzel J**, Domschke W. Intraductal ultrasonography (IDUS) of the pancreato-biliary duct system. Personal experience and review of literature. *Eur J Ultrasound* 1999; **10**: 105-115 [PMID: 10586015]
4 **Farrell RJ**, Agarwal B, Brandwein SL, Underhill J, Chuttani R, Pleskow DK. Intraductal US is a useful adjunct to ERCP for distinguishing malignant from benign biliary strictures. *Gastrointest Endosc* 2002; **56**: 681-687 [PMID: 12397276]
5 **Levy MJ**, Baron TH, Clayton AC, Enders FB, Gostout CJ, Halling KC, Kipp BR, Petersen BT, Roberts LR, Rumalla A, Sebo TJ, Topazian MD, Wiersema MJ, Gores GJ. Prospective evaluation of advanced molecular markers and imaging techniques in patients with indeterminate bile duct strictures. *Am J Gastroenterol* 2008; **103**: 1263-1273 [PMID: 18477350]
6 **Vazquez-Sequeiros E**, Baron TH, Clain JE, Gostout CJ, Norton ID, Petersen BT, Levy MJ, Jondal ML, Wiersema MJ. Evaluation of indeterminate bile duct strictures by intraductal US. *Gastrointest Endosc* 2002; **56**: 372-379 [PMID: 12196775]
7 **Chen YK**, Pleskow DK. SpyGlass single-operator peroral cholangiopancreatoscopy system for the diagnosis and therapy of bile-duct disorders: a clinical feasibility study (with video). *Gastrointest Endosc* 2007; **65**: 832-841 [PMID: 17466202 DOI: 10.1016/j.gie.2007.01.025]
8 **Tamada K**, Ushio J, Sugano K. Endoscopic diagnosis of extrahepatic bile duct carcinoma: Advances and current limitations. *World J Clin Oncol* 2011; **2**: 203-216 [PMID: 21611097 DOI: 10.5306/wjco.v2.i5.203]
9 **Faigel DO**, Baron TH, Lewis B, Petersen B, Petrini J. Ensuring competence in endoscopy. Prepared by the ASGE taskforce on ensuring competence in endoscopy and American College of Gastroenterology executive and practice management committees. ASGE policy and procedures manual for gastrointestinal endoscopy: guidelines for training and practice on CD-ROM. *ASGE* 2005: 1-36
10 **Riphaus A**, Wehrmann T, Weber B, Arnold J, Beilenhoff U, Bitter H, von Delius S, Domagk D, Ehlers A, Faiss S, Hartmann D, Heinrichs W, Hermans M, Hofmann C, in der Smitten S, Jung M, Kähler G, Kraus M, Martin J, Meining A, Radke J, Rösch T, Seifert H, Sieg A, Wigginghaus B, Kopp I. S3 Guideline: Sedation for gastrointestinal endoscopy. *Endoscopy* 2009; **41**: 787-815
11 **Heinzow H**, Woestmeyer C, Domschke W, Domagk D, Meister T. Endoscopic transpapillary biopsies are of limited value in the diagnostics of bile duct strictures of unknown etiology - results of a histopathologically controlled study in 312 patients. *Hepatogastroenterology* 2012; In press

12 **Edge S**, Byrd D, Compton C, Fritz A, Greene F, Trotti A. AJCC cancer staging manual. 7th ed. New York: Springer-Verlag, 2010

13 **Rösch T**, Lorenz R, Braig C, Classen M. Endoscopic ultrasonography in diagnosis and staging of pancreatic and biliary tumors. *Endoscopy* 1992; **24** Suppl 1: 304-308 [PMID: 1633770]

14 **Domagk D**, Poremba C, Dietl KH, Senninger N, Heinecke A, Domschke W, Menzel J. Endoscopic transpapillary biopsies and intraductal ultrasonography in the diagnostics of bile duct strictures: a prospective study. *Gut* 2002; **51**: 240-244 [PMID: 12117887]

15 **Menzel J**, Poremba C, Dietl KH, Domschke W. Preoperative diagnosis of bile duct strictures--comparison of intraductal ultrasonography with conventional endosonography. *Scand J Gastroenterol* 2000; **35**: 77-82 [PMID: 10672839]

16 **Tamada K**, Ido K, Ueno N, Kimura K, Ichiyama M, Tomiyama T. Preoperative staging of extrahepatic bile duct cancer with intraductal ultrasonography. *Am J Gastroenterol* 1995; **90**: 239-246 [PMID: 7847293]

17 **Menzel J**, Domschke W. Gastrointestinal miniprobe sonography: the current status. *Am J Gastroenterol* 2000; **95**: 605-616 [PMID: 10710047]

18 **Meyer J**, Sulkowski U, Preusser P, Bünte H. Clinical aspects and surgical therapy of papillary cancer. *Zentralbl Chir* 1987; **112**: 20-26 [PMID: 3564744]

19 **Itoh A**, Goto H, Naitoh Y, Hirooka Y, Furukawa T, Hayakawa T. Intraductal ultrasonography in diagnosing tumor extension of cancer of the papilla of Vater. *Gastrointest Endosc* 1997; **45**: 251-260 [PMID: 9087831]

20 **Menzel J**, Hoepffner N, Sulkowski U, Reimer P, Heinecke A, Poremba C, Domschke W. Polypoid tumors of the major duodenal papilla: preoperative staging with intraductal US, EUS, and CT--a prospective, histopathologically controlled study. *Gastrointest Endosc* 1999; **49**: 349-357 [PMID: 10049419]

21 **Kalaitzakis E**, Webster GJ, Oppong KW, Kallis Y, Vlavianos P, Huggett M, Dawwas MF, Lekharaju V, Hatfield A, Westaby D, Sturgess R. Diagnostic and therapeutic utility of single-operator peroral cholangioscopy for indeterminate biliary lesions and bile duct stones. *Eur J Gastroenterol Hepatol* 2012; **24**: 656-664 [PMID: 22433791 DOI: 10.1097/MEG.0b013e3283526fa1]

22 **Ramchandani M**, Reddy DN, Gupta R, Lakhtakia S, Tandan M, Darisetty S, Sekaran A, Rao GV. Role of single-operator peroral cholangioscopy in the diagnosis of indeterminate biliary lesions: a single-center, prospective study. *Gastrointest Endosc* 2011; **74**: 511-519 [PMID: 21737076 DOI: 10.1016/j.gie.2011.04.034]

23 **Siddiqui AA**, Mehendiratta V, Jackson W, Loren DE, Kowalski TE, Eloubeidi MA. Identification of cholangiocarcinoma by using the Spyglass Spyscope system for peroral cholangioscopy and biopsy collection. *Clin Gastroenterol Hepatol* 2012; **10**: 466-471; quiz e48 [PMID: 22178463 DOI: 10.1016/j.cgh.2011.12.021]

24 **Tamada K**, Kanai N, Ueno N, Ichiyama M, Tomiyama T, Wada S, Oohashi A, Nishizono T, Tano S, Aizawa T, Ido K, Kimura K. Limitations of intraductal ultrasonography in differentiating between bile duct cancer in stage T1 and stage T2: in-vitro and in-vivo studies. *Endoscopy* 1997; **29**: 721-725 [PMID: 9427490]

25 **Domagk D**, Diallo R, Menzel J, Schleicher C, Bankfalvi A, Gabbert HE, Domschke W, Poremba C. Endosonographic and histopathological staging of extrahepatic bile duct cancer: time to leave the present TNM-classification? *Am J Gastroenterol* 2005; **100**: 594-600 [PMID: 15743357]

26 **Tamada K**, Ido K, Ueno N, Ichiyama M, Tomiyama T, Nishizono T, Wada S, Noda T, Tano S, Aizawa T. Assessment of hepatic artery invasion by bile duct cancer using intraductal ultrasonography. *Endoscopy* 1995; **27**: 579-583 [PMID: 8608750]

27 **Tamada K**, Ido K, Ueno N, Ichiyama M, Tomiyama T, Nishizono T, Wada S, Noda T, Tano S, Aizawa T. Assessment of portal vein invasion by bile duct cancer using intraductal ultrasonography. *Endoscopy* 1995; **27**: 573-578 [PMID: 8608749]

28 **Rösch T**, Meining A, Frühmorgen S, Zillinger C, Schusdziarra V, Hellerhoff K, Classen M, Helmberger H. A prospective comparison of the diagnostic accuracy of ERCP, MRCP, CT, and EUS in biliary strictures. *Gastrointest Endosc* 2002; **55**: 870-876 [PMID: 12024143]

29 **Domagk D**, Wessling J, Conrad B, Fischbach R, Schleicher C, Böcker W, Senninger N, Heinecke A, Heindel W, Domschke W, Kucharzik T. Which imaging modalities should be used for biliary strictures of unknown aetiology? *Gut* 2007; **56**: 1032 [PMID: 17566044 DOI: 10.1136/gut.2007.123315]

30 **Tamada K**, Nagai H, Yasuda Y, Tomiyama T, Ohashi A, Wada S, Kanai N, Satoh Y, Ido K, Sugano K. Transpapillary intraductal US prior to biliary drainage in the assessment of longitudinal spread of extrahepatic bile duct carcinoma. *Gastrointest Endosc* 2001; **53**: 300-307 [PMID: 11231387]

31 **Domagk D**, Wessling J, Reimer P, Hertel L, Poremba C, Senninger N, Heinecke A, Domschke W, Menzel J. Endoscopic retrograde cholangiopancreatography, intraductal ultrasonography, and magnetic resonance cholangiopancreatography in bile duct strictures: a prospective comparison of imaging diagnostics with histopathological correlation. *Am J Gastroenterol* 2004; **99**: 1684-1689 [PMID: 15330902 DOI: 10.1111/j.1572-0241.2004.30347.x]

P- Reviewers Sharma M, Mehrabi A, Oh HC **S- Editor** Song XX
L- Editor A **E- Editor** Li JY



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i6.882

World J Gastroenterol 2013 February 14; 19(6): 882-888
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Chronic intestinal ischemia and splanchnic blood-flow: Reference values and correlation with body-composition

Helle Damgaard Zacho, Jens Henrik Henriksen, Jan Abrahamsen

Helle Damgaard Zacho, Jan Abrahamsen, Department of Clinical Physiology, Viborg Regional Hospital, 8800 Viborg, Denmark
Jens Henrik Henriksen, Department of Clinical Physiology, Hvidovre Hospital, University of Copenhagen, 2650 Hvidovre, Denmark
Author contributions: Zacho HD designed and performed the experiments, analysed data, wrote the manuscript and has approved of the final draft submitted; Henriksen JH analyzed data, edited the manuscript, and has approved of the final draft submitted; Abrahamsen J designed and performed the experiments, supervised the analysis of data, edited the manuscript, and has approved of the final draft submitted; all authors discussed the results and implications and commented on the manuscript at all stages.
Supported by The Rosa and Asta Jensen Foundation; the Danielsen Foundation and the Region Midts Research Foundation
Correspondence to: Dr. Helle Damgaard Zacho, Department of Clinical Physiology, Viborg Regional Hospital, Heibergs Allé 4, 8800 Viborg, Denmark. helle.damgaard@viborg.rm.dk
Telephone: +45-78-443306 Fax: +45-78-443399
Received: July 25, 2012 Revised: November 5, 2012
Accepted: November 24, 2012
Published online: February 14, 2013

Abstract

AIM: To determine the splanchnic blood flow and oxygen uptake in healthy-subjects and patients and to relate the findings to body-composition.

METHODS: The total splanchnic blood flow (SBF) and oxygen uptake (SO_2U) were measured in 20 healthy volunteers (10 women) and 29 patients with suspected chronic intestinal ischemia (15 women), age 40-85 years, prior to and after a standard meal. The method is based on the Fick principle using the continuous infusion of an indicator (99mTechnetium-labelled mebrofenin) and catheterization of an artery and the hepatic vein. An angiography of the intestinal arteries was performed during the same investigation. A whole-body dual-energy x-ray absorptiometry scan was performed in healthy volunteers to determine body composition.

RESULTS: Angiography revealed no atherosclerotic lesions in the intestinal arteries. The mean baseline SBF was 1087 mL/min (731-1390), and this value increased significantly to 1787 mL/min after the meal in healthy volunteers ($P < 0.001$). The baseline SBF in patients was 1080 mL/min, which increased to 1718 mL/min postprandially ($P < 0.001$). The baseline SBF was independent of age, sex, lean body mass and percentage of body fat. The mean meal-induced increase in SBF was equal to 282 mL/min + 5.4 mL/min × bodyweight, ($P = 0.025$). The SO_2U in healthy volunteers and patients was 50.7 mL/min and 48.0 mL/min, respectively, and these values increased to 77.5 mL/min and 75 mL/min postprandially, respectively. Both baseline and postprandial SO_2U were directly related to lean body mass. Age and sex exerted no impact on SO_2U.

CONCLUSION: A direct correlation between body weight and the postprandial increase in SBF was observed. The effect of body weight should be considered in the diagnosis of chronic intestinal ischemia.

© 2013 Baishideng. All rights reserved.

Key words: Splanchnic circulation; Postprandial period; Body composition; Mesenteric vascular occlusion; Middle aged

Zacho HD, Henriksen JH, Abrahamsen J. Chronic intestinal ischemia and splanchnic blood-flow: Reference values and correlation with body-composition. *World J Gastroenterol* 2013; 19(6): 882-888 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i6/882.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i6.882

INTRODUCTION

Hepatic and total splanchnic blood flow (SBF) can be

quantified using the Fick principle with the continuous infusion of an indicator, the catheterization of the liver vein and subsequent blood sampling. This method has been thoroughly described in the literature[1,2], and it has been validated[3]. SBF is used for the investigation of splanchnic oxygen uptake (SO_2U) or liver metabolism[4] and the examination of the liver clearance of endogen substances and pharmaceuticals[5]. This method is used in Scandinavian countries as a diagnostic tool for chronic intestinal ischemia (CII)[6,7]. SBF is measured at baseline and after a meal to quantify the meal-induced SBF response.

The diagnosis of CII is based on the inability to enhance SBF after a standard meal, and an increase less than 250 mL/min is abnormal[6-8]. Reference values for SBF are based on few and often young individuals without consideration of their body composition or the morphology of their intestinal arteries[6,8,9]. The lack of this information is deplorable because atherosclerotic lesions in the intestinal arteries are commonly observed in a population of otherwise healthy middle-aged individuals[10,11]. Furthermore, patients suffering from CII are frequently severely underweight[7,12], and the large variation in the anthropometrics of individuals may influence SBF values. Therefore, investigations are required to explore the association between SBF, SO_2U, age, and body composition.

The present study determined the SBF and SO_2U prior to and after a standard meal in a group of middle-aged healthy volunteers with angiography-proven normal intestinal arteries. This study also related SBF and SO_2U values to anthropometric measures of the body in healthy volunteers and in a cohort of patients with suspected chronic intestinal ischemia due to weight loss and abdominal pain but with angiography-proven normal intestinal arteries.

MATERIALS AND METHODS

Ethical approval

The protocol was performed under a license from the Ethical Committee, Central Denmark Region, and the Danish Data Protection Agency. Individually signed informed consent forms were obtained according to the Helsinki II declaration. No complications or side effects were encountered in the study. Twenty healthy volunteers aged 40-70 year (10 women) participated in the present study. None of the volunteers exhibited any signs of cardiovascular disease, abdominal complaints or weight loss one year prior to the investigation. Apart from appendectomy $n = 2$, no former abdominal surgery was performed in any of the volunteers. A total of 180 patients with suspected CII were routinely referred for SBF measurements due to weight loss and abdominal pain from June 2002 to October 2011. Patients with a digital subtraction angiography that revealed three normal intestinal arteries were included in the present study for comparison only. Therefore, 32 patients participated in the study; 31 (97%) patients suffered from postprandial abdominal pain, and 25/32 had experienced unintentional weight loss (mean 10.4 kg). Three patients were excluded: two patients due to ischemic colitis as verified on biopsy and one patient due to pulmonary cancer that had metastasized to the ventricle and colon. Table 1 presents the anthropometric data of the healthy volunteers and patients.

Table 1 Demographic data from 20 healthy volunteers and 29 patients suspected of chronic intestinal ischemia but normal intestinal arteries

	Healthy volunteers		Patients suspected of CII, normal angiography		
	Female $n = 10$	Male $n = 10$	Female $n = 15$	Male $n = 14$	*P* value
Age (yr)	53.8 (40-64)	54.5 (43-69)	63 (44-85)	60.4 (45-76)	0.01
Weight (kg)	72 (55.0-92.0)	83 (65.0-94.8)	57.9 (34.0-114)	74.9 (52.0-98.0)	0.02
Height (cm)	165 (160-170)	177 (170-188)	165 (153-172)	176 (170-186)	0.88
BMI (kg/m^2)	26.5 (21.2-34.5)	26.6 (21.7-30.6)	21.4 (12.5-41.9)	24 (15.0-31.6)	0.02
Body fat (%)	34.4 (28.0-48.2)	21.9 (13.1-29.4)	NA	NA	
LBM (kg)	47.4 (39.6-54.6)	65.5 (55.8-70.6)	NA	NA	
BSA (m^2)	1.81 (1.56-2.07)	2.01 (1.77-2.16)	1.61 (1.25-2.29)	1.89 (1.64-2.19)	0.02

All results are given as mean and range. The *P* value is the difference between healthy volunteers and patients. CII: Chronic intestinal ischemia; LBM: Lean body mass; BSA: Body surface area; BMI: Body mass index; NA: Not available.

All of the healthy volunteers and patients underwent the following protocol.

Catheterization

Subjects underwent catheterization of the femoral artery and vein in the morning following an overnight fast using the Seldinger technique under local analgesia. A 5F sheath was used for the artery, and a 7F sheath was used for the vein. The venous catheter (Schwan-Ganz, Edwards, CA, United States) was placed in a central hepatic vein using fluoroscopy, and the arterial catheter (pigtail catheter, Cordis, NJ, United States) was positioned in the abdominal aorta.

SBF and SO_2U

The SBF equals the hepatic blood flow in normal subjects, and it was measured using a standardized protocol of the indirect Fick principle with 99mTechnetium labeled Bridatec (Mebrofenin®, GE Healthcare, Suluggia, Italy) [99mTc-Mebrofenin (MBF)] as the indicator. This method was originally introduced by Bradley[1], and it was used with corrections for unsteady state and small urinary excretion of 99mTc-MBF, as recommend by Henriksen *et al*[2].

Briefly, a bolus injection of 99mTc-MBF was administered followed by a constant infusion at 2.02 mL/min (range: 1.96-2.17 mL/min), which equaled an infusion rate of 0.323 MBq/min. Less than 80 MBq was used in total. An equilibration period of 20 min was interposed

before blood samples were collected to obtain steady state measurements. Splanchnic plasma flow (SPF) was calculated as SPF = E/(Ca-Cv), where E is the hepatobiliary excretion rate of 99mTc-MBF, which equals the corrected infusion rate[2]. Ca and Cv are the concentrations of 99mTc-MBF in the abdominal aorta and the hepatic vein, respectively. The level of 99mTc-MBF in plasma samples was determined using a Cobra Ⅱ Auto-Gamma counter (Packard Bioscience Company, Frankfurt, Germany). At least 10 000 counts were obtained and corrected for decay, background and dead time.

SBF was calculated as SPF/(1-hematocrit fraction). The splanchnic oxygen uptake (SO_2U) was calculated as hemoglobin × (arterial oxygen saturation-hepatic venous oxygen saturation) × SBF × 1.34 mL O_2/g of hemoglobin. The blood samples were analyzed using an ABL 700 series (Radiometer Medical A/S, Brønshøj, Denmark), which was operated according to the manufacturer's instructions.

Blood samples were taken from a central hepatic vein and the abdominal aorta simultaneously every ten minutes during the first hour. A mean baseline value was calculated. The participants ingested a 4000 kJ/400 mL standard liquid meal that consisted of 33% protein, 33% carbohydrates and 33% fat after one hour. Blood samples were collected every ten minutes for an additional hour, and blood glucose levels were measured at each sampling time as a control of gastric emptying and intestinal absorption. The extraction fraction (EF) of 99mTc-MBF was calculated as (Ca-Cv)/Ca.

The wedged and free hepatic vein pressures were measured using a capacitance transducer at the end of the session to exclude the presence of portal hypertension and resulting porto-systemic shunting. The mean gradient wedged-to-free hepatic vein pressure was 2.5 mmHg (range 1-5 mmHg).

Angiography

An angiography was performed during the same session *via* a 4F pigtail catheter in the abdominal aorta as a control for the morphology of the intestinal arterial blood supply. The angiography included an antero-posterior and lateral horizontal abdominal projection to visualize the individual origin of the mesenteric artery branches. Iomeprol (Iomeron® 200 mg iodine/mL, Bracco, Milan, Italy; 15 mL for each image) was used as a contrast agent, and a total of 45 mL was administered on average.

Each artery was classified as normal (0%-9% lumen reduction) or exhibiting slight stenosis (10%-49% lumen reduction), moderate stenosis (50%-69% lumen reduction), significant stenosis (70%-99% lumen reduction) or occlusion. The investigator who evaluated the angiographies was blinded to the results of the SBF, and both positive and negative controls were included.

Dual-energy x-ray absorptiometry-scanning

Only healthy volunteers were subjected to a dual-energy x-ray absorptiometry (DEXA) scan. Body composition, which was evaluated as the percentage of body fat and fat-free mass, was measured using whole-body scanning in a Delfi Discovery A S/N 70879 and software version 12.6 (Hologic, MA, United Stastes) for quantification. Each participant was immobilized in supine position during evaluation with the arms and legs away from the body. The equipment was calibrated daily according to the manufacturer's instructions.

Statistical analysis

Statistical analysis was performed using STATA®11 (StataCorp LP, Station, Texas, United States). All results are presented as mean values, SD and ranges. Paired and unpaired Student's *t* tests were used for intergroup comparisons. Relationships between variables were analyzed using linear or multiple linear regression analysis and repeated measurements when appropriate. A P value < 0.05 was considered statistically significant.

RESULTS

The average values and ranges of SBF and SO_2U during fasting and after the meal are presented in Table 2. Significant differences ($P < 0.01$) between these values were observed at baseline and after the standard meal. The mean body weight among patients (65.4 kg) was significantly ($P = 0.016$) smaller than the group of healthy volunteers (77.5 kg). Eleven of the 29 patients were underweight [defined as body mass index (BMI) < 20]. None of the healthy volunteers were underweight. However, no differences in the mean baseline SBF were observed between patients (1080 mL/min) and healthy volunteers (1087 mL/min) ($P = 0.95$) or the mean postprandial SBFs, which were 1718 mL/min in patients and 1787 mL/min in healthy volunteers. Figure 1 presents the mean SBF and standard deviation as a function of time in healthy volunteers and the patient group.

Healthy volunteers

Visceral arteriography revealed no significant stenotic vessels or occlusions in the group of healthy volunteers. A slight stenosis (10%-49% lumen reduction) of the celiac artery was present in five cases. The presence of these minor stenoses did not impact the SBF at baseline (1001 mL/min, $P = 0.31$) or alter the postprandial increase (648 mL/min, $P = 0.49$). Baseline SBF and SO_2U values did not change over time (1045 mL/min to 1068 mL/min, $P = 0.89$ and 48 mL O_2/min to 50 mL O_2/min, $P = 0.66$, respectively). Variations in SBF were significantly larger between individuals than within individuals ($P = 0.002$), and the mean baseline SBF was 1087 mL/min (range 731-1390 mL/min), which increased to 1787 mL/min (range 1387-2343 mL/min) postprandially ($P < 0.001$). The corresponding SO_2U increased from 50.7 mL O_2/min (range 32.1-84.5 mL O_2/min) to 77.5 mL O_2/min (range 49.1-118.9 mL O_2/min) ($P < 0.01$).

The baseline values and the increase in SBF after meals were independent of age, sex, body weight, body surface area, and lean body mass in the group of healthy volunteers. However, SO_2U differed significantly between gen-

Table 2 Results of glucose, splanchnic blood flow and oxygen consumption from 20 healthy volunteers and 29 patients suspected of chronic intestinal ischemia but normal intestinal arteries

	Healthy volunteers		Patients suspected of CII, normal angiography		
	Female n = 10	Male n = 10	Female n = 15	Male n = 14	*P* value
Fasting plasma glucose (mmol/L)	5.7 (5.1-6.9)	5.8 (5.2-6.9)	5.7 (4.5-11.6)	5.6 (4.7-7.5)	0.59
Postprandial plasma glucose (mmol/L)	6.8[b] (5.9-7.8)	7.2[b] (6.3-8.8)	7.0[b] (5.4-12.1)	7.2[b] (5.9-9.9)	0.27
Mean fasting SBF (mL/min)	1044 (731-1319)	1129 (800-1390)	1040 (619-2781)	1123 (429-2740)	0.95
Mean postprandial SBF (mL/min)	1731[b] (1386-1987)	1844[b] (1485-2343)	1581[b] (906-2340)	1863[b] (1332-3163)	0.54
Postprandial rise in SBF (mL/min)	686 (515-915)	714 (314-1145)	542 (-441-1014)	707 (293-1325)	0.47
Mean fasting oxygen consumption (mL/min)	43.4 (32.1-53.8)	58.1 (40.5-84.5)	44.5 (34.6-76.1)	51.8 (15.2-78.2)	0.48
Mean postprandial oxygen consumption (mL/min)	65.5[b] (49.1-84.0)	89.6[b] (60.1-118.9)	68.3[b] (46.2-104.7)	82.2[b] (45.0-118.2)	0.63

All results are given as mean and range. The *P* value is the comparison difference between healthy volunteers and patients. [b]$P < 0.001$ *vs* fasting values. CII: Chronic intestinal ischemia; SBF: Splanchnic blood flow.

ders ($P = 0.001$). This difference disappeared when gender was corrected for lean body mass ($P = 0.99$). Therefore, the gender-related differences in baseline SO_2U and the postprandial increase were solely attributed to the gender-related difference in lean body mass ($P = 0.001$). Lean body mass did not influence SBF. Therefore, the SO_2U increase was accommodated by a larger oxygen extraction from the blood, which lowered hepatic venous blood saturation (data not shown).

The percentage of body fat influenced baseline SO_2U, but it did not influence the postprandial increase ($P = 0.003$). The baseline SO_2U decreased by 4.4 mL/min for a five percent increase in body fat percentage.

Figure 2 illustrates the EF as a function of time in each healthy volunteer. The arrow indicates the time of the standard meal. The mean EF of 99mTc-MBF decreased significantly ($P < 0.01$) from 0.46 to 0.39 during the baseline period. A steep drop in EF from 0.39 to 0.24 ($P < 0.01$) and an increase in SBF were observed 20 min after the meal. The EF did not change thereafter.

Patients

Figure 3A presents the mean baseline SBF for individual patients and healthy volunteers as a function of weight. Two of the patients exhibited high baseline SBF values (2740 and 2781 mL/min). Figure 3B presents the postprandial increase in SBF in individuals as a function of weight. One of the outliers with hyperperfusion at baseline experienced a decreased SBF after the meal; this individual had the negative increase in Figure 3B.

The increase in baseline and postprandial SBF did not depend on weight ($P = 0.47$ and $P = 0.12$, respectively). However, weight did exert a significant ($P = 0.025$) impact on the postprandial increase but not the baseline SBF in all individuals ($n = 49$) independent of health status. Therefore, an average person weighing 60 kg would demonstrate a 605 mL/min (95%CI: 510-701 mL/min) postprandial increase in SBF. Each additional kg in bodyweight would augment the postprandial increase by 5.4 mL/min. No significant difference between genders or any relationship to age was observed. Baseline and postprandial SO_2U values were 48.0 mL O_2/min and 75.0 mL O_2/min, respectively, and these values in patients did not differ significantly from the healthy volunteers ($P = 0.48$ and $P = 0.68$). Patients also exhibited a significant difference between genders in postprandial SO_2U ($P = 0.03$).

DISCUSSION

Despite the redundancy in the visceral circulation, with its numerous interconnections between the three abdominal arteries, the celiac trunk, and the superior and inferior mesenteric arteries, symptoms of CII ultimately arise when the genuine and collateral arteries can no longer accommodate the postprandial oxygen demand. Therefore, investigations of the morphology of the intestinal arteries cannot stand alone; functional tests that evaluate the physiological consequences are required[12]. An understanding of splanchnic hemodynamics and SO_2U in healthy volunteers is crucial. None of the healthy volunteers with stable weight exhibited a BMI < 20. Therefore, a group of patients who underwent SBF measurements due to a suspicion of CII with weights of 34 to 114 kg and angiography-proven normal intestinal arteries served as a control group. The mean baseline SBF of 1087 mL/min and the increase to 1787 mL/min after the ingestion of a standard meal that was observed in this study are consistent with Madsen *et al*[8], who performed a study in a comparable population of healthy volunteers but without knowledge of arterial morphology.

Previous investigations of SBF have been performed in volunteers who were 20 to 30 years of age. This age group is not representative of the population of interest for CII. These studies reported a total splanchnic

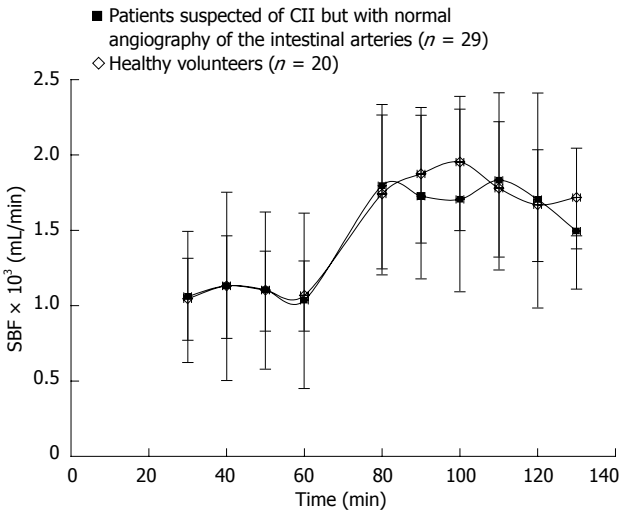


Figure 1 Splanchnic blood flow ± SD at each sampling time. The standard meal of 4000 kJ was served after 60 min. CII: Chronic intestinal ischemia; SBF: Splanchnic blood flow.

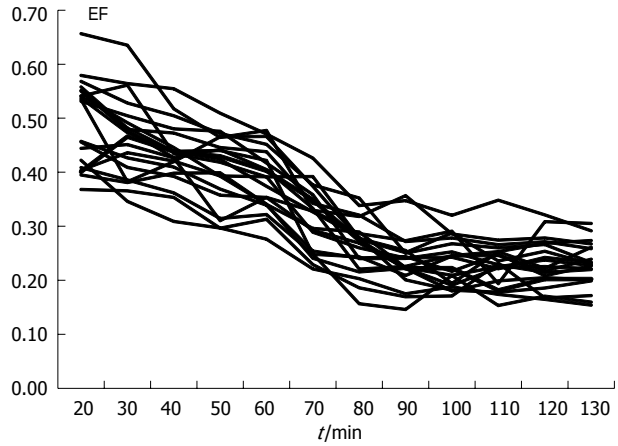


Figure 2 Extraction fraction as a function of time for each of the healthy individuals. The standard meal is served after 60 min as marked by the line, which causes a sharper decrease in extraction fraction (EF) during the first 20 min after the meal.

plasma flow of 900 mL/min during fasting[4,9,13], which corresponds to an SBF of 1500 mL/min given a normal hematocrit. These flow values are 35% higher than the results in the present study. Although we did not observe an age-related effect in participants aged 40 to 85 years, age may be a factor in comparisons of young adults to middle-aged and elderly individuals.

The frequent presence of atherosclerotic changes in asymptomatic 60- to 70-year-old adults renders the knowledge of arterial morphology in a study population essential. However, this knowledge has been neglected in previous studies. The present study demonstrated no atherosclerosis in any mesenteric arteries in healthy volunteers, and only patients with normal intestinal arteries were included.

Healthy volunteers demonstrated no correlation between SBF and body composition, which was described as body weight, body surface area and lean body mass. This result contrasts a previous study[8] in which both baseline SBF and SO_2U were directly related to the body surface area. One reason for this discrepancy may be that the ranges of body surface area or lean body mass in the healthy volunteers in the present study were too small to detect a correlation. This limitation was encountered because of the use of a group of patients with a weight range from 34 to 114 kg. The inclusion of weight in the analysis further augmented the postprandial increase by 5.4 mL/min for each kg bodyweight. Even patients who weighed 34 kg, which represented the lightest patient at our clinic, should have experienced a mean postprandial SBF increase of 465 mL/min (95%CI: 275-656) using this calculation. The exclusion of two patients with hyperperfusion at baseline slightly changed these values (the mean postprandial SBF increased 4.9 mL/min for each kg of bodyweight). These results support previous studies[6,8], which have reported that all individuals should demonstrate a postprandial response larger than 250 mL/min independent of body weight. However, the present study recommends the consideration of body weight when postprandial SBF is used to diagnose CII.

Interestingly, weight did not influence baseline SBF. A minor tendency toward an increase in baseline SBF of 2.2 mL/min (95%CI: -1.6 to 6.1, $P = 0.25$) for each kg increase in body weight was observed. However, a significant correlation between weight and baseline SBF must be rejected.

A significant difference in SO_2U between genders was observed in both groups. These differences between genders were uniquely correlated to the differences in lean body mass in the healthy volunteers who underwent a DEXA scan. This difference may also apply to the patient group, but this group did not undergo DEXA scans. Therefore, the correlation of gender differences in SO_2U to differences in body mass remains unknown in this group. The whole-body DEXA scan can reveal the body composition in each region of the body. A large total lean body mass is closely associated with a large truncal lean body mass, which may explain the increased demand for oxygen in the splanchnic territory that was observed in the present study.

The observed decrease in the EF of 99mTc-MBF in healthy volunteers was most pronounced in the 20 min following the meal. This decrease is an inherent consequence of the Fick principle in which the EF decreases with an increase in flow according to the following formula: SPF = E/(Ca-Cv). However, the decrease in EF throughout the baseline period, which was characterized by a constant SBF, remains unexplained. The decrease in EF during continuous infusion has been observed previously in pigs[3] and patients with fatty liver and cirrhosis[2]. This observation may be due to the limited capacity of hepatocytes to process 99mTc-MBF from the blood to the bile.

International guidelines[14,15] on CII suggest the exclusive use of investigations that describe the morphology of the intestinal arteries for the diagnosis of CII. The diagnostic criteria include a combination of relevant symptoms, such as postprandial abdominal pain and

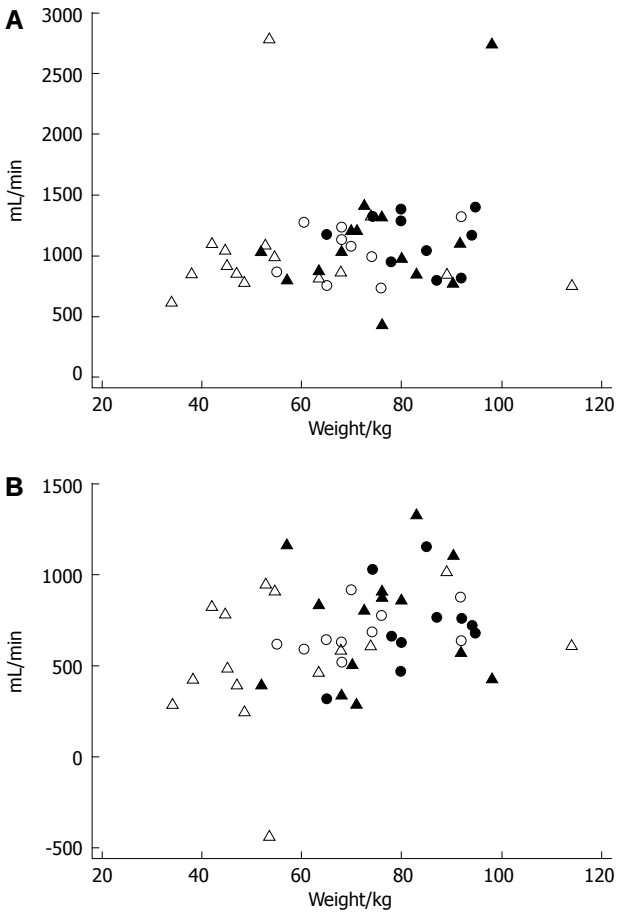


Figure 3 Postprandial increase in splanchnic blood flow for each individual as a function of bodyweight. A: Baseline splanchnic blood flow (SBF): the linear correlation between bodyweight and baseline SBF is given by the equation. SBF = 858 mL/min + 3.2 mL/min × bodyweight, the correlation is however not significant (P = 0.35); B: Postprandial increase in SBF: the linear correlation between bodyweight and the postprandial increase in SBF can be described by the equation: SBF (increase) = 282 mL/min + bodyweight × 5.4 mL/min (P = 0.025). △: Female patients with normal angiography; ▲: Male patients with normal angiography; ○: Healthy female volunteers; ●: Healthy male volunteers.

weight loss, and at least two significant stenoses/occlusions in the intestinal arteries. This approach ignores the well-documented knowledge of the frequent presence of inconsequential arterial stenosis[11,16,17]. Furthermore, patients with single-vessel disease or non-occlusive mesenteric ischemia are neglected.

The major drawbacks of physiological investigations, such as SBF measurements or tonometry, are that these techniques are often time consuming[18,19] and invasive. Therefore, the use of non-invasive investigations, such as duplex ultrasound (DUS) and MRI, are widely used. The wide availability and non-invasive nature of DUS make it an attractive screening tool. The overall accuracy, sensitivity and specificity for the identification of stenotic or occluded vessels for both the CA and the SMA are above 90% compared to DSA[20]. The meal-induced differences in blood flow velocity responses and the resistivity index in the celiac and superior mesenteric arteries can be assessed using DUS[21] as a surrogate marker for blood flow in healthy young volunteers. However, the calculation of blood flow volume is dependent on an accurate cross-sectional area of the vessel, which is difficult to obtain using DUS, especially in the intestinal arteries, and this area is almost impossible to obtain in patients with disseminated atherosclerosis. Therefore, DUS exhibits a disadvantage because only the morphology of the vessels is examined. Another major disadvantage of DUS is the requirement of an experienced examiner because obesity and overlying bowel gas may compromise the results and the lack of the visualization of the inferior mesenteric artery.

MRI has demonstrated promising results for the quantification of blood flow in the portal and superior mesenteric veins[22,23], and MR angiography has a short acquisition time (17 s)[24]. Another promising study reported the possible quantification of small bowel perfusion for the differentiation of normal individuals from patients with CII[25]. However, the capability of a functional assessment of CII using MRI has not been applied in a clinical setting despite the promise of previous reports. This fact assigns the measurement of SBF as one of a few clinical applicable investigations for the functional evaluation of CII.

In conclusion, the present study recommends the consideration of body weight in the diagnosis of CII using the postprandial increase in SBF.

COMMENTS

Background

Chronic intestinal ischemia is characterized by postprandial abdominal pain and weight loss. Atherosclerosis of the intestinal arteries is the most common cause of chronic intestinal ischemia. However, the rich collateral blood supply in the intestines may further develop in the presence of arterial stenosis to provide an adequate blood flow despite the significant stenosis of the intestinal arteries. Therefore, the application of physiological testing is required. The measurement of the splanchnic blood flow prior to and after a meal allows for an assessment of the physiological consequences of arterial stenosis in the intestines.

Research frontiers

Reference values for the total splanchnic blood flow and splanchnic oxygen uptake are lacking. The effect of body size and body composition should be considered because the majority of patients are underweight.

Innovations and breakthroughs

Previous investigations of the splanchnic blood flow have not considered the morphology of the intestinal arteries or the possible effect of body composition. An intestinal angiography and a whole-body dual-energy X-ray absorptiometry scan were performed in each of the healthy volunteers in the present study. A group of patients was included as a second group to achieve a weight range that represented individuals with very low bodyweight. All patients exhibited a completely normal angiography of the intestinal arteries. The splanchnic blood flow during fasting was independent of age, sex, body weight and body composition, but the meal-induced increase was directly related to body weight.

Applications

The study suggests that the meal-induced increase in splanchnic blood flow is directly related to body weight. This relationship should be considered in the diagnosis of chronic intestinal ischemia based on splanchnic blood flow measurements.

Terminology

Chronic intestinal ischemia is a clinical entity that is characterized by abdominal pain after eating. The pain causes the patient to eat smaller meals at longer intervals, which often leads to weight loss. The total splanchnic blood flow equals the blood flow through the liver, which can be calculated using the Fick principle.

Peer review

The paper makes sense to search for and refine new diagnostic tools, espe-

cially with regard to the functional aspects of the chronic mesenteric ischemia. The study design is valid and the data is sufficient.

REFERENCES

1 **Bradley SE**, Ingelfinger FJ, Bradley GP, Curry JJ. The estimation of hepatic blood flow in man. *J Clin Invest* 1945; **24**: 890-897 [PMID: 16695286]

2 **Henriksen JH**, Winkler K. Hepatic blood flow determination. A comparison of 99mTc-diethyl-IDA and indocyanine green as hepatic blood flow indicators in man. *J Hepatol* 1987; **4**: 66-70 [PMID: 3571934]

3 **Zacho HD**, Kristensen NB, Henriksen JH, Abrahamsen J. Validation of mTechnetium-labeled mebrofenin hepatic extraction method to quantify meal-induced splanchnic blood flow responses using a porcine model. *J Appl Physiol* 2012; **112**: 877-882 [PMID: 22174389 DOI: 10.1152/japplphysiol.01125.2011]

4 **Enevoldsen LH**, Simonsen L, Macdonald IA, Bülow J. The combined effects of exercise and food intake on adipose tissue and splanchnic metabolism. *J Physiol* 2004; **561**: 871-882 [PMID: 15498802 DOI: 10.1113/jphysiol.2004.076588]

5 **Peeters MY**, Aarts LP, Boom FA, Bras LJ, Tibboel D, Danhof M, Knibbe CA. Pilot study on the influence of liver blood flow and cardiac output on the clearance of propofol in critically ill patients. *Eur J Clin Pharmacol* 2008; **64**: 329-334 [PMID: 17994316 DOI: 10.1007/s00228-007-0399-9]

6 **Hansen HJ**, Engell HC, Ring-Larsen H, Ranek L. Splanchnic blood flow in patients with abdominal angina before and after arterial reconstruction. A proposal for a diagnostic test. *Ann Surg* 1977; **186**: 216-220 [PMID: 889368]

7 **Zacho HD**, Abrahamsen J. Functional versus radiological assessment of chronic intestinal ischaemia. *Clin Physiol Funct Imaging* 2010; **30**: 116-121 [PMID: 19968673]

8 **Madsen JL**, Søndergaard SB, Møller S. Meal-induced changes in splanchnic blood flow and oxygen uptake in middle-aged healthy humans. *Scand J Gastroenterol* 2006; **41**: 87-92 [PMID: 16373281]

9 **Simonsen L**, Coker R, A L Mulla N, Kjaer M, Bülow J. The effect of insulin and glucagon on splanchnic oxygen consumption. *Liver* 2002; **22**: 459-466 [PMID: 12445170]

10 **Chandra A**, Quinones-Baldrich WJ. Chronic mesenteric ischemia: how to select patients for invasive treatment. *Semin Vasc Surg* 2010; **23**: 21-28 [PMID: 20298946 DOI: 10.1053/j.semvascsurg.2009.12.003]

11 **Järvinen O**, Laurikka J, Sisto T, Salenius JP, Tarkka MR. Atherosclerosis of the visceral arteries. *Vasa* 1995; **24**: 9-14 [PMID: 7725785]

12 **Mensink PB**, Moons LM, Kuipers EJ. Chronic gastrointestinal ischaemia: shifting paradigms. *Gut* 2011; **60**: 722-737 [PMID: 21115543 DOI: 10.1136/gut.2009.199695]

13 **Jørgensen M**, Tønnesen KH, Petersen KR, Jespersen J, Gram J, Vinberg N. Splanchnic extraction and clearance of tissue plasminogen activator (t-PA) after injection of recombinant t-PA (Actilyse) in resting healthy subjects is proportional to the arterial concentration. *Blood Coagul Fibrinolysis* 2002; **13**: 331-338 [PMID: 12032399]

14 American Gastroenterological Association Medical Position Statement: guidelines on intestinal ischemia. *Gastroenterology* 2000; **118**: 951-953 [PMID: 10784595]

15 **Hirsch AT**, Haskal ZJ, Hertzer NR, Bakal CW, Creager MA, Halperin JL, Hiratzka LF, Murphy WR, Olin JW, Puschett JB, Rosenfield KA, Sacks D, Stanley JC, Taylor LM, White CJ, White J, White RA, Antman EM, Smith SC, Adams CD, Anderson JL, Faxon DP, Fuster V, Gibbons RJ, Hunt SA, Jacobs AK, Nishimura R, Ornato JP, Page RL, Riegel B. ACC/AHA Guidelines for the Management of Patients with Peripheral Arterial Disease (lower extremity, renal, mesenteric, and abdominal aortic): a collaborative report from the American Associations for Vascular Surgery/Society for Vascular Surgery, Society for Cardiovascular Angiography and Interventions, Society for Vascular Medicine and Biology, Society of Interventional Radiology, and the ACC/AHA Task Force on Practice Guidelines (writing committee to develop guidelines for the management of patients with peripheral arterial disease)--summary of recommendations. *J Vasc Interv Radiol* 2006; **17**: 1383-1397; quiz 1398 [PMID: 16990459 DOI: 10.1097/01.RVI.0000240426.53079.46]

16 **Korotinski S**, Katz A, Malnick SD. Chronic ischaemic bowel diseases in the aged--go with the flow. *Age Ageing* 2005; **34**: 10-16 [PMID: 15591479]

17 **Zeller T**, Macharzina R. Management of chronic atherosclerotic mesenteric ischemia. *Vasa* 2011; **40**: 99-107 [PMID: 21500174 DOI: 10.1024/0301-1526/a000079]

18 **Mensink PB**, Geelkerken RH, Huisman AB, Kuipers EJ, Kolkman JJ. Twenty-four hour tonometry in patients suspected of chronic gastrointestinal ischemia. *Dig Dis Sci* 2008; **53**: 133-139 [PMID: 17530402 DOI: 10.1007/s10620-007-9833-1]

19 **Zacho HD**, Abrahamsen J. Chronic intestinal ischaemia: diagnosis. *Clin Physiol Funct Imaging* 2008; **28**: 71-75 [PMID: 18005164 DOI: 10.1111/j.1475-097X.2007.00779.x]

20 **Zwolak RM**, Fillinger MF, Walsh DB, LaBombard FE, Musson A, Darling CE, Cronenwett JL. Mesenteric and celiac duplex scanning: a validation study. *J Vasc Surg* 1998; **27**: 1078-1087; discussion 1088 [PMID: 9652470]

21 **Someya N**, Endo MY, Fukuba Y, Hayashi N. Blood flow responses in celiac and superior mesenteric arteries in the initial phase of digestion. *Am J Physiol Regul Integr Comp Physiol* 2008; **294**: R1790-R1796 [PMID: 18385466 DOI: 10.1152/ajpregu.00553.2007]

22 **Burkart DJ**, Johnson CD, Reading CC, Ehman RL. MR measurements of mesenteric venous flow: prospective evaluation in healthy volunteers and patients with suspected chronic mesenteric ischemia. *Radiology* 1995; **194**: 801-806 [PMID: 7862982]

23 **Laissy JP**, Trillaud H, Douek P. MR angiography: noninvasive vascular imaging of the abdomen. *Abdom Imaging* 2002; **27**: 488-506 [PMID: 12172987 DOI: 10.1007/s00261-001-0063-2]

24 **Sutter R**, Heilmaier C, Lutz AM, Weishaupt D, Seifert B, Willmann JK. MR angiography with parallel acquisition for assessment of the visceral arteries: comparison with conventional MR angiography and 64-detector-row computed tomography. *Eur Radiol* 2009; **19**: 2679-2688 [PMID: 19526242 DOI: 10.1007/s00330-009-1473-8]

25 **Lauenstein TC**, Ajaj W, Narin B, Göhde SC, Kröger K, Debatin JF, Rühm SG. MR imaging of apparent small-bowel perfusion for diagnosing mesenteric ischemia: feasibility study. *Radiology* 2005; **234**: 569-575 [PMID: 15601890]

P- Reviewers Mann O, Kopacova M **S- Editor** Zhai HH
L- Editor A **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i6.889

World J Gastroenterol 2013 February 14; 19(6): 889-896
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Mucosal healing effect of mesalazine granules in naproxen-induced small bowel enteropathy

István Rácz, Milán Szalai, Valéria Kovács, Henriett Regőczi, Gyöngyi Kiss, Zoltán Horváth

István Rácz, Milán Szalai, Valéria Kovács, Henriett Regőczi, Gyöngyi Kiss, Department of Gastroenterology, Petz Aladár County and Teaching Hospital, 9024 Győr, Hungary
Zoltán Horváth, Department of Mathematics and Computational Sciences, Széchenyi István University, 9026 Győr, Hungary
Author contributions: Rácz I designed the research; Szalai M, Kovács V, Regőczi H and Kiss Gy performed the research; Horváth Z analyzed the data; and Rácz I wrote the paper.
Supported by Dr. Falk Pharma GmbH, in part, study code: SAG-43/SBE
Correspondence to: István Rácz, MD, PhD, Department of Gastroenterology, Petz Aladár County and Teaching Hospital, 9024 Győr, Hungary. raczi@petz.gyor.hu
Telephone: +36-96-418244 Fax: +36-96-418486
Received: August 29, 2012 Revised: November 1, 2012
Accepted: November 14, 2012
Published online: February 14, 2013

Abstract

AIM: To investigate the effect of mesalazine granules on small intestinal injury induced by naproxen using capsule endoscopy (CE).

METHODS: This was a single center, non-randomized, open-label, uncontrolled pilot study, using the PillCam SB CE system with RAPID 5 software. The Lewis Index Score (LIS) for small bowel injury was investigated to evaluate the severity of mucosal injury. Arthropathy patients with at least one month history of daily naproxen use of 1000 mg and proton pump inhibitor co-therapy were screened. Patients with a minimum LIS of 135 were eligible to enter the 4-wk treatment phase of the study. During this treatment period, 3 × 1000 mg/d mesalazine granules were added to ongoing therapies of 1000 mg/d naproxen and 20 mg/d omeprazole. At the end of the 4-wk combined treatment period, a second small bowel CE was performed to re-evaluate the enteropathy according to the LIS results. The primary objective of this study was to assess the mucosal changes after 4 wk of mesalazine treatment.

RESULTS: A total of 18 patients (16 females), ranging in age from 46 to 78 years (mean age 60.3 years) were screened, all had been taking 1000 mg/d naproxen for at least one month. Eight patients were excluded from the mesalazine therapeutic phase of the study for the following reasons: the screening CE showed normal small bowel mucosa or only insignificant damages (LIS < 135) in five patients, the screening esophagogastroduodenoscopy revealed gastric ulcer in one patient, capsule technical failure and incomplete CE due to poor small bowel cleanliness in two patients. Ten patients (9 female, mean age 56.2 years) whose initial LIS reached mild and moderate-to-severe enteropathy grades (between 135 and 790 and ⩾ 790) entered the 4-wk therapeutic phase and a repeat CE was performed. When comparing the change in LIS from baseline to end of treatment in all patients, a marked decrease was seen (mean LIS: 1236.4 ± 821.9 *vs* 925.2 ± 543.4, $P = 0.271$). Moreover, a significant difference between pre- and post-treatment mean total LIS was detected in 7 patients who had moderate-to-severe enteropathy gradings at the inclusion CE (mean LIS: 1615 ± 672 *vs* 1064 ± 424, $P = 0.033$).

CONCLUSION: According to the small bowel CE evaluation mesalazine granules significantly attenuated mucosal injuries in patients with moderate-to-severe enteropathies induced by naproxen.

© 2013 Baishideng. All rights reserved.

Key words: Nonsteroidal anti-inflammatory drug; Small bowel enteropathy; Mesalazine granules; Mucosal healing; Capsule endoscopy

Rácz I, Szalai M, Kovács V, Regőczi H, Kiss Gy, Horváth Z. Mucosal healing effect of mesalazine granules in naproxen-induced small bowel enteropathy. *World J Gastroenterol* 2013; 19(6): 889-896 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i6/889.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i6.889

INTRODUCTION

Non-steroidal anti-inflammatory drugs (NSAIDs) are among the most commonly prescribed medications worldwide[1,2]. The side effects of NSAIDs on the upper gastrointestinal tract are well-known, however, toxicity to the small bowel mucosa is an increasingly recognized problem and is emerging as a more prevalent site of injury than the stomach[3-7].

Advances in small bowel imaging have led to direct observation at enteroscopy and during the last decade by video capsule endoscopy (CE) allowing a full investigation of the entire small intestine[8-15].

Capsule endoscopy is capable of demonstrating NSAIDs-induced pathology in the small bowel. CE studies comparing NSAIDs users and healthy volunteers found a higher rate of lesions in the former group (55%-76% *vs* 7%-11%)[16-19]. The most common NSAID-induced lesions in the small bowel are mucosal breaks, reddened folds, petechial or red spots, and blood in the lumen without a visualized source of bleeding. The conclusion from these initial studies was that CE is the optimal diagnostic tool to obtain direct evidence of macroscopic injury of the small intestine, resulting from short-term (2-4 wk) administration of conventional NSAIDs.

Studies in long-term NSAID users suggest that 60%-70% have an asymptomatic enteropathy characterized by increased intestinal permeability and mild mucosal inflammation[18,20]. Occult intestinal bleeding resulting from long-term NSAID usage is an increasing clinical challenge, especially in elderly patients[21,22].

Management options for NSAID enteropathy are limited. Co-administration of proton pump inhibitors failed to prevent NSAID-induced small intestinal damage[7,8]. Cessation of NSAIDs is not tolerable for most arthropathy patients. Cyclooxygenase (COX)-2 inhibitors have fewer toxic effects in the small bowel[23-25]. However, due to increased cardiovascular event risk many physicians prefer to prescribe conventional NSAIDs[17]. Although a recently published short-term study showed promising results indicating that prostaglandin (PG) reduced the incidence of small-intestinal lesions induced by diclofenac sodium[19], there are no convincing long-term clinical data on the usefulness of PG co-therapy with NSAIDs. Sulfasalazine significantly reduced NSAID-provoked intestinal permeability in an experimental study[26].

Mesalazine has not yet been studied in the treatment of NSAID-induced enteropathy. However, according to the release patterns of the oral preparations of mesalazine some therapeutic efficacy was noted in Crohn's disease, *e.g.*, with Salofalk® tablets from the duodenum to the ileum[27-29]. Pharmacokinetic data indicate a release pattern of 5-aminosalicylate from Salofalk® granules preparation starting in the same intestinal region as that for Salofalk® tablets[30].

Thus, it would be valuable in a clinical setting to determine the therapeutic effect of mesalazine granules on NSAID-induced small intestine injury. Therefore, we investigated the effect of mesalazine granules on small intestinal injury induced by NSAIDs in an open label, uncontrolled pilot study. We assessed naproxen-induced mucosal changes using CE before and after 4 wk of treatment with 3 g mesalazine granules.

MATERIALS AND METHODS

This was a single center, non-randomized, open label, uncontrolled pilot study. Entry criteria included men and women, 18-75 years of age, with at least one month history of daily naproxen use of 1000 mg for osteoarthrosis, rheumatoid arthritis, or nonspecific arthritis. To avoid gastroduodenal mucosal injury, the patients were treated with a standard dose of proton pump inhibitor. A complete small bowel CE examination within 7 d of inclusion was performed to diagnose small bowel NSAID-induced enteropathy. Patients with a Lewis CE scoring index of at least 135 were eligible to enter the 4-wk treatment phase of the study. During this treatment period 3 × 1000 mg/d mesalazine granules (Salofalk® Granu-Stix®) medication was added to ongoing 1000 mg/d naproxen and 20 mg/d omeprazole therapy. At the end of the 4-wk combined therapy period, a second small bowel CE examination was performed to re-evaluate the CE enteropathy scoring index.

Patients with evidence of small bowel strictures as previously proven by barium small bowel follow through, enteroclysis or patency test capsule, or with swallowing disorders, gastroduodenal ulcer in the patient history or proven by upper endoscopy were excluded. Further exclusion criteria were a history of IBD and colon diverticulosis, mesalazine treatment within 12 wk and corticosteroid treatment within 30 d prior to baseline endoscopy. Known previous or concurrent malignancy was also an exclusion criterion.

This study was approved by the central ethics committee of the National Health Scientific Board, and all patients provided written informed consent before their enrollment in this study.

Capsule endoscopy

We used the PillCam SB capsule endoscopy system with RAPID 5 software (Given Imaging Ltd, Yokneam, Israel). The patients had a liquid diet on the day before the CE examination. For preparation of the small bowel, 2 L of polyethylene glycol (PEG) solution on the day before and 1 L of PEG on the morning of the CE examination were administered. The CE procedure and methodology for review of images were conducted as previously described[5,7]. Briefly, the patients were equipped with a sensor array and recorder-battery belt pack, and then swallowed a capsule, which continuously transmitted video images at 2 frames per second for 8 h, at which point, the apparatus was disconnected and the images were processed. All video images were independently analyzed by 2 reviewers per video for pathology detection. All images were saved for final comprehensive analysis.

Evaluation of small bowel injury

We investigated the Lewis Index Score (LIS) for small

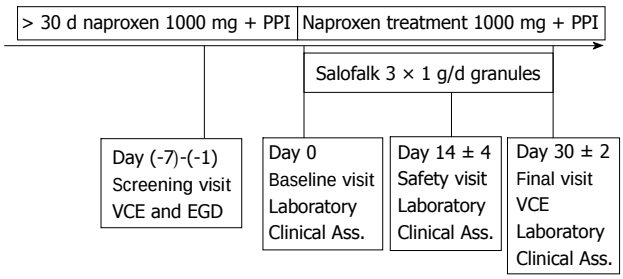


Figure 1 Study design. PPI: Proton pump inhibitor; VCE: Video capsule endoscopy; EGD: Esophagogastroduodenoscopy; ASS.: Assessment.

bowel enteropathy alterations to strengthen the validity of the results[31,32]. The LIS is incorporated as an integral part of Given's RAPID 5 version. The scoring index is based on three capsule endoscopy variables: villous appearance, ulceration, and stenosis. The changes in villous appearance and ulceration were assessed by tertiles, dividing the small bowel transit time into three equal time allotments. Villous appearance was defined as edema where villous width was equal or greater than villous height. Ulcerations were defined as mucosal breaks with white or yellow bases surrounded by red or pink collars. Ulcer size was based on the entire lesion including its surrounding collar and was measured according to the percentage of the capsule image occupied by the ulcerated lesion. The evaluation of stenosis was carried out for one entire study. The total score was the sum of the highest tertile score plus the stenosis score. The results were classified into the three categories by the final numerical score: normal or clinically insignificant change (< 135), mild change (between 135 and 790), and moderate-to-severe change (≥ 790).

The rate of successful arrival of the capsule in the cecum was assessed on the capsule endoscopic images. Gastric transit time was defined as the time taken from the first gastric image to the first duodenal image. Small bowel transit time was defined as the time from the first duodenal image to the first cecal image.

Small bowel cleanliness was graded using a 4-point scale (excellent[1] or good[2], fair[3] or poor[4]) for each tertile. Each small bowel tertile was scored separately, with the final cleansing score being the average of the sum of the three tertile cleansing scores[31].

Study protocol

At the screening visit, patients taking 1000 mg/d naproxen and 20 mg/d omeprazole first underwent esophagogastroduodenoscopy (EGD) to exclude active peptic ulcer. Those without active ulcer underwent the baseline CE examination. Eligible patients with LSI higher than 135 were administered 1000 mg mesalazine granules three times daily and 500 mg naproxen two times daily, and 20 mg omeprazole once daily immediately after meals for a period of 4 wk. Post-treatment CE was performed within 24 h after completion of the drug regimen (Figure 1). Patients who developed symptoms that warranted withdrawal from the study or with incomplete CE were excluded from the study and analysis.

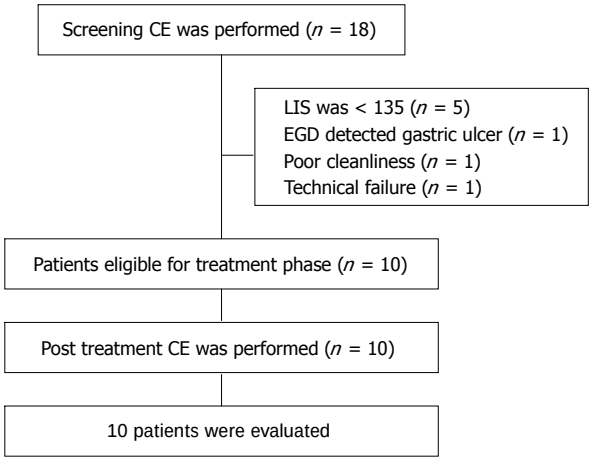


Figure 2 Flow chart of the study patients. LIS: Lewis Index Score; EGD: Esophagogastroduodenoscopy; CE: Capsule endoscopy.

Statistical analysis

The Lewis Index Score results, gastric transit time, small bowel transit time, cleanliness scores, hemoglobin results, results of baseline CE and the 4 wk control were compared and tested using the Wilcoxon signed rank test. In addition, paired *t* tests were also performed. Data were expressed as mean ± SD. *P* values < 0.05 were considered statistically significant. The effect of treatment on the presence of ulcers with a diameter of at least 3 mm was tested using the binomial sign test. McNemar tests were also performed but due to the small sample size, the binomial test was more relevant.

RESULTS

A total of 18 patients (16 females), ranging in age from 46 to 78 years (mean age 60.3 years) were screened, all had been taking 1000 mg/d naproxen for at least one month. Eight patients were excluded from the mesalazine therapeutic phase of the study for the following reasons: the screening CE showed normal small bowel mucosa or only insignificant damage (LIS < 135) in five patients, the screening EGD revealed gastric ulcer in one patient, and capsule technical failure and incomplete CE due to poor small bowel cleanliness in two patients (Figure 2). Ten patients whose initial LIS reached the mild and moderate-to-severe enteropathy grades (between 135 and 790, and ≥ 790, respectively) entered the therapeutic study phase and a second CE was carried out 4 wk later (Table 1). The CE enteropathy scores for small bowel are presented in Table 2.

At the screening CE, seven out of the ten enteropathy patients showed moderate-to-severe abnormalities according to the LIS grades (≥ 790).

In six of these seven moderate-to-severe small bowel enteropathy cases, the characteristic alterations were already seen in the first tertile, and in only three patients in the third tertile.

Table 1 Baseline characteristics of the intent-to-treat patient population (n = 10)

Characteristic	
Sex (female)	9
Age, yr (mean ± SD)	56.2 ± 9.7
Reason for NSAID therapy	
Rheumatoid arthritis	2
Osteoarthritis	8
Medication use	
Anti-hypertensive agents	3
Anti-diabetic agents	2
Low dose aspirin	NA
Statin	2

NA: Not available; NSAID: Non-steroidal anti-inflammatory drugs.

Table 2 Capsule endoscopy results according to Lewis Index Score evaluation of the small bowel mucosa at inclusion and 4 wk after mesalazine granules treatment

Subject No.	First tertile	Second tertile	Third tertile	Total LIS score
NSAID				
1	429	3040	1404	3040
2	225	450	0	450
3	1630	225	225	1630
4	1462	204	1462	1462
5	247	247	0	247
6	1104	654	562	1104
7	1312	712	135	1312
8	361	0	0	361
9	1068	1068	225	1068
10	1690	393	135	1690
NSAID + mesalazine				
1	900	1208	1237	1208
2	1462	165	0	1462
3	247	0	247	247
4	1068	339	1012	1068
5	112	0	0	112
6	1068	900	225	1068
7	1068	1068	233	1068
8	225	225	135	225
9	1104	0	0	1104
10	1690	1554	1104	1690

NSAID: Non-steroidal anti-inflammatory drugs; LIS: Lewis Index Score.

The average small bowel cleanliness grades were 1.3 ± 0.67 at the screening CE, and 1.4 ± 0.69 at the control CE examinations, respectively. The mean hemoglobin levels at screening and at the end of therapy were 131.1 ± 5.8 g/L and 127.1 ± 7.8 g/L, respectively (Table 3).

The most frequently observed NSAID-induced morphological alterations were villous edema which was present in all 10 patients at inclusion, and 19 ulcers were detected at baseline CE examinations (Figure 3). No stenosis was seen in any of the patients, either at inclusion or at the control CE examinations.

No dyspepsia or other significant abdominal symptoms were reported by the patients at any of the study visits. When comparing the LIS results assessed at inclusion and at the control CE after treatment with mesalazine granules, a marked decrease was seen (mean LIS: 1236.4 ± 821.9 *vs* 925.2 ± 543.4), however, the difference was not significant (P = 0.271).

Table 3 Capsule endoscopy results of the patients with naproxen and naproxen + mesalazine granules therapy (n = 10)

	Inclusion	After mesalazine gran therapy	P value
Total Lewis Index Score	1236.4 ± 821.9	925.2 ± 543.4	0.271
1st tertile	952.8 ± 584.9	894.4 ± 534.3	0.569
2nd tertile	699.3 ± 877.9	545.9 ± 580.9	0.649
3rd tertile	415.3 ± 561.5	319.3 ± 403.3	0.247
Ulcers with a diameter of > 3 mm, n			
1st tertile	8	7	1.000
2nd tertile	8	6	0.625
3rd tertile	3	4	1.000
Gastric transit time (min)	43.8 ± 32	49.8 ± 35.6	0.470
Small bowel transit time (min)	261.9 ± 108	250.9 ± 93.9	0.470
Cleanliness score	1.3 ± 0.67	1.4 ± 0.69	0.345
Hemoglobin	131.1 ± 5.8	127.1 ± 7.8	0.103

In contrast, a significant difference in pre- and post-treatment LIS was detected in the seven patients who had moderate-to-severe enteropathy gradings at the inclusion CE visit (mean LIS: 1615 ± 672 *vs* 1064 ± 424, P = 0.033). Further analysis showed that two patients (subject number 9 and 10) in the group with moderate-to-severe NSAID-induced enteropathy at inclusion, the total LIS score was unchanged or worsened after mesalazine treatment. However, even in these two patients a decrease in LIS scores was detected either in the first or second small bowel tertile which was also seen in the other 5 cases (Figure 4).

DISCUSSION

In the present pilot study we found that mesalazine granules reduced the severity of small intestine lesions induced by the administration of naproxen, a non-selective NSAID. Patients, who received naproxen therapy with mesalazine granules showed markedly lower enteropathy scores compared with the period when naproxen was taken alone. Importantly, the co-administration of mesalazine granules significantly attenuated the mucosal damage caused by naproxen in patients with moderate-to-severe enteropathy.

NSAIDs are known to increase intestinal permeability; the severity of the mucosal injury is dose-dependently related to the potency of NSAIDs to inhibit COX-1. The increase in intestinal permeability is the most important factor which provokes NSAID-induced inflammation and injury in the small intestine[2,3].

Previous studies evaluating NSAID-associated mucosal injury have shown various ways to interpret and compare CE data. Goldstein *et al*[17], who compared the effects of naproxen versus celecoxib on the small bowel, simply counted the number of mucosal breaks per tertile to measure adverse drug effects. Graham *et al*[18] assessed small bowel mucosal injury in chronic NSAIDs users. In their study, CE lesions were scored as normal, red spots, small erosions, large erosions or ulcers. Maiden *et al*[16]

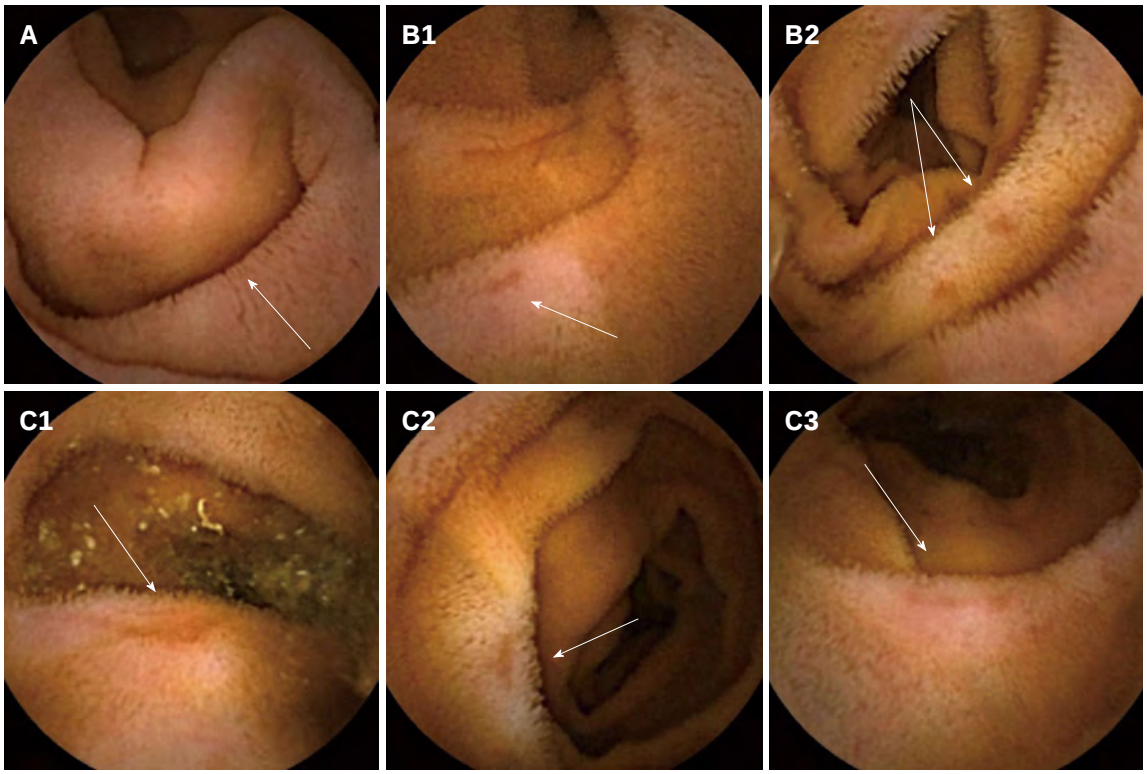


Figure 3 Examples of capsule endoscopy images in non-steroidal anti-inflammatory drug-induced enteropathy patients. A: Villous edema (arrow); B: Erosions (arrows); C: Non-steroidal anti-inflammatory drug- induced ulcers (arrows).

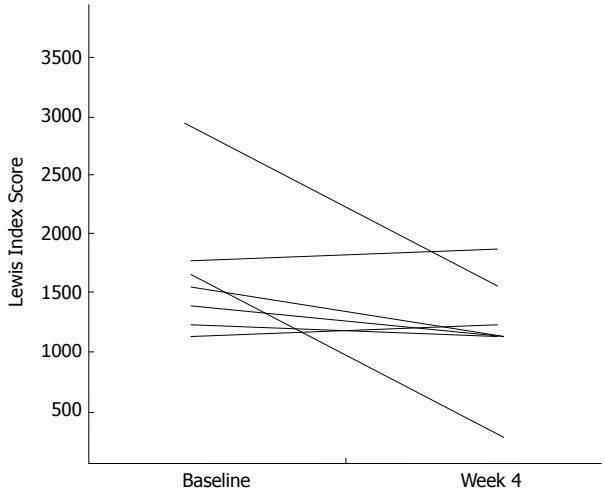


Figure 4 Lewis Index Score results before and after mesalazine granules treatment in patients with moderate-to-severe naproxen-induced enteropathy (n = 7).

graded diclofenac-induced lesions as category 1 (reddened folds), category 2 (denuded area), category 3 (petechia/red spot), category 4 (mucosal break), or category 5 (presence of blood without a visualized lesion).

A new score index has been developed by Gralnek *et al*[32] to assess mucosal changes in the small bowel detected by CE. The LIS offers objective scoring of small bowel injuries and has the potential to measure and document mucosal healing in response to therapy[33-36]. Therefore, in our study we used the LIS to assess the mucosal effect of naproxen as well as the effects of mesalazine granules therapy.

In the present pilot study we found mild and moderate-to-severe changes in the LIS in 66% of patients (10/15) who received NSAID and proton pump inhibitor therapy. These results are in a good correlation with the data from other CE studies in which NSAID-induced intestinal injury was directly evaluated. Maiden *et al*[16] found intestinal lesions using CE in 68% of healthy volunteers; Goldstein *et al*[17] reported that 55% of subjects on naproxen developed small-bowel injuries, while Fujimori *et al*[19] found small bowel mucosal breaks in 53% of subjects taking diclofenac. In these three studies, CE examinations were performed in healthy volunteers after two wk of NSAID medication. Graham *et al*[18] performed small bowel CE in chronic NSAID users with at least 3 mo history of daily NSAIDs for rheumatologic diseases. Small bowel injury was seen in 71% of these patients. Similar to this study we also recruited patients taking NSAIDs chronically which better reflects the real life situation in the small bowel compared to short-term NSAID exposure.

We analyzed the LIS results for NSAID-induced mucosal changes according to the small bowel tertiles. This analysis revealed that mucosal injuries tended to decrease in severity in the distal part of the small bowel. This result is inconsistent with a previous report in which chronic low dose aspirin-associated ulcers were observed mainly in the distal part of the small bowel[36]. However, there are no other available data regarding the topical dis-

tribution of NSAID-induced small bowel changes.

Management of NSAID-induced enteropathy is an unsolved problem. Moreover, the clinical significance of small bowel lesions is also a challenge. Proton-pump-inhibitors have been proved to be ineffective for the prevention of NSAID-induced injury[16-19]. Celecoxib, a selective COX-2 inhibitor effectively decreased the number of mucosal breaks and the percentage of subjects with at least one mucosal break[17]. Nevertheless, due to the risk of cardiovascular events, the use of COX-2 inhibitors is limited. In a recent pilot study co-administration of misoprostol was effective in preventing small bowel injury induced by NSAID medication in healthy volunteers[19]. Although the results of this short-term trial are promising, the clinical outcome of misoprostol therapy in typical chronic NSAIDs users is still an open question.

The role of sulfasalazine has been evaluated in 40 patients with rheumatoid arthritis on NSAIDs[26]. After 3-9 mo treatment, sulfasalazine (1.5-3.5 g/d) significantly reduced intestinal permeability, as measured by ^{111}In-labelled leucocytes.

Mesalazine has not yet been studied for the prevention or treatment of NSAID-induced enteropathy. Based on the hypothesis that mesalazine granules may have some therapeutic effect against NSAID-induced small bowel injury we designed a short-term therapeutic trial instead of a preventive trial. The primary objective of our pilot study was to assess the mucosal changes according to the LIS results detected by CE after 4 wk of treatment with mesalazine granules in patients receiving naproxen therapy. To avoid to inclusion of patients with clinically insignificant small bowel injuries, only patients with a LIS > 135 were recruited into the mesalazine granules therapeutic phase. When comparing the pre- and post-therapeutic LIS results in all 10 patients, a marked but not significant improvement in NSAID-induced enteropathy was observed. However, in the analysis of the 7 patients with moderate-to-severe initial enteropathy a significant improvement in the LIS was seen after treatment with mesalazine granules. These results demonstrate that patients whose initial enteropathy was more advanced benefited most from mesalazine treatment. Importantly, the improvement in LIS results was detected mainly in the first and second small bowel tertiles indicating that the mesalazine granules were effective even in the upper part of the small intestine. Although this is in contrast to the normal release profile of mesalazine, this may be due to the concomitant therapy with omeprazole.

According to the patient diaries and despite continuous NSAID treatment, our patients did not have dyspeptic symptoms or any other adverse effects. Significant anemia was not detected, either at inclusion or at the controls during the treatment phase. Ulcer-like clinical symptoms and dyspepsia are driven most probably by the gastroduodenal effect of NSAIDs which can be abolished or prevented by omeprazole co-therapy.

This is the first study to demonstrate using CE that mesalazine granules can reduce NSAID-induced macroscopic damage throughout the small intestine. This was also the first study to use the LIS system to evaluate the therapeutic effect of mesalazine granules on NSAID-induced small bowel injury. However, the trial has some inherent limitations. First, our study included only a small number of patients. Second, there was no parallel control group included to test the mucosal effect of the NSAID without mesalazine treatment. Third, this study was an open-label trial, which has a potential bias against neutrality.

Consequently, further studies are necessary to confirm the beneficial effect of mesalazine granules detected in the present pilot study.

ACKNOWLEDGMENTS

We thank Dr. Ralph Müller for help during our clinical research and also for the advices during the manuscript preparation.

COMMENTS

Background

The side effects of non-steroidal anti-inflammatory drugs (NSAIDs) on the upper gastrointestinal tract are well-known, however, toxicity to the small bowel mucosa is an increasingly recognized problem and is emerging as a more prevalent site of injury than the stomach. Capsule endoscopy (CE) is capable of demonstrating NSAID-induced pathology in the small bowel. CE is the optimal diagnostic tool to obtain direct evidence of macroscopic injury of the small intestine, resulting from short-term (2-4 wk) administration of conventional NSAIDs. Studies in long-term NSAID users suggest that 60%-70% have an asymptomatic enteropathy characterized by increased intestinal permeability and mild mucosal inflammation. Management options for NSAID-induced enteropathy are limited. Mesalazine has not yet been studied in the treatment of NSAID-induced enteropathy. In this article the authors investigated the effect of mesalazine granules on small intestinal injury induced by naproxen in an open label, uncontrolled pilot study. The authors assessed naproxen-induced mucosal changes using CE before and after 4 wk of treatment with 3 g mesalazine granules.

Research frontiers

Entry criteria included men and women, 18-75 years of age, with at least one month history of daily naproxen use of 1000 mg for osteoarthrosis, rheumatoid arthritis, or nonspecific arthritis. To avoid gastroduodenal mucosal injury, the patients were treated with a standard dose of proton pump inhibitor. A complete small bowel CE examination within 7 d of inclusion was performed to diagnose small bowel NSAID-induced enteropathy. Patients with a Lewis capsule endoscopy scoring index of at least 135 were eligible to enter the 4-wk treatment phase of the study. During this treatment period, 3 × 1000 mg/d mesalazine granules (Salofalk® Granu-Stix®) medication was added to ongoing 1000 mg/d naproxen and 20 mg/d omeprazole therapy. At the end of the 4-wk combined therapy period, a second small bowel CE examination was performed to re-evaluate the capsule endoscopy enteropathy scoring index.

Innovations and breakthroughs

The authors found that mesalazine granules reduced the severity of small intestine lesions induced by the administration of naproxen, a non-selective NSAID. Patients, who received naproxen therapy with mesalazine granules showed markedly lower enteropathy scores compared with the period when naproxen was administered alone. Importantly, the co-administration of mesalazine granules significantly attenuated the mucosal damage caused by naproxen in patients with moderate-to-severe enteropathy.

Applications

This study offers a better understanding of the NSAID-induced enteropathy changes evaluated by the Lewis Index Score (LIS). The results demonstrated that patients whose initial naproxen-induced enteropathy was the most advanced benefited most from treatment with mesalazine granules.

Terminology

The LIS was used to investigate small bowel enteropathy alterations to

strengthen the validity of the results. The LIS is incorporated as an integral part of Given's RAPID 5 version. The scoring index is based on three capsule endoscopy variables: villous appearance, ulceration, and stenosis. The changes in villous appearance and ulceration were assessed by tertiles, dividing the small bowel transit time into three equal time allotments. The results were classified into the three categories by the final numerical score: normal or clinically insignificant change (< 135), mild change (between 135 and 790), and moderate-to-severe change (⩾ 790).

Peer review

Management of NSAID enteropathy is an unsolved problem. The authors present a pilot study in a group of patients with NSAID macroscopic lesions found in small intestine. The authors evaluate the effect of treatment by mesalazine in this group of patients using CE and an objective score (LIS). The result has limited statistical power due to the scarce number of patients included in the study. Nevertheless, the figures are promising and, as said by the authors, open an investigational field on NSAID side effects prevention/treatment, needing further investigation.

REFERENCES

1 **Fries JF**, Williams CA, Bloch DA, Michel BA. Nonsteroidal anti-inflammatory drug-associated gastropathy: incidence and risk factor models. *Am J Med* 1991; **91**: 213-222 [PMID: 1892140 DOI: 10.1016/0002-9343(91)90118-H]

2 **Allison MC**, Howatson AG, Torrance CJ, Lee FD, Russell RI. Gastrointestinal damage associated with the use of nonsteroidal antiinflammatory drugs. *N Engl J Med* 1992; **327**: 749-754 [PMID: 1501650 DOI: 10.1056/NEJM199209103271101]

3 **Fortun PJ**, Hawkey CJ. Nonsteroidal antiinflammatory drugs and the small intestine. *Curr Opin Gastroenterol* 2005; **21**: 169-175 [PMID: 15711208]

4 **Fujimori S**, Seo T, Gudis K, Tanaka S, Mitsui K, Kobayashi T, Ehara A, Yonezawa M, Tatsuguchi A, Sakamoto C. Diagnosis and treatment of obscure gastrointestinal bleeding using combined capsule endoscopy and double balloon endoscopy: 1-year follow-up study. *Endoscopy* 2007; **39**: 1053-1058 [PMID: 18072055 DOI: 10.1055/s-2007-967014]

5 **Hawkey CJ**. Nonsteroidal anti-inflammatory drug gastropathy. *Gastroenterology* 2000; **119**: 521-535 [PMID: 10930388]

6 **Langman MJ**, Morgan L, Worrall A. Use of anti-inflammatory drugs by patients admitted with small or large bowel perforations and haemorrhage. *Br Med J* (Clin Res Ed) 1985; **290**: 347-349 [PMID: 3917814]

7 **Manetas M**, O'Loughlin C, Kelemen K, Barkin JS. Multiple small-bowel diaphragms: a cause of obscure GI bleeding diagnosed by capsule endoscopy. *Gastrointest Endosc* 2004; **60**: 848-851 [PMID: 15557976 DOI: 10.1016/S0016-5107(04)02174-1]

8 **Iddan G**, Meron G, Glukhovsky A, Swain P. Wireless capsule endoscopy. *Nature* 2000; **405**: 417 [PMID: 10839527 DOI: 10.1038/35013140]

9 **Yamamoto H**, Sekine Y, Sato Y, Higashizawa T, Miyata T, Iino S, Ido K, Sugano K. Total enteroscopy with a nonsurgical steerable double-balloon method. *Gastrointest Endosc* 2001; **53**: 216-220 [PMID: 11174299 DOI: 10.1067/mge.2001.112181]

10 **Lewis BS**. Expanding role of capsule endoscopy in inflammatory bowel disease. *World J Gastroenterol* 2008; **14**: 4137-4141 [PMID: 18636657 DOI: 10.3748/wjg.14.4137]

11 **Lucendo AJ**, Guagnozzi D. Small bowel video capsule endoscopy in Crohn's disease: What have we learned in the last ten years? *World J Gastrointest Endosc* 2011; **3**: 23-29 [PMID: 21403813 DOI: 10.4253/wjge.v3.i2.23]

12 **Eliakim R**, Fischer D, Suissa A, Yassin K, Katz D, Guttman N, Migdal M. Wireless capsule video endoscopy is a superior diagnostic tool in comparison to barium follow-through and computerized tomography in patients with suspected Crohn's disease. *Eur J Gastroenterol Hepatol* 2003; **15**: 363-367 [PMID: 12655255 DOI: 10.1097/01.meg.0000050000.68425.7e]

13 **Costamagna G**, Shah SK, Riccioni ME, Foschia F, Mutignani M, Perri V, Vecchioli A, Brizi MG, Picciocchi A, Marano P. A prospective trial comparing small bowel radiographs and video capsule endoscopy for suspected small bowel disease. *Gastroenterology* 2002; **123**: 999-1005 [PMID: 12360460 DOI: 10.1053/gast.2002.35988]

14 **Friedman S**. Comparison of capsule endoscopy to other modalities in small bowel. *Gastrointest Endosc Clin N Am* 2004; **14**: 51-60 [PMID: 15062380]

15 **Chan FK**, Graham DY. Review article: prevention of non-steroidal anti-inflammatory drug gastrointestinal complications--review and recommendations based on risk assessment. *Aliment Pharmacol Ther* 2004; **19**: 1051-1061 [PMID: 15142194 DOI: 10.1111/j.1365-2036.2004.01935.x]

16 **Maiden L**, Thjodleifsson B, Seigal A, Bjarnason II, Scott D, Birgisson S, Bjarnason I. Long-term effects of nonsteroidal anti-inflammatory drugs and cyclooxygenase-2 selective agents on the small bowel: a cross-sectional capsule enteroscopy study. *Clin Gastroenterol Hepatol* 2007; **5**: 1040-1045 [PMID: 17625980]

17 **Goldstein JL**, Eisen GM, Lewis B, Gralnek IM, Zlotnick S, Fort JG. Video capsule endoscopy to prospectively assess small bowel injury with celecoxib, naproxen plus omeprazole, and placebo. *Clin Gastroenterol Hepatol* 2005; **3**: 133-141 [PMID: 15704047]

18 **Graham DY**, Opekun AR, Willingham FF, Qureshi WA. Visible small-intestinal mucosal injury in chronic NSAID users. *Clin Gastroenterol Hepatol* 2005; **3**: 55-59 [PMID: 15645405]

19 **Fujimori S**, Seo T, Gudis K, Ehara A, Kobayashi T, Mitsui K, Yonezawa M, Tanaka S, Tatsuguchi A, Sakamoto C. Prevention of nonsteroidal anti-inflammatory drug-induced small-intestinal injury by prostaglandin: a pilot randomized controlled trial evaluated by capsule endoscopy. *Gastrointest Endosc* 2009; **69**: 1339-1346 [PMID: 19243767 DOI: 10.1016/j.gie.2008.08.017]

20 **Sigthorsson G**, Tibble J, Hayllar J, Menzies I, Macpherson A, Moots R, Scott D, Gumpel MJ, Bjarnason I. Intestinal permeability and inflammation in patients on NSAIDs. *Gut* 1998; **43**: 506-511 [PMID: 9824578 DOI: 10.1136/gut.43.4.506]

21 **Smalley WE**, Ray WA, Daugherty JR, Griffin MR. Nonsteroidal anti-inflammatory drugs and the incidence of hospitalizations for peptic ulcer disease in elderly persons. *Am J Epidemiol* 1995; **141**: 539-545 [PMID: 7900721]

22 **Laine L**, Connors LG, Reicin A, Hawkey CJ, Burgos-Vargas R, Schnitzer TJ, Yu Q, Bombardier C. Serious lower gastrointestinal clinical events with nonselective NSAID or coxib use. *Gastroenterology* 2003; **124**: 288-292 [PMID: 12557133 DOI: 10.1053/gast.2003.50054]

23 **Sigthorsson G**, Crane R, Simon T, Hoover M, Quan H, Bolognese J, Bjarnason I. COX-2 inhibition with rofecoxib does not increase intestinal permeability in healthy subjects: a double blind crossover study comparing rofecoxib with placebo and indomethacin. *Gut* 2000; **47**: 527-532 [PMID: 10986213 DOI: 10.1136/gut.47.4.527]

24 **Hawkey CJ**, Ell C, Simon B, Albert J, Keuchel M, McAlindon M, Fortun P, Schumann S, Bolten W, Shonde A, Hugot JL, Yu V, Arulmani U, Krammer G, Rebuli R, Toth E. Less small-bowel injury with lumiracoxib compared with naproxen plus omeprazole. *Clin Gastroenterol Hepatol* 2008; **6**: 536-544 [PMID: 18242145 DOI: 10.1016/j.cgh.2007.12.023]

25 **Goldstein JL**, Eisen GM, Lewis B, Gralnek IM, Aisenberg J, Bhadra P, Berger MF. Small bowel mucosal injury is reduced in healthy subjects treated with celecoxib compared with ibuprofen plus omeprazole, as assessed by video capsule endoscopy. *Aliment Pharmacol Ther* 2007; **25**: 1211-1222 [PMID: 17451567 DOI: 10.1111/j.1365-2036.2007.03312.x]

26 **Bjarnason I**, Hopkinson N, Zanelli G, Prouse P, Smethurst P, Gumpel JM, Levi AJ. Treatment of non-steroidal anti-inflammatory drug induced enteropathy. *Gut* 1990; **31**: 777-780 [PMID: 1973396 DOI: 10.1136/gut.31.7.777]

27 **Gross V**, Andus T, Fischbach W, Weber A, Gierend M, Hartmann F, Schölmerich J. Comparison between high dose

5-aminosalicylic acid and 6-methylprednisolone in active Crohn's ileocolitis. A multicenter randomized double-blind study. German 5-ASA Study Group. *Z Gastroenterol* 1995; **33**: 581-584 [PMID: 7502549]
28 **Schreiber S**, Howaldt S, Raedler A. Oral 4-aminosalicylic acid versus 5-aminosalicylic acid slow release tablets. Double blind, controlled pilot study in the maintenance treatment of Crohn's ileocolitis. *Gut* 1994; **35**: 1081-1085 [PMID: 7926910 DOI: 10.1136/gut.35.8.1081]
29 **Tromm A**, Bunganič I, Tomsová E, Tulassay Z, Lukáš M, Kykal J, Bátovský M, Fixa B, Gabalec L, Safadi R, Kramm HJ, Altorjay I, Löhr H, Koutroubakis I, Bar-Meir S, Stimac D, Schäffeler E, Glasmacher C, Dilger K, Mohrbacher R, Greinwald R. Budesonide 9 mg is at least as effective as mesalamine 4.5 g in patients with mildly to moderately active Crohn's disease. *Gastroenterology* 2011; **140**: 425-434.e1; quiz e13-14 [PMID: 21070781 DOI: 10.1053/j.gastro.2010.11.004]
30 **Brunner M**, Greinwald R, Kletter K, Kvaternik H, Corrado ME, Eichler HG, Müller M. Gastrointestinal transit and release of 5-aminosalicylic acid from 153Sm-labelled mesalazine pellets vs. tablets in male healthy volunteers. *Aliment Pharmacol Ther* 2003; **17**: 1163-1169 [PMID: 12752353 DOI: 10.1046/j.1365-2036.2003.01564.x]
31 **Koulaouzidis A**, Douglas S, Plevris JN. Lewis score correlates more closely with fecal calprotectin than Capsule Endoscopy Crohn's Disease Activity Index. *Dig Dis Sci* 2012; **57**: 987-993 [PMID: 22057284 DOI: 10.1007/s10620-011-1956-8]
32 **Gralnek IM**, Defranchis R, Seidman E, Leighton JA, Legnani P, Lewis BS. Development of a capsule endoscopy scoring index for small bowel mucosal inflammatory change. *Aliment Pharmacol Ther* 2008; **27**: 146-154 [PMID: 17956598 DOI: 10.1111/j.1365-2036.2007.03556.x]
33 **Koulaouzidis A**, Douglas S, Plevris JN. Blue mode does not offer any benefit over white light when calculating Lewis score in small-bowel capsule endoscopy. *World J Gastrointest Endosc* 2012; **4**: 33-37 [PMID: 22347530 DOI: 10.4253/wjge.v4.i2.33]
34 **Rácz I**. Is low-dose aspirin really harmful to the small bowel? *Digestion* 2009; **79**: 42-43 [PMID: 19246921 DOI: 101159/000204464]
35 **Endo H**, Hosono K, Inamori M, Kato S, Nozaki Y, Yoneda K, Akiyama T, Fujita K, Takahashi H, Yoneda M, Abe Y, Kirikoshi H, Kobayashi N, Kubota K, Saito S, Matsuhashi N, Nakajima A. Incidence of small bowel injury induced by low-dose aspirin: a crossover study using capsule endoscopy in healthy volunteers. *Digestion* 2009; **79**: 44-51 [PMID: 19246922 DOI: 10.1159/000204465]
36 **Endo H**, Hosono K, Higurashi T, Sakai E, Iida H, Sakamoto Y, Fujita K, Takahashi H, Koide T, Yoneda M, Tokoro C, Inamori M, Abe Y, Matsuhashi N, Nakajima A. Quantitative analysis of low-dose aspirin-associated small bowel injury using a capsule endoscopy scoring index. *Dig Endosc* 2011; **23**: 56-61 [PMID: 21198918 DOI: 10.1111/j.1443-1661.2010.01044.x]

P- Reviewers Koulaouzidis A, Bordas JM **S- Editor** Gou SX
L- Editor Webster JR **E- Editor** Li JY



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i6.897

World J Gastroenterol 2013 February 14; 19(6): 897-902
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Effect of *Gongronema latifolium* on gastric emptying in healthy dogs

Sylvester O Ogbu, Kenneth K Agwu, Isaac U Asuzu

Sylvester O Ogbu, Kenneth K Agwu, Department of Medical Radiography and Radiological Sciences, Faculty of Health Sciences and Technology, College of Medicine, University of Nigeria Enugu Campus, Enugu State 400001, Nigeria
Isaac U Asuzu, Department of Veterinary Physiology and Pharmacology, Faculty of Veterinary Medicine, University of Nigeria Nsukka Campus, Enugu State 400001, Nigeria
Author contributions: Ogbu SO, Agwu KK and Asuzu IU contributed equally to this work; Ogbu SO participated in the design of the study, collected the data, performed the statistical calculations and drafted the manuscript; Agwu KK participated in the design of the study and in drafting the manuscript; Asuzu IU participated in the design of the study, clinical examination and management of the animals and in drafting the manuscript; and all authors read and approved the final manuscript.
Correspondence to: Dr. Sylvester O Ogbu, Department of Medical Radiography and Radiological Sciences, Faculty of Health Sciences and Technology, College of Medicine, University of Nigeria Enugu Campus, Enugu State 400001, Nigeria. sylvester.ogbu@unn.edu.ng
Telephone: +234-80-33760550 Fax: +234-42-771977
Received: July 20, 2012 Revised: October 31, 2012
Accepted: November 24, 2012
Published online: February 14, 2013

Abstract

AIM: To investigate sonographically the effect of *Gonogronema latifolium* (*G. latifolium*) on gastric emptying of semi-solid meals in healthy dogs.

METHODS: In a randomized, placebo-controlled experiment, twenty-five clinically healthy dogs were randomly allotted into five groups of five dogs in each group. The placebo group served as the control, and the low, moderate and high dose groups ingested the methanolic leaf extract of *G. latifolium* in capsules at 100 mg/kg, 250 mg/kg and 500 mg/kg, respectively, while the prokinetic group ingested 0.5 mg/kg capsules of metoclopramide. After a 12-h fast, each group ingested its treatment capsules 30 min before the administration of a test meal. Measurements of gastric emptying and blood glucose levels were obtained 30 min before and immediately after the ingestion of the test meal and thereafter every 15 min for 4 h. This was followed by further measurements every 30 min for another 2 h.

RESULTS: The gastric emptying times of the placebo, low dose, moderate dose, high dose and prokinetic dose groups were 127.0 ± 8.2 min, 135.5 ± 3.7 min, 155.5 ± 3.9 min, 198.0 ± 5.3 min and 59.0 ± 2.5 min, respectively. Gastric emptying times of the moderate and high dose groups were significantly slower than in the placebo control group (155.5 ± 3.9 min, 198.0 ± 5.3 min *vs* 127.0 ± 8.2 min, $P = 0.000$). No significant difference in gastric emptying between the low dose and placebo control groups was noted (135.5 ± 3.7 min *vs* 127.0 ± 8.2 min, $P = 0.072$). Gastric emptying of the prokinetic group was significantly faster than that of the control group (59.0 ± 2.5 min *vs* 127.0 ± 8.2 min, $P = 0.000$). The hypoglycaemic effect of *G. latifolium* and gastric emptying were inversely related ($r = -0.95$, $P = 0.000$).

CONCLUSION: *G. latifolium* delays gastric emptying and lowers postprandial blood glucose in healthy dogs. It reduces the postprandial blood glucose by delaying gastric emptying.

© 2013 Baishideng. All rights reserved.

Key words: *Gonogronema latifolium*; Gastric emptying; Sonography; Postprandial blood glucose; Semi-solid meals

Ogbu SO, Agwu KK, Asuzu IU. Effect of *Gongronema latifolium* on gastric emptying in healthy dogs. *World J Gastroenterol* 2013; 19(6): 897-902 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i6/897.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i6.897

INTRODUCTION

Gastric emptying (GE) is the rate with which substances

leave the stomach after ingestion[1]. The process involves the storage of food, mixing with gastric secretions, grinding the solid food into particles of 1-2 mm in diameter, and subsequent delivery of the chyme into the small intestine at a rate designed to optimize digestion and absorption[2]. GE is one of the factors that affect the rate and completeness of intestinal nutrient absorption[3] and is a major determinant of postprandial glycaemic excursions not only in healthy subjects but in type 1 and type 2 diabetic patients[2].

The GE process can be influenced by a variety of physiological, pathological, pharmacological and dietary factors[4]. The effect on GE of *Gongronema latifolium* (*G. latifolium*) (Asclepiadaceae), which is used as a dietary and pharmacological agent for the control of postprandial blood glucose excursions[5,6], has, as far as we know, not been investigated previously. None of the relatively scanty information available on the mechanism(s) of action of *G. latifolium* relates to its effect on GE. We hypothesized that ingestion of a hypoglycaemic agent like *G. latifolium* might accelerate gastric emptying in healthy individuals just as insulin does[7].

In this study we investigated with the use of ultrasonography the effect of the methanolic leaf extract of *G. latifolium* on GE in health, as well as the relationship between its hypoglycaemic effect and GE, using the dog as an animal model. The study further investigated whether the effect of *G. latifolium* on GE in healthy individuals is dose dependent. This study is important as new therapies that aim to change blood glucose by modulating GE are being actively explored and evaluated.

MATERIALS AND METHODS

Plant preparation

Fresh *Gongronema latifolium* leaves were supplied, identified and authenticated by Mr. Ozioko AO, a taxonomist with the Bioresources Development and Conservation Programme Center, Nsukka, South Eastern Nigeria, an International Centre for Enthomedicine and Drug Development (INTERCEDD). A voucher specimen (INTERCEDD/170) was deposited for reference at the centre. The fresh *G. latifolium* leaves were air-dried, pulverized and the extract was prepared according to the previously described method by Ugochukwu and Babady[5]. The 2.2 kg dried powder of *G. latifolium* was extracted with 80% Romil-SA Methanol (MRS Scientific Ltd Essex United Kingdom) and dried in a hot air oven (Gallenkamp, England) at 40 ℃. The filtrates were concentrated at 40 ℃ using a vacuum rotary evaporator and freeze-dried to yield about 146.6 g of green coarse powder. The powder was further pulverized and encapsulated in doses of 100 mg, 250 mg and 500 mg for use in the study.

Animals

Clinically healthy mongrel dogs with no clinical and laboratory evidence of gastrointestinal disease, diabetes, gastroparesis, cardiovascular, pulmonary, renal, and hepatic diseases as ascertained by a veterinary doctor were used in this study. Pregnant female dogs confirmed by palpation and ultrasound were excluded. The dogs were dewormed with 5 mg/kg Levamizole® (Levamisole hydrochloride, Eagle chemical Co. Ltd, N. Korea) one week prior to the GE study. Food was withheld from the dogs for 12 h while water was withheld for 2 h before the study. The age, weight and fasting blood glucose concentration of the dogs did not differ between the control and the treatment groups ($P = 0.7$; $P = 0.2$; $P = 0.7$) (Table 1).

Table 1 Demographic and baseline clinical characteristics of the healthy groups (mean ± SD)

Characteristics	Placebo control	Low dose	Moderate dose	High dose	Prokinetic dose
Age (mo)	6.6 ± 0.7	6.5 ± 1.1	5.9 ± 0.8	6.4 ± 1.1	6.1 ± 0.3
Weight (kg)	6.2 ± 0.7	5.3 ± 0.3	5.6 ± 0.4	5.8 ± 0.7	5.7 ± 0.5
FBG (mmol/L)	4.0 ± 0.6	3.9 ± 0.1	4.0 ± 0.5	3.9 ± 0.2	4.2 ± 0.1

FBG: Fasting blood glucose.

Test meal

The test meal used consisted of 100 g proprietary canned Nestle Cerelac (Maize and Milk infant cereal, Nestle Nigeria plc) food and 150 mL of water. The calories and nutritional components in the 100 g food and 150 mL water are: Calories 1730 KJ (414 kcal); Protein 15 g; Fat 9 g; Carbohydrates 68.2 g; Dietary fibre 2 g; Minerals (ash) 3.3 g; Moisture 2.5 g.

Study design

The study was approved by the University of Nigeria Ethical Committee UNTH Enugu. The guidelines of the National Institutes of Health (NIH) *Principles of Laboratory Animal Care* (NIH Publication No. 86-23, revised 1985) were followed. This clinic-based experimental study was carried out in the University of Nigeria Veterinary Teaching Hospital, Nsukka. A randomized, placebo-controlled experimental design was adopted in this study and the dog was used as an animal model because of its established performance in the assessment of gastrointestinal motility[3] and in many physiological and pharmacological studies[8]. The dogs were randomly allotted into five groups of five dogs in each group. The placebo group served as the control; the low, moderate and high dose groups ingested the *G. latifolium* leaf extract capsules at 100 mg/kg, 250 mg/kg, 500 mg/kg, respectively, while the prokinetic dose group ingested 0.5 mg/kg capsules of metoclopramide (Mederax® 10 mg, Jiangsu Peng YAO Pharmaceutical Inc China). The prokinetic dose group served as prokinetic control. The prokinetic effect of metoclopramide is comparable to the insulin effect on gastrointestinal motility[7].

After a 12-h fast, each group ingested its treatment capsules 30 min before the administration of the test meal. Measurements of GE and blood glucose levels were obtained 30 min before and immediately after the ingestion of the test meal and then every 15 min for 4 h for each dog. Further measurements were made every 30 min for another 2 h. The three doses of *G. latifolium* were

Table 2 Gastric emptying and incremental postprandial blood glucose concentration of the healthy groups (mean ± SD)

Group	GE (min)	AUC of *i*PPBG (mmol/L × min)
Placebo control	127.0 ± 8.2	1028.6 ± 204.2
Low dose	135.5 ± 3.7	938.1 ± 40.0
Moderate dose	155.5 ± 3.9[b]	559.1 ± 101.8[b]
High dose	198.0 ± 5.3[b]	223.5 ± 52.2[b]
Prokinetic dose	59.0 ± 2.5[b]	1426 ± 108.2[b]

[b]$P < 0.0001$ *vs* control group. *i*PPBG: Incremental postprandial blood glucose concentrations; GE: Gastric emptying; AUC: Area under the curve.

introduced to assess its dose-dependent effect. The minimum dose of 100 mg/kg was based on the dose used in mice and rats[5,9]. All the treatment capsules ingested by the dogs were visually identical. The dogs ingested the test meal under natural free-feeding circumstances.

Measurement of gastric emptying

Gastric emptying was measured using an ultrasound technique as described by Chalmers *et al*[10] and McLellan *et al*[11]. The examinations were performed using a veterinary ultrasound machine with a 6-8 MHz microconvex transducer (Medison SA-600 v; 2006; Medison Co., Ltd., South Korea). Each dog was gently restrained while erect and the transducer was placed in a longitudinal orientation on the ventral midline, caudal to the xiphoid. The ultrasound beam was maintained in the sagittal plane and directed cranially until the liver was located and the stomach identified immediately caudal to it. The stomach was observed using real-time imaging, allowing the image to be frozen between peristaltic contractions when it was at a constant, maximal distension. Electronic callipers were used to measure the craniocaudal and ventrodorsal diameters of the antrum between the serosal margins. The antral area was calculated by using the software incorporated in the ultrasound machine to predict the area inside the elliptical shape defined by the craniocaudal and ventrodorsal diameters of the stomach. Three measurements of antral area were taken at each time, and their mean was used for further calculations. Baseline values were subtracted from the measurements made at each subsequent time point and the values expressed as a percentage of maximal antral area. The percentages of the maximal antral areas measured during each test were plotted against time. The gastric half-emptying time with ultrasonography (T50) that correlated significantly with $t_{1/2}$ of carbon 13-labelled octanoic acid breath test in dogs[11] was used to describe the rate of GE. The T50 was defined as the time at which the antral area decreased to 50 percent of its maximal area. T50 was calculated by linear interpolation between two points in the curve.

Measurement of blood glucose

Five millilitres of blood was drawn from each dog's ear at specific time intervals indicated in the study design. Blood glucose was determined by using a portable Accu-chek® Advantage glucometer (Roche Diagnostics GmbH Mannheim Germany). The incremental blood glucose concentrations were computed and plotted against time, and the blood glucose area under the curve (AUC) was calculated from the blood glucose curve.

Statistical analysis

All the data were expressed as mean ± SD. Dunnett's test was used for parametric multiple comparisons between the control and the treatment groups. Analysis of variance linear trend test was used to assess the dose trend. Pearson correlation was used to assess the linear association between the values of two variables. The values were considered to be significant when the P value was less than 0.05. Graphpad prism version 5.03 for windows (Graphpad Software San Diego California United States) and SPSS 15.0 for Windows Evaluation Version (United States) were used for statistical analysis.

RESULTS

Effect of G. latifolium leaf extract on GE

The GE times of the moderate and high dose groups were significantly ($P = 0.000$) slower than in the placebo control, while the GE of the prokinetic group was significantly ($P = 0.000$) faster than in the placebo control group (Table 2). No significant ($P = 0.072$) difference in GE was observed between the low dose and control groups. The effect of *G. latifolium* on GE was also significantly ($P = 0.000$) dose dependent (Figure 1A).

Effect of G. latifolium leaf extract on postprandial blood glucose concentration

The AUC values of incremental postprandial blood glucose concentrations (*i*PPBG) of the moderate and high dose groups were significantly ($P = 0.000$) smaller than in the placebo control, while the AUC of *i*PPBG of the prokinetic group was significantly ($P = 0.000$) larger than that of the placebo control (Table 2). No significant ($P = 0.442$) difference in AUC of *i*PPBG was observed between the low dose and control groups. The effect of *G. latifolium* on AUC of *i*PPBG of healthy dogs was significantly ($P = 0.000$) dose-dependent (Figure 1B).

The hypoglycaemic effect of G. latifolium and GE

The hypoglycaemic effect of *G. latifolium* and GE were inversely related ($r^2 = 0.95$) (Figure 2).

DISCUSSION

The gastric emptying process can be influenced by a variety of physiological, pathological, pharmacological and dietary factors[4] but the effect on GE of *G. latifolium*, which is used as a dietary and pharmacological agent for the control of postprandial blood glucose excursions[5,6], has, as far as we know, not been investigated in animals/humans previously. In this study, we demonstrate that the ingestion of the methanolic extract of *G. latifolium* delayed the gastric emptying of a semi-solid meal dose-dependently.

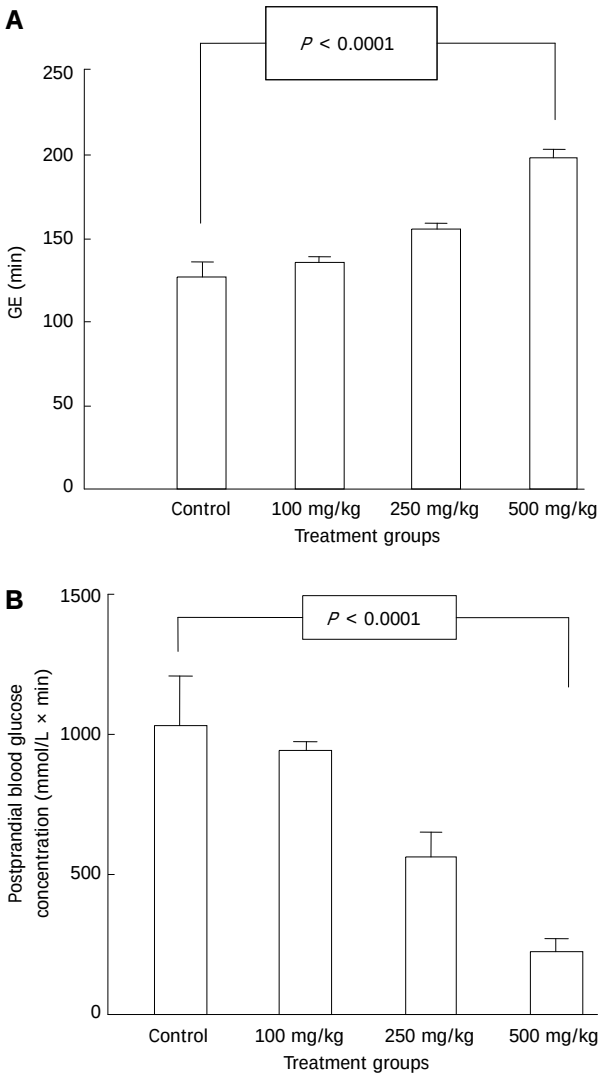


Figure 1 Dose-dependent effect of *Gongronema latifolium* on gastric emptying and postprandial incremental blood glucose concentrations. A: Dose-dependent effect of *Gongronema latifolium* (*G. latifolium*) on gastric emptying; B: Dose-dependent effect of *G. latifolium* on postprandial incremental blood glucose concentrations. GE: Gastric emptying.

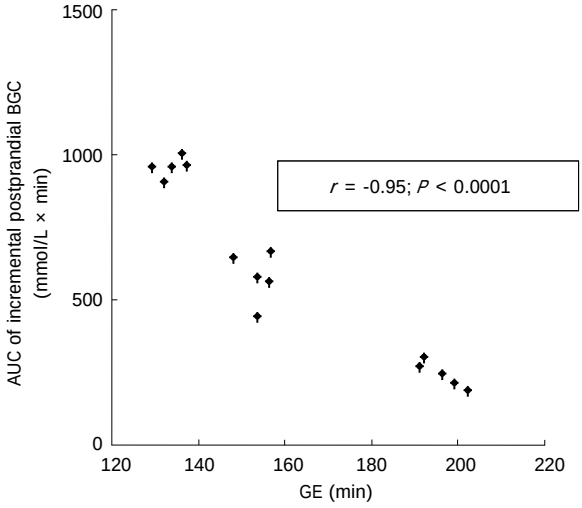


Figure 2 Relationship between the hypoglycaemic and gastric emptying effects of *Gongronema latifolium*. GE: Gastric emptying; AUC: Area under the curve; BGC: Blood glucose concentration.

The effect is similar to that of *Trigonella foenum-graecum*[12] and that of *Gymnema sylvestre*[13,14] which belong to the same Asclepiadaceae plant family as *G. latifolium*[15,16], but opposite to that of metoclopramide as noted in this study.

The presence of saponins in *G. latifolium*[17] and *G. sylvestre*[13,14] may be responsible for the similarity in their GE effects. Saponins significantly and dose-dependently delay gastric emptying[12-14,18]. GE is linearly associated with changes in the antral area[11,19,20]; therefore, our data suggest that *G. latifolium* causes less rapid reductions in antral areas and an increase in antral areas. This agrees with works which associated substances that slow GE with a relative increase in the content of the distal stomach[21,22] and inhibition of antral motility[23]. The more rapid reductions in antral areas and a decrease in antral areas observed with metoclopramide in this study are because it improves GE by increasing amplitude and frequency of antral contractions[4]. Therefore, the observed difference between the GE effect of metoclopramide and that of *G. latifolium* in this study may be due to their different mechanisms of action.

The mechanisms underlying the delayed effects of *G. latifolium* on GE were not evaluated in this study, but we speculate that the effect may be mediated by vagal mechanisms[21,24-26] and/or the release of gastrointestinal hormones[27]. *G. latifolium* may have elicited a gastrointestinal motor and/or sensory function that caused antral distension and subsequently suppressed antral contractions to result in a slower rate of antral delivery of the ingested semi-solid meals. The demonstration of the potential of *G. latifolium* to slow the rate of antral delivery of the ingested semi-solid meals from the stomach into the small intestine by this study is very important as new therapies that aim to change blood glucose by modulating GE are being actively explored and evaluated. The effect has hitherto not been demonstrated in animals or humans.

This study also demonstrates that there is an inverse relationship between the GE effect of *G. latifolium* and blood glucose concentration. It agrees with the fact that the pharmacologic acceleration of GE results in higher postprandial glucose concentrations, while delaying GE results in lower postprandial glucose concentrations after a physiologic meal[28]. Although the demonstration of a correlation does not establish causation, this finding suggests that *G. latifolium* when ingested with a meal may, through the mechanism of delayed GE, slow digestion and prolong the postprandial absorption of food, with a resultant improvement or reduction in postprandial blood glucose concentrations after a semi-solid meal. The rates of meal-derived glucose appearance in the systemic circulation are determined mainly by GE[29,30]. A previous work indicates that saponins with hypoglycaemic activity also inhibited GE while other saponins that have no hypoglycaemic activity did not affect GE[31]. Thus, saponins in *G. latifolium* may be responsible for the correlation between GE and its hypoglycaemic effects in healthy dogs. Some authors have proposed that saponin compounds act as hypogly-

caemic agents by delaying the transfer of glucose from the stomach to the small intestine, the main site of glucose absorption, and by inhibiting the glucose transport at the site of intestinal brush border membranes[32,33].

In this study, 100 mg/kg of *G. latifolium* did not significantly slow GE, probably due to low dose-response effect. The 100 mg/kg dose of *G. latifolium* might be below the threshold required to cause a significant delay on GE. Our findings that *G. latifolium* in a dose-dependent manner affects both the rate and extent of carbohydrate absorption by slowing the transfer of food from the stomach into the small intestine and thereby reducing or delaying exposure of nutrient to small bowel mucosa are clinically relevant with regard to improving postprandial blood glucose and triglycerides and consequently lowering the risk of chronic disease. Distension of the stomach is one factor that promotes the feeling of satiety[34]. Therefore, *G. latifolium* may, through the mechanism of delayed GE, play an important role in the regulation of appetite and energy intake. Finally, since interventions directed at modulating upper gastrointestinal motor and absorptive functions have a major effect on postprandial blood glucose excursion and are likely soon to enter the mainstream of therapy for diabetes[2,35], *G. latifolium* may be relevant in the current treatment and management of diabetes. Alternatively hyperglycaemia can cause a decrease in GE rate[36,37]. This factor is unlikely to have influenced the result of this study much since there was no statistically significant difference between the preprandial blood glucose concentrations in the subgroups when compared with the placebo control.

The neural, humoral and cellular mechanisms by which *G. latifolium* affects GE were not investigated, therefore further studies are required to elucidate them. Although the dog is an animal model for the study of human GE in many physiological and pharmacological studies[8] and an established model for the assessment of gastrointestinal motility[3], the pattern of findings may not be exactly the same in humans as demonstrated in this study.

Gongronema latifolium delays GE and lowers postprandial blood glucose in healthy dogs. It reduces the postprandial blood glucose by delaying GE. The effect has also been noted to be dose-dependent. Therefore it can play an important role in the current dietary and pharmacological approaches in the prevention, treatment and management of metabolic diseases like diabetes.

ACKNOWLEDGMENTS

We would like to thank Dr. Anya KO of the University of Nigeria Veterinary Teaching Hospital, Nsukka for the assistance in taking care of the dogs.

COMMENTS

Background

Gastric emptying is a major determinant of postprandial glycaemic excursions not only in healthy subjects but in diabetic patients. Interventions directed at modulating gastric emptying have a major effect on postprandial blood glucose excursion. *Gongronema latifolium* (*G. latifolium*) (Asclepiadaceae) is currently used as a dietary and pharmacological agent for the control of postprandial blood glucose excursions, but its effect on gastric emptying has, as far as we know, not been reported.

Research frontiers

Previous studies have demonstrated the hypoglycaemic effect of *G. latifolium*, but none of the relatively scanty information available on its mechanism(s) of action relates to its effect on gastric emptying. In this study, the authors demonstrated that the gastric emptying delaying effect of *G. latifolium* could be one of the pathways for reducing the postprandial blood glucose level.

Innovations and breakthroughs

New therapies that aim to change blood glucose by modulating gastric emptying are being actively explored and evaluated. This is believed to be the first study to explore the effect of *G. latifolium* on gastric emptying in health as well as the relationship between its hypoglycaemic effect and gastric emptying.

Applications

The findings that *G. latifolium* in a dose-dependent manner slows the rate of antral delivery of the ingested semi-solid meals from the stomach into the small intestine may be clinically relevant in the management approach of postprandial blood glucose and triglycerides and consequently in diabetics.

Terminology

Gastric emptying is the rate with which substances leave the stomach after ingestion. The process involves the storage of food, mixing with gastric secretions, grinding the solid food into particles of 1-2 mm in diameter, and subsequent delivery of the chyme into the small intestine at a rate designed to optimize digestion and absorption. *G. latifolium* (Asclepiadaceae) is an edible tropical rainforest plant that is widely used in folk medicine.

Peer review

The authors present data on the effects of various doses of the methanolic leaf extract of *G. latiifolium* on gastric emptying and postprandial plasma glucose levels in a dog animal model. Gastric emptying was measured by ultrasonography. The data demonstrate dose related effects on inhibiting gastric emptying and plasma glucose.

REFERENCES

1 **Bourreau J**, Hernot D, Bailhache E, Weber M, Ferchaud V, Biourge V, Martin L, Dumon H, Nguyen P. Gastric emptying rate is inversely related to body weight in dog breeds of different sizes. *J Nutr* 2004; **134**: 2039S-2041S [PMID: 15284397]

2 **Horowitz M**, O'Donovan D, Jones KL, Feinle C, Rayner CK, Samsom M. Gastric emptying in diabetes: clinical significance and treatment. *Diabet Med* 2002; **19**: 177-194 [PMID: 11918620 DOI: 10.1046/j.1464-5491.2002.00658.x]

3 **Xu X**, Brining D, Rafiq A, Hayes J, Chen JD. Effects of enhanced viscosity on canine gastric and intestinal motility. *J Gastroenterol Hepatol* 2005; **20**: 387-394 [PMID: 15740481 DOI: 10.1111/j.1440-1746.2005.03604.x]

4 **Schmitz S**, Neiger R. Gastric emptying-physiology, pathology, diagnostic procedures and therapeutic approaches in the dog. *Eur J Companion Anim Pract* 2009; **19**: 67-74

5 **Ugochukwu NH**, Babady NE. Antioxidant effects of Gongronema latifolium in hepatocytes of rat models of non-insulin dependent diabetes mellitus. *Fitoterapia* 2002; **73**: 612-618 [PMID: 12490219 DOI: 10.1016/S0367-326x]

6 **Ugochukwu NH**, Babady NE, Cobourne M, Gasset SR. The effect of Gongronema latifolium extracts on serum lipid profile and oxidative stress in hepatocytes of diabetic rats. *J Biosci* 2003; **28**: 1-5 [PMID: 12682417 DOI: 10.1007/BF02970124]

7 **Reddy PM**, Dkhar SA, Subramanian R. Effect of insulin on small intestinal transit in normal mice is independent of blood glucose level. *BMC Pharmacol* 2006; **6**: 4 [PMID: 16448577 DOI: 10.1186/1471-2210-6-4]

8 **Wyse CA**, McLellan J, Dickie AM, Sutton DG, Preston T, Yam PS. A review of methods for assessment of the rate of gastric emptying in the dog and cat: 1898-2002. *J Vet Intern Med* 2003; **17**: 609-621 [PMID: 14529126 DOI: 10.1111/j.1939-1676.2003.tb02491.x]

9 **Ogundipe OO**, Moody JO, Akinyemi TO, Raman A. Hypoglycemic potentials of methanolic extracts of selected plant foods in alloxanized mice. *Plant Foods Hum Nutr* 2003; **58**:

1-7 [DOI: 10.1023/B: QUAL.0000040321.56831.c4]
10 **Chalmers AF**, Kirton R, Wyse CA, Dickie A, Cumming D, Cooper JM, Preston T, Yam PS. Ultrasonographic assessment of the rate of solid-phase gastric emptying in dogs. *Vet Rec* 2005; **157**: 649-652 [PMID: 16299365 DOI: 10.1136/vr.157.21.649]
11 **McLellan J**, Wyse CA, Dickie A, Preston T, Yam PS. Comparison of the carbon 13-labeled octanoic acid breath test and ultrasonography for assessment of gastric emptying of a semisolid meal in dogs. *Am J Vet Res* 2004; **65**: 1557-1562 [PMID: 15566095 DOI: 10.2460/ajvr.2004.65.1557]
12 **Madar Z**, Abel R, Samish S, Arad J. Glucose-lowering effect of fenugreek in non-insulin dependent diabetics. *Eur J Clin Nutr* 1988; **42**: 51-54 [PMID: 3286242]
13 **Matsuda H**, Li Y, Murakami T, Matsumura N, Yamahara J, Yoshikawa M. Antidiabetic principles of natural medicines. III. Structure-related inhibitory activity and action mode of oleanolic acid glycosides on hypoglycemic activity. *Chem Pharm Bull* (Tokyo) 1998; **46**: 1399-1403 [PMID: 9775435 DOI: 10.1248/cpb.46.1399]
14 **Matsuda H**, Murakami T, Li Y, Yamahara J, Yoshikawa M. Mode of action of escins Ia and IIa and E,Z-senegin II on glucose absorption in gastrointestinal tract. *Bioorg Med Chem* 1998; **6**: 1019-1023 [PMID: 9730238 DOI: 10.1016/S0968-0896(98)00054-6]
15 **Chattopadhyay RR**. Possible mechanism of antihyperglycemic effect of Gymnema sylvestre leaf extract, part I. *Gen Pharmacol* 1998; **31**: 495-496 [PMID: 9703226 DOI: 10.1016/S0306-3623(97)00450-3]
16 **Chattopadhyay RR**. A comparative evaluation of some blood sugar lowering agents of plant origin. *J Ethnopharmacol* 1999; **67**: 367-372 [PMID: 10617074 DOI: 10.1016/S0378-8741(9)00095-1]
17 **Ugochukwu NH**, Babady NE. Antihyperglycemic effect of aqueous and ethanolic extracts of Gongronema latifolium leaves on glucose and glycogen metabolism in livers of normal and streptozotocin-induced diabetic rats. *Life Sci* 2003; **73**: 1925-1938 [PMID: 12899918 DOI: 10.1016/S0024-3205(03)00543-5]
18 **Matsuda H**, Li Y, Yamahara J, Yoshikawa M. Inhibition of gastric emptying by triterpene saponin, momordin Ic, in mice: roles of blood glucose, capsaicin-sensitive sensory nerves, and central nervous system. *J Pharmacol Exp Ther* 1999; **289**: 729-734 [PMID: 10215646]
19 **Bolondi L**, Bortolotti M, Santi V, Calletti T, Gaiani S, Labò G. Measurement of gastric emptying time by real-time ultrasonography. *Gastroenterology* 1985; **89**: 752-759 [PMID: 3896910]
20 **Darwiche G**, Björgell O, Thorsson O, Almér LO. Correlation between simultaneous scintigraphic and ultrasonographic measurement of gastric emptying in patients with type 1 diabetes mellitus. *J Ultrasound Med* 2003; **22**: 459-466 [PMID: 12751857]
21 **Little TJ**, Pilichiewicz AN, Russo A, Phillips L, Jones KL, Nauck MA, Wishart J, Horowitz M, Feinle-Bisset C. Effects of intravenous glucagon-like peptide-1 on gastric emptying and intragastric distribution in healthy subjects: relationships with postprandial glycemic and insulinemic responses. *J Clin Endocrinol Metab* 2006; **91**: 1916-1923 [PMID: 16492694 DOI: 10.1210/jc.2005-2220]
22 **Gentilcore D**, Chaikomin R, Jones KL, Russo A, Feinle-Bisset C, Wishart JM, Rayner CK, Horowitz M. Effects of fat on gastric emptying of and the glycemic, insulin, and incretin responses to a carbohydrate meal in type 2 diabetes. *J Clin Endocrinol Metab* 2006; **91**: 2062-2067 [PMID: 16537685 DOI: 10.1210/jc.2005-2644]
23 **Schirra J**, Houck P, Wank U, Arnold R, Göke B, Katschinski M. Effects of glucagon-like peptide-1(7-36)amide on antropyloro-duodenal motility in the interdigestive state and with duodenal lipid perfusion in humans. *Gut* 2000; **46**: 622-631 [PMID: 10764704 DOI: 10.1136/gut.46.5.622]
24 **Schirra J**, Leicht P, Hildebrand P, Beglinger C, Arnold R, Göke B, Katschinski M. Mechanisms of the antidiabetic action of subcutaneous glucagon-like peptide-1(7-36)amide in non-insulin dependent diabetes mellitus. *J Endocrinol* 1998; **156**: 177-186 [PMID: 9496247 DOI: 10.1677/joe.0.1560177]
25 **Imeryüz N**, Yeğen BC, Bozkurt A, Coşkun T, Villanueva-Peñacarrillo ML, Ulusoy NB. Glucagon-like peptide-1 inhibits gastric emptying via vagal afferent-mediated central mechanisms. *Am J Physiol* 1997; **273**: G920-G927 [PMID: 9357836]
26 **Delgado-Aros S**, Vella A, Camilleri M, Low PA, Burton DD, Thomforde GM, Stephens D. Effects of glucagon-like peptide-1 and feeding on gastric volumes in diabetes mellitus with cardio-vagal dysfunction. *Neurogastroenterol Motil* 2003; **15**: 435-443 [PMID: 12846732 DOI: 10.1046/j.1365-2982.2003.00422.x]
27 **Caspary WF**. Physiology and pathophysiology of intestinal absorption. *Am J Clin Nutr* 1992; **55**: 299S-308S [PMID: 1728844]
28 **Gonlachanvit S**, Hsu CW, Boden GH, Knight LC, Maurer AH, Fisher RS, Parkman HP. Effect of altering gastric emptying on postprandial plasma glucose concentrations following a physiologic meal in type-II diabetic patients. *Dig Dis Sci* 2003; **48**: 488-497 [PMID: 12757160 DOI: 10.1023/A: /022528414264]
29 **Woerle HJ**, Meyer C, Dostou JM, Gosmanov NR, Islam N, Popa E, Wittlin SD, Welle SL, Gerich JE. Pathways for glucose disposal after meal ingestion in humans. *Am J Physiol Endocrinol Metab* 2003; **284**: E716-E725 [PMID: 12475753 DOI: 10.1152/ajpendo.00365.2002]
30 **Woerle HJ**, Albrecht M, Linke R, Zschau S, Neumann C, Nicolaus M, Gerich J, Göke B, Schirra J. Importance of changes in gastric emptying for postprandial plasma glucose fluxes in healthy humans. *Am J Physiol Endocrinol Metab* 2008; **294**: E103-E109 [PMID: 17971511 DOI: 10.1152/ajpendo.00514.2007]
31 **Yoshikawa M**, Murakami T, Kishi A, Kageura T, Matsuda H. Medicinal flowers. III. Marigold. (1): hypoglycemic, gastric emptying inhibitory, and gastroprotective principles and new oleanane-type triterpene oligoglycosides, calendasaponins A, B, C, and D, from Egyptian Calendula officinalis. *Chem Pharm Bull* (Tokyo) 2001; **49**: 863-870 [PMID: 11456093 DOI: 10.1248/cpb.49.863]
32 **Tiwari AK**, Rao JM Diabetes mellitus and multiple therapeutic approaches of phytochemicals: Present status and future prospects. *Curr Sci* 2002; **83**: 30-37
33 **Francis G**, Kerem Z, Makkar HP, Becker K. The biological action of saponins in animal systems: a review. *Br J Nutr* 2002; **88**: 587-605 [PMID: 12493081 DOI: 10.1079/BJN2002725]
34 **Hlebowicz J**, Hlebowicz A, Lindstedt S, Björgell O, Höglund P, Holst JJ, Darwiche G, Almér LO. Effects of 1 and 3 g cinnamon on gastric emptying, satiety, and postprandial blood glucose, insulin, glucose-dependent insulinotropic polypeptide, glucagon-like peptide 1, and ghrelin concentrations in healthy subjects. *Am J Clin Nutr* 2009; **89**: 815-821 [PMID: 19158209 DOI: 10.3945/ajcn.2008.26807]
35 **Rayner CK**, Samsom M, Jones KL, Horowitz M. Relationships of upper gastrointestinal motor and sensory function with glycemic control. *Diabetes Care* 2001; **24**: 371-381 [PMID: 11213895 DOI: 10.2337/diacare.24.2.371]
36 **Koch KL**. Diabetic gastropathy: gastric neuromuscular dysfunction in diabetes mellitus: a review of symptoms, pathophysiology, and treatment. *Dig Dis Sci* 1999; **44**: 1061-1075 [PMID: 10389675 DOI: 10.1023/A: 1026647417465]
37 **Vinik A**, Erbas T, Stansberry K. Gastrointestinal, genitourinary and neurovascular disturbances in diabetes. *Diabetes Rev* 1999; **7**: 358-378

P- Reviewer Moran TH **S- Editor** Gou SX **L- Editor** Logan S
E- Editor Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i6.903

World J Gastroenterol 2013 February 14; 19(6): 903-908
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Ampulla dilation with different sized balloons to remove common bile duct stones

Neng-Ping Li, Jiang-Qi Liu, Zhi-Qiang Zhou, Tao-Ying Ji, Xiao-Yan Cai, Qing-Yun Zhu

Neng-Ping Li, Jiang-Qi Liu, Zhi-Qiang Zhou, Tao-Ying Ji, Xiao-Yan Cai, Qing-Yun Zhu, Department of General Surgery, Shanghai Gongli Hospital, Shanghai 200135, China
Author contributions: Li NP designed and performed the study, analyzed data and wrote the manuscript; Liu JQ instructed the study and revised the manuscript; Zhou ZQ performed the study and collected and analyzed data; Ji TY followed up the patients and collected and analyzed data; Cai XY collected data and supplemented references; and Zhu QY revised the manuscript.
Supported by Shanghai Municipal Health Bureau, No. 201104369
Correspondence to: Neng-Ping Li, MS, Chief Doctor, Department of General Surgery, Shanghai Gongli Hospital, No. 219, Miaopu Road, Pudong New Area, Shanghai 200135, China. linengp@163.com
Telephone: +86-21-58858730 Fax: +86-21-38821635
Received: November 27, 2012 Revised: January 15, 2013
Accepted: January 29, 2013
Published online: February 14, 2013

Abstract

AIM: To assess the outcomes of ampulla dilation with different sized balloons to remove common bile duct (CBD) stones.

METHODS: Patients (n = 208) were divided into five groups based on the largest CBD stone size of < 5, 6-8, 8-12, 12-14, and > 14 mm. Patients underwent limited endoscopic sphincterotomy (EST) alone or limited EST followed by endoscopic papillary balloon dilation with 8, 10, 12 and 14 mm balloons, such that the size of each balloon did not exceed the size of the CBD. Short- and long-term outcomes, such as post-endoscopic retrograde cholangiopancreatography (ERCP) pancreatitis, perforation, bleeding, and pneumobilia were compared among the five groups.

RESULTS: The overall rate of successful stone removal in all groups was 100%, and all patients were cured. Eight (3.85%) patients had post-ERCP pancreatitis, none had perforations, and 6 (2.9%) had bleeding requiring transfusion. There were no significant differences in early complication rates among the five groups. We observed significant correlations between increased balloon size and the short- and long-term rates of post-ERCP pneumobilia. Post-ERCP pancreatitis and bleeding correlated significantly with age, with post-ERCP pancreatitis occurring more frequently in patients aged < 60 years, and bleeding occurring more frequently in patients aged > 70 years. We observed a significant correlation between patient age and the diameter of the largest CBD stone, with stones > 12 mm occurring more frequently in patients > 60 years old.

CONCLUSION: Choosing a balloon size based on the largest stone diameter is safe and effective for removing CBD stones. Balloon size should not exceed 15 mm.

© 2013 Baishideng. All rights reserved.

Key words: Endoscopic papillary balloon dilation; Endoscopic sphincterotomy; Common bile duct stone; Endoscopic retrograde cholangiopancreatography; Pancreatitis

Li NP, Liu JQ, Zhou ZQ, Ji TY, Cai XY, Zhu QY. Ampulla dilation with different sized balloons to remove common bile duct stones. *World J Gastroenterol* 2013; 19(6): 903-908 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i6/903.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i6.903

INTRODUCTION

Endoscopic sphincterotomy (EST) and endoscopic papillary balloon dilation (EPBD) with balloons < 12 mm in diameter are the methods of choice for the removal of stones from the common bile duct (CBD)[1-4]. Both methods, however, have distinct advantages and disadvantages. EST is associated with serious short-term complications, such as hemorrhage, perforation, and pancreatitis,

and long-term complications such as permanent loss of sphincter of Oddi (SO) function and recurrent bile duct infection[5-7]. Although complications of bleeding and perforation seldom occur during EPBD[8-10] and SO function can be preserved[11,12], it is difficult to remove large CBD stones using EPBD because the biliary opening is not as enlarged as it is with EST, and EPBD is associated with a higher rate of post-endoscopic retrograde cholangiopancreatographic (ERCP) pancreatitis.

More recently, EPBD with large sized balloons (12-20 mm) has been used to remove large CBD stones following limited EST[13-16]. It is unclear, however, whether the increase in balloon size is associated with increased rates of short-term complications, such as perforation and hemorrhage, or with preservation of SO function. Combining the advantages of EST and EPBD, by selecting the correct sized balloon and EST incision length to achieve a high rate of stone extraction, while minimizing complications of both procedures, would be of great benefit to the patients. We therefore prospectively investigated the short- and long-term outcomes of different sized balloons, chosen according to each patient's maximum CBD stone size, to dilate the papilla following limited EST.

MATERIALS AND METHODS

Patients

Patients with possible CBD stones, as diagnosed by biliary symptoms and abnormality of biliary enzymes, or whose presence was suspected through imaging modalities, such as ultrasound (US), computed tomography (CT) and/or magnetic resonance imaging (MRI) were screened. Patients were excluded if they had (1) severe acute pancreatitis (APACHE Ⅱ $\geqslant$ 8, or Balthazar CT score $\geqslant$ 4); (2) severe cholangitis with disturbance of consciousness and shock; (3) coagulopathies; (4) malignant diseases; (5) a history of previous EPBD or EST; (6) age > 85 years; (7) a CBD filled with stones; and (8) had undergone a Billroth Ⅱ gastrectomy. Patients who met these criteria and lived in Shanghai, enabling follow-up, were fully informed about the methods and possible complications of the procedure, and were asked to provide written informed consent before ERCP. The study was approved by the Ethics Committee of Shanghai Gongli Hospital, and was supported by the Shanghai Municipal Health Bureau.

Patients were enrolled if selective deep cannulation to the CBD was successful, CBD stones were diagnosed by ERC, and an incision was made to the mid-portion of the papilla with a pull-type sphincterotomy. The diameter of the largest stone was determined by comparing it with the size of the endoscope tip. Patients were divided into five groups based on the largest CBD stone size, of (1) < 5 mm, (2) 6-8 mm, (3) 8-12 mm, (4) 12-14 mm, and (5) > 14 mm. These groups underwent limited EST alone without EPBD, and EPBD with balloons of 8, 10, 12 and 14 mm in diameter, respectively, such that the size of each balloon did not exceed the size of the CBD.

Methods

Pharyngeal anesthesia and premedication before the procedure, including the intravenous administration of diazepam, meperidine hydrochloride, and scopolamine, were performed in the same manner as for general endoscopy. ERCP was performed with a side-viewing endoscope (JF240; JF260V; Olympus, Tokyo, Japan).

Limited EST was performed according to the standard methods using a pull-type sphincterotomy. The incision was made up to the mid-portion of the papilla.

Endoscopic papillary balloon dilation

A balloon dilation catheter of 8, 10, 12 or 14 mm in diameter (Wilson-Cook Medical Inc., NC, United States), was inserted and inflated slowly with diluted contrast fluid until the waistline was obliterated under fluoroscopic monitoring and maintained for one min at 6 atm or 8 atm as required. After the balloon was deflated, the stones were extracted using a retrieval basket (Wilson-Cook Medical Inc., NC, United States) and/or a retrieval balloon (Extracter XL; Boston Scientific Corporation, MA, United States).

When the stone diameter was > 16 mm, as shown by diagnostic ERCP, a mechanical lithotripter (ML; BML-4Q; Olympus Corporation, Tokyo, Japan) was used to break the stones into fragments.

Follow-up

All patients were seen at the outpatient clinic six months to one year after discharge and every year thereafter. At each visit, blood and liver function tests, abdominal US and CT were performed. Other relevant examinations were performed when deemed necessary. If stone recurrence was suspected from symptoms, laboratory data, and/or images, ERCP was performed, and the recurrent stone was removed.

Outcome measures

Short-term outcomes included the rates of post-ERCP pancreatitis, bleeding requiring transfusion, perforation, pneumobilia and mortality. Long-term (2-5 years) outcomes included the rates of reflux cholangitis, pneumobilia, and recurrence of CBD stones.

Statistical analysis

Statistical analyses were performed using statistical software (SPSS 12.0 for Windows; SPSS Inc., Chicago, IL, United States). Quantitative data were presented as the mean ± SD. The χ^2 test or Fisher's exact test was used to compare sex distribution, and rates of mechanical lithotripter (ML) use, gallbladder *in situ*, concomitant gallbladder stones, and early and later complications in the 5 groups. ANOVA was used to compare age, number of stones, diameter of largest stone, and relationships between age and post-ERCP pancreatitis and bleeding in the 5 groups. A P value < 0.05 was considered statistically significant.

Table 1 Demographic data and baseline characteristics of the 5 patient groups

	Balloon diameter					
	EST alone (n = 42)	8 mm (n = 35)	10 mm (n = 87)	12 mm (n = 29)	14 mm (n = 15)	P value
Sex (F/M)	20/22	19/16	50/37	15/14	9/6	0.832
Age (yr)	55.6 ± 13.1	59.5 ± 14.2	66.8 ± 15.5	72.8 ± 11.8	74 ± 5.3	0.003
No. of stones	2.0 ± 1.0	2.2 ± 1.0	2.1 ± 1.4	2.1 ± 1.0	2.3 ± 1.0	0.994
Diameter of largest stone (mm)	5.5 ± 1.5	7.0 ± 2.0	9.3 ± 2.1	10.3 ± 2.7	14.7 ± 1.2	0.000
Use of mechanical lithotripter	0	0	0	0	4	0.000
Gallbladder *in situ*	33	29	74	21	10	0.358
Concomitant gallbladder stones	26	22	61	18	7	0.795
Sessions required for complete stone removal						
Single session	42	35	87	28	13	
Two sessions	0	0	0	1	2	

F: Female; M: Male; EST: Endoscopic sphincterotomy.

RESULTS

Patient characteristics and early complications

We enrolled 208 consecutive patients (95 males and 113 females), all of whom were diagnosed with CBD stones by ERC and underwent successful selective deep cannulation to the CBD at our institution between January 1, 2006, and January 1, 2008. Mean patient age was 62.4 ± 15.0 years. Stones were successfully removed from all the patients in all 5 groups. The demographic data and baseline characteristics of the 5 groups are shown in Table 1.

We observed a significant correlation between patient age and the diameter of the largest stone, with stones > 12 mm occurring more frequently in patients > 60 years old. The ML was used more often in patients with larger CBD stones, especially for stones > 16 mm in diameter. Although the overall success rate of stone removal was 100% in all groups, two patients in the 14 mm balloon group and one in the 12 mm group each required 2 sessions for stone removal due to patient intolerance of a long operation time to remove large stones.

Early complications

All patients were cured, none died, and none had a perforation. We found that 8 (3.85%) patients had post-ERCP pancreatitis, including 1 in the 10 mm balloon dilation group who had severe pancreatitis. All patients were cured by conservative treatments. Six (2.9%) patients experienced upper gastrointestinal bleeding requiring transfusions, including 2 patients with bleeding in the stomach and 4 with bleeding in the duodenal papilla. Two patients were cured by angiographic embolization and 1 by laparotomy to ligate the bleeding vessel after 2 attempts of endoscopic clamping and 1 of angiographic embolization all failed. The other 3 patients were cured conservatively. There were no significant differences in early complication rates among the five groups.

Pneumobilia occurred in 55 (26.4%) patients at a mean of 4.9 ± 0.7 d (range, 3-7 d) after ERCP. We observed a significant correlation between increased balloon size and the incidence of pneumobilia, suggesting that dilation with a large balloon may cause more damage to SO function. Details of early complications are described in Table 2.

Interestingly, post-ERCP pancreatitis and bleeding correlated significantly with age, with post-ERCP pancreatitis occurring more frequently in patients aged < 60 years, and bleeding occurring more frequently in patients aged > 70 years. The 8 patients with post-ERCP pancreatitis were significantly younger than the 200 who did not develop post-ERCP pancreatitis (51.1 ± 8.3 years *vs* 63.5 ± 15.1 years, P = 0.026). Conversely, the 6 patients with bleeding were significantly older than the 202 who did not develop bleeding (75.7 ± 7.1 years *vs* 61.4 ± 15.0 years, P = 0.024).

Later complications

Of the 208 patients, 192 (92.3%) were followed up for at least 2 years, with a mean follow-up time of 3.2 ± 1.1 years (range, 2-5 years). There were no significant differences in the rates of later complications, including reflux cholangitis and recurrence of CBD stones, among the 5 patient groups.

The incidence of pneumobilia one year after ERCP was significantly lower than shortly after ERCP, suggesting that SO function had recovered, at least partially, in these patients. We observed a significant correlation between the size of the dilation balloon and the 1-year incidence of pneumobilia, suggesting that larger balloons may cause more damage to SO function. The details of later complications in each group are shown in Table 2.

DISCUSSION

As no standard endoscopic procedure has been developed to date to maximize the effects and minimize the complications of EST and EPBD[17,18], we prospectively assessed a method combining EPBD with limited EST. CBD stone sizes vary, from 3-5 mm in diameter to 15-30 mm in diameter, or even larger, suggesting that an endoscopic treatment method should be based on stone size. We therefore utilized limited EST alone for CBD stones < 5 mm in diameter, and limited EST followed by EPBD with balloons of 8, 10, 12 and 14 mm for CBD stones 6-8, 8-12, 12-14 and > 14 mm, respectively. We found that tailoring balloon size to stone size was safe and effective,

Table 2 Early and later complications in the 5 patient groups

	Balloon size					
	EST alone (n = 42)	8 mm (n = 35)	10 mm (n = 87)	12 mm (n = 29)	14 mm (n = 15)	P value
Early complications						
Post-ERCP pancreatitis	1	2	3	1	1	0.918
Perforation	0	0	0	0	0	
Bleeding	1	1	3	1	0	0.961
Incidence of pneumobilia	7	5	25	11	7	0.039
Later complications						
Long term outcome						
Incidence of pneumobilia	2	1	10	5	4	0.029
Reflux cholangitis	0	0	2	2	1	0.235
Recurrence of CBD stones	0	0	2	1	0	0.624

ERCP: Endoscopic retrograde cholangiopancreatography; EST: Endoscopic sphincterotomy; CBD: Common bile duct.

with low rates of short- and long-term complications.

Limited EST was sufficient to remove CBD stones < 5 mm in diameter, as the biliary opening was large enough to remove these stones. EBPD was not required as balloons larger than CBD stones can cause more damage to SO function. We found that limited EST did not cause any perforations, an often fatal complication and even more serious than pancreatitis and bleeding, and preserved SO function.

We also found that limited EST followed by EPBD with balloons < 12 mm in size could partially preserve SO function. Although limited EST plus EPBD with balloons 12-14 mm in size did not cause any perforations, it was associated with higher rates of pneumobilia, both shortly after ERCP and ⩾ 2 years later, compared with limited EST alone or followed by EPBD with smaller balloons, suggesting that large balloons result in greater damage to SO function.

Limited EST followed by EPBD has several benefits, including a lower incidence of post-ERCP pancreatitis. After EST, the openings of the pancreatic duct and common bile duct separate, decreasing the pressure on the pancreatic duct caused by EPBD and papillary edema[19]. Using limited EST in all our patients with CBD stones, we found that the overall incidence of post-ERCP pancreatitis was 3.85%, lower than previously reported[5-7,10]. Limited EST combined with EPBD can also make cannulation easier and reduce the procedure and fluoroscopy times[20] by shortening the cannulation length, and is safer than full EST or EPBD alone, because full EST may lead to perforation, while EPBD alone may lead to post-ERCP pancreatitis. Furthermore, limited EST is easier to perform than full EST. We have successfully utilized this method to remove large CBD stones since 1999[21], and have found that it is a good choice for different sized CBD stones.

Large balloon dilation of the papilla may make the removal of CBD stones easier, reducing the need for an ML, and shortening cannulation and stone removal times, thus decreasing the incidence of post-ERCP pancreatitis. However, we did not use a balloon > 15 mm. A recent study in animals showed that EPBD with balloons < 15 mm was safe, with no perforations, whereas balloons > 15 mm was associated with a significantly higher rate of perforation[22]. In contrast, the use of 8 mm balloons in animals showed that EPBD was not associated with fibrosis or altered papillary architecture[23] and many clinical reports have shown that EPBD with large balloons > 15 mm was effective and safe[13-16]. However, the risk of perforation is higher with large balloons, prolonging hospitalization and increasing costs. We also found that increased dilation size was significantly associated with an increased incidence of pneumobilia, indicating that dilation with large balloons may cause more damage to SO function. Although we found no significant differences in later complications, such as reflux cholangitis and recurrence of CBD stones among the 5 patient groups, follow-up time may not have been sufficiently long. SO function is important in preventing biliary diseases, such as acute cholecystitis, cholangitis and recurrence of CBD stones[24], suggesting that preserving SO physiological function may be advantageous, especially in younger patients. Our findings also indicate that 14 mm balloons were large enough to remove CBD stones > 15 mm, assisted by an ML. Taken together, our findings indicate that limited EST, followed by EPBD with balloons < 15 mm is safe.

We found that the rate of post-ERCP pancreatitis and bleeding correlated with patient age, with patients < 60 years more frequently having post-ERCP pancreatitis and patients > 70 years more prone to bleeding. The progressive decline in pancreatic exocrine function with age may protect older patients from pancreatic injury[25,26]. In contrast, the Oddi muscle may be stronger in younger than in older patients, resulting in more difficult dilation in the former and a higher rate of pancreatitis. Although bleeding has seldom been reported after EBPD, we found that 6 (2.9%) of our patients had upper gastrointestinal bleeding requiring transfusions. All 6 were > 65 years old, with a mean age of 76 years. Older patients may be more prone to bleeding due to the relative inelasticity of their blood vessels. We also observed a correlation between CBD stone size and patient age, with stones > 12 mm occurring more frequently in patients > 60 years old.

The main limitation of this study was that we evaluated SO function by pneumobilia incidence, and not by endoscopic manometry. Manometry requires cannulation to the CBD, making it painful for patients and unacceptable during follow-up. Other limitations include the performance of this study at a single center, the relatively small number of patients, and the relatively short follow-up period.

In conclusion, limited EST, alone or followed by EPBD with balloons 8-14 mm, is safe and effective for the removal of different sized CBD stones. Choosing balloon size based on CBD stone size can maximize outcomes and minimize the complications of both EST and EPBD. Balloons > 15 mm in size are not necessary.

ACKNOWLEDGMENTS

We thank Medjaden Bioscience Limited for assisting in the preparation of this manuscript.

COMMENTS

Background

Common bile duct (CBD) stones are very common, and patients are traditionally treated by open CBD exploration. With the advent of invasive endoscopic techniques, more patients are being treated endoscopically. However, endoscopists are often faced with difficult treatment decisions: endoscopic sphincterotomy (EST) or endoscopic papillary balloon dilation (EPBD)? Both methods have distinct advantages and disadvantages. The authors of this study prospectively investigated the short-and long-term outcomes of different sized balloons to dilate the papilla following limited EST.

Research frontiers

Both EST and EPBD are used for patients with CBD stones. Limited EST combined with EPBD has become popular for the removal of CBD stones in recent years, as it is thought to maximize outcomes and minimize the complications of both EST and EPBD.

Innovations and breakthroughs

This study investigated the short- and long-term outcomes of different sized balloons, chosen according to each patient's maximum CBD stone size, to dilate the papilla following limited EST. The authors found that choosing a balloon size no more than 15 mm based on the diameter of each patient's largest CBD stone is a good choice for removing CBD stones.

Applications

EPBD using a balloon size no more than 15 mm based on the diameter of patient's largest CBD stone following limited EST was proven to be a safe and effective treatment for CBD stones, and should be recommended for patients with CBD stones.

Terminology

ERCP: endoscopic retrograde cholangiopancreatography, a technique that combines the use of endoscopy and fluoroscopy to diagnose and treat certain problems of the biliary or pancreatic ductal systems; EST: endoscopic sphincterotomy, a minimally invasive surgery that was developed on the basis of ERCP to treat biliary or pancreatic ductal disease, the incision is made up to the full-portion of the papilla; Limited EST: like EST, the incision is made up to the mid-portion of the papilla; EPBD: endoscopic papillary balloon dilation, the papilla is dilated with a balloon to facilitate the removal of CBD stones.

Peer review

This is a good study in which authors evaluate the short- and long-term outcomes of different sized balloons to dilate the papilla following limited EST. The results are interesting and suggest that choosing balloon size no more than 15 mm based on the diameter of each patient's largest CBD stone is a good choice for removing CBD stones.

REFERENCES

1 **Ikeda S**, Tanaka M, Matsumoto S, Yoshimoto H, Itoh H. Endoscopic sphincterotomy: long-term results in 408 patients with complete follow-up. *Endoscopy* 1988; **20**: 13-17 [PMID: 3342766 DOI: 10.1055/s-2007-1018117]

2 **Rabenstein T**, Schneider HT, Hahn EG, Ell C. 25 years of endoscopic sphincterotomy in Erlangen: assessment of the experience in 3498 patients. *Endoscopy* 1998; **30**: A194-A201 [PMID: 9932780]

3 **Mac Mathuna P**, White P, Clarke E, Lennon J, Crowe J. Endoscopic sphincteroplasty: a novel and safe alternative to papillotomy in the management of bile duct stones. *Gut* 1994; **35**: 127-129 [PMID: 8307433]

4 **Komatsu Y**, Kawabe T, Toda N, Ohashi M, Isayama M, Tateishi K, Sato S, Koike Y, Yamagata M, Tada M, Shiratori Y, Yamada H, Ihori M, Kawase T, Omata M. Endoscopic papillary balloon dilation for the management of common bile duct stones: experience of 226 cases. *Endoscopy* 1998; **30**: 12-17 [PMID: 9548037 DOI: 10.1055/s-2007-993721]

5 **Cotton PB**. Endoscopic management of bile duct stones; (apples and oranges). *Gut* 1984; **25**: 587-597 [PMID: 6376290]

6 **Freeman ML**, Nelson DB, Sherman S, Haber GB, Herman ME, Dorsher PJ, Moore JP, Fennerty MB, Ryan ME, Shaw MJ, Lande JD, Pheley AM. Complications of endoscopic biliary sphincterotomy. *N Engl J Med* 1996; **335**: 909-918 [PMID: 8782497 DOI: 10.1056/NEJM199609263351301]

7 **Lauri A**, Horton RC, Davidson BR, Burroughs AK, Dooley JS. Endoscopic extraction of bile duct stones: management related to stone size. *Gut* 1993; **34**: 1718-1721 [PMID: 8282260]

8 **Bergman JJ**, Rauws EA, Fockens P, van Berkel AM, Bossuyt PM, Tijssen JG, Tytgat GN, Huibregtse K. Randomised trial of endoscopic balloon dilation versus endoscopic sphincterotomy for removal of bileduct stones. *Lancet* 1997; **349**: 1124-1129 [PMID: 9113010]

9 **Watanabe H**, Yoneda M, Tominaga K, Monma T, Kanke K, Shimada T, Terano A, Hiraishi H. Comparison between endoscopic papillary balloon dilatation and endoscopic sphincterotomy for the treatment of common bile duct stones. *J Gastroenterol* 2007; **42**: 56-62 [PMID: 17322994 DOI: 10.1007/s00535-006-1969-9]

10 **Mathuna PM**, White P, Clarke E, Merriman R, Lennon JR, Crowe J. Endoscopic balloon sphincteroplasty (papillary dilation) for bile duct stones: efficacy, safety, and follow-up in 100 patients. *Gastrointest Endosc* 1995; **42**: 468-474 [PMID: 8566640]

11 **Sato H**, Kodama T, Takaaki J, Tatsumi Y, Maeda T, Fujita S, Fukui Y, Ogasawara H, Mitsufuji S. Endoscopic papillary balloon dilatation may preserve sphincter of Oddi function after common bile duct stone management: evaluation from the viewpoint of endoscopic manometry. *Gut* 1997; **41**: 541-544 [PMID: 9391256]

12 **Yasuda I**, Tomita E, Enya M, Kato T, Moriwaki H. Can endoscopic papillary balloon dilation really preserve sphincter of Oddi function? *Gut* 2001; **49**: 686-691 [PMID: 11600473]

13 **Ersoz G**, Tekesin O, Ozutemiz AO, Gunsar F. Biliary sphincterotomy plus dilation with a large balloon for bile duct stones that are difficult to extract. *Gastrointest Endosc* 2003; **57**: 156-159 [PMID: 12556775 DOI: 10.1067/mge.2003.52]

14 **Kim HG**, Cheon YK, Cho YD, Moon JH, Park do H, Lee TH, Choi HJ, Park SH, Lee JS, Lee MS. Small sphincterotomy combined with endoscopic papillary large balloon dilation versus sphincterotomy. *World J Gastroenterol* 2009; **15**: 4298-4304 [PMID: 19750573]

15 **Rebelo A**, Ribeiro PM, Correia AP, Cotter J. Endoscopic papillary large balloon dilation after limited sphincterotomy for difficult biliary stones. *World J Gastrointest Endosc* 2012; **4**:

180-184 [PMID: 22624069 DOI: 10.4253/wjge.v4.i5.180]
16 **Youn YH**, Lim HC, Jahng JH, Jang SI, You JH, Park JS, Lee SJ, Lee DK. The increase in balloon size to over 15 mm does not affect the development of pancreatitis after endoscopic papillary large balloon dilatation for bile duct stone removal. *Dig Dis Sci* 2011; **56**: 1572-1577 [PMID: 20945093 DOI: 10.1007/s10620-010-1438-4]
17 **Aiura K**, Kitagawa Y. Current status of endoscopic papillary balloon dilation for the treatment of bile duct stones. *J Hepatobiliary Pancreat Sci* 2011; **18**: 339-345 [PMID: 21161289 DOI: 10.1007/s00534-010-0362-5]
18 **Chung JW**, Chung JB. Endoscopic papillary balloon dilation for removal of choledocholithiasis: indications, advantages, complications, and long-term follow-up results. *Gut Liver* 2011; **5**: 1-14 [PMID: 21461066 DOI: 10.5009/gnl.2011.5.1.1]
19 **Stefanidis G**, Christodoulou C, Manolakopoulos S, Chuttani R. Endoscopic extraction of large common bile duct stones: A review article. *World J Gastrointest Endosc* 2012; **4**: 167-179 [PMID: 22624068 DOI: 10.4253/wjge.v4.i5.167]
20 **Itoi T**, Itokawa F, Sofuni A, Kurihara T, Tsuchiya T, Ishii K, Tsuji S, Ikeuchi N, Moriyasu F. Endoscopic sphincterotomy combined with large balloon dilation can reduce the procedure time and fluoroscopy time for removal of large bile duct stones. *Am J Gastroenterol* 2009; **104**: 560-565 [PMID: 19174779 DOI: 10.1038/ajg.2008.67]
21 **Li NP**, Yang WX, Liu JQ, Yang ZQ, Geng ZJ, Zhou MQ, Lu DR. Endoscopic papillary balloon dilation in the treatment of common bile duct stones. *Zhongguo Weichuang Waike Zazhi* 2003; **3**: 28-29
22 **Hisatomi K**, Ohno A, Tabei K, Kubota K, Matsuhashi N. Effects of large-balloon dilation on the major duodenal papilla and the lower bile duct: histological evaluation by using an ex vivo adult porcine model. *Gastrointest Endosc* 2010; **72**: 366-372 [PMID: 20674625]
23 **Mac Mathuna P**, Siegenberg D, Gibbons D, Gorin D, O'Brien M, Afdhal NA, Chuttani R. The acute and long-term effect of balloon sphincteroplasty on papillary structure in pigs. *Gastrointest Endosc* 1996; **44**: 650-655 [PMID: 8979052]
24 **Yasuda I**, Fujita N, Maguchi H, Hasebe O, Igarashi Y, Murakami A, Mukai H, Fujii T, Yamao K, Maeshiro K, Tada T, Tsujino T, Komatsu Y. Long-term outcomes after endoscopic sphincterotomy versus endoscopic papillary balloon dilation for bile duct stones. *Gastrointest Endosc* 2010; **72**: 1185-1191 [PMID: 20869711 DOI: 10.1016/j.gie.2010.07.006.]
25 **Wang P**, Li ZS, Liu F, Ren X, Lu NH, Fan ZN, Huang Q, Zhang X, He LP, Sun WS, Zhao Q, Shi RH, Tian ZB, Li YQ, Li W, Zhi FC. Risk factors for ERCP-related complications: a prospective multicenter study. *Am J Gastroenterol* 2009; **104**: 31-40 [PMID: 19098846 DOI: 10.1038/ajg.2008.5.]
26 **Weinberg BM**, Shindy W, Lo S. Endoscopic balloon sphincter dilation (sphincteroplasty) versus sphincterotomy for common bile duct stones. *Cochrane Database Syst Rev* 2006; (4): CD004890 [PMID: 17054222 DOI: 10.1002/14651858.CD004890.pub2]

P- Reviewer Martin JS **S- Editor** Song XX **L- Editor** A **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i6.909

World J Gastroenterol 2013 February 14; 19(6): 909-916
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Novel *CDH1* germline mutations identified in Chinese gastric cancer patients

Qin-Hua Chen, Wei Deng, Xiao-Wei Li, Xiu-Fang Liu, Jing-Mei Wang, Li-Feng Wang, Nong Xiao, Qiong He, Ya-Ping Wang, Yi-Mei Fan

Qin-Hua Chen, Wei Deng, Xiao-Wei Li, Xiu-Fang Liu, Ya-Ping Wang, Yi-Mei Fan, Department of Medical Genetics, Medical School, Nanjing University, Nanjing 210093, Jiangsu Province, China
Qin-Hua Chen, Wei Deng, Xiao-Wei Li, Xiu-Fang Liu, Ya-Ping Wang, Yi-Mei Fan, Jiangsu Key Laboratory of Molecular Medicine, Nanjing 210093, Jiangsu Province, China
Jing-Mei Wang, Department of Pathology, Drum Tower Hospital Affiliated to Medical School, Nanjing University, Nanjing 210093, Jiangsu Province, China
Li-Feng Wang, Department of Oncology, Drum Tower Hospital Affiliated to Medical School, Nanjing University, Nanjing 210093, Jiangsu Province, China
Nong Xiao, Qiong He, Lujiang Hospital, Lujiang 231500, Anhui Province, China
Author contributions: Chen QH and Deng W contributed equally to this work; Fan YM conceived and designed the study, and drafted the manuscript; Deng W also helped to draft the manuscript; Chen QH and Deng W carried out mutation screening, case-control and bioinformatics analysis; Chen QH and Liu XF performed promoter luciferase activity assay; Li XW carried out RNA splicing analysis; Wang JM, Wang LF, Xiao N and He Q contributed to the collection of samples and clinical data; Wang YP reviewed and modified the paper; and all of the authors have read and approved the final manuscript.
Supported by National Natural Science Foundation of China, No. 30972535; the Natural Science Foundation of Jiangsu, China, No. BK2012724; the Fundamental Research Funds for the Central Universities of China, 1112021402
Correspondence to: Dr. Yi-Mei Fan, Associate Professor, Department of Medical Genetics, Medical School, Nanjing University, Hankou Road 22, Nanjing 210093, Jiangsu Province, China. ymfan@nju.edu.cn
Telephone: +86-25-83593374 Fax: +86-25-83686559
Received: July 9, 2012 Revised: December 4, 2012
Accepted: December 27, 2012
Published online: February 14, 2013

Abstract

AIM: To give a comprehensive report of E-cadherin gene (*CDH1*) variations in a population at a high risk for gastric cancer (GC).

METHODS: The samples consisted of 178 men and 58 women with a mean age of 62.3 ± 9.4 years and an age range of 30-84 years. A total of 240 cancer-free controls were recruited (mean age of 61.8 ± 10.1 years, age range of 26-82 years). Samples were screened for *CDH1* germline mutations by high-resolution melting analysis or directly sequencing. Luciferase reporter assay, RNA splicing assay and bioinformatic analysis were used to evaluate the effect of mutations.

RESULTS: Four novel *CDH1* sequence alterations were identified in GC patients including a G>T transition 49 bp before the start codon; a three-nucleotide deletion, c.44_46del TGC; one missense mutation, c.604G>A (V202I); and one variation in the intron, c.1320+7A>G. In addition, polymorphism frequencies were observed for *CDH1*-164delT, -161C>A, -73A>C, c.48+6C>T, c.48+62_48+63delinsCGTGCCCCAGCCC, c.894C>T (A298A), c.1224G>A (A408A), c.1888C>G (L630V), c.2076T>C (A692A), and c.2253C>T (N751N) which is similar to the data reported in http://www.ncbi.nlm.nih.gov/projects/SNP/. RNA splicing analysis suggested that the c.1320+7A>G and c.1224G>A variations did not affect exon splicing ability. Luciferase reporter assay demonstrated that the c.-49T variation might be helpful for *E-cadherin* transcription, though the increase in transcription activity is limited (only 33%). SIFT score and PolyPhen analysis both demonstrated that the L630V missense mutation probably damages protein function, while the V202I variant does not.

CONCLUSION: This study reveals novel mutations in sporadic GC patients which had been poorly investigated for susceptibility genes.

© 2013 Baishideng. All rights reserved.

Key words: Gastric cancer; Germline mutation; *CDH1*; Luciferase reporter assay; RNA splicing analysis

Chen QH, Deng W, Li XW, Liu XF, Wang JM, Wang LF, Xiao N, He Q, Wang YP, Fan YM. Novel *CDH1* germline mutations identified in Chinese gastric cancer patients. *World J Gastroenterol* 2013; 19(6): 909-916 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i6/909.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i6.909

INTRODUCTION

Gastric cancer (GC) is one of the most common malignancies worldwide and the leading cancer in East Asian countries[1]. There are two histopathological types of gastric cancer, differentiated and undifferentiated[2], or intestinal and diffuse, respectively[3]. Genetic factors are important for the etiology of GC. The E-cadherin gene (*CDH1*), a calcium-dependent transmembrane glycoprotein, is critical for epithelial architecture, intercellular adhesion, and cell invasion[4]. E-cadherin consists of a large extracellular domain composed of five repeat domains and smaller transmembrane and cytoplasmic domains[5]. Mutations in the *CDH1* gene and perturbation of E-cadherin expression are the most frequent genetic alterations in hereditary diffuse gastric cancer (HDGC)[6,7]. The *CDH1* germline mutation spectrum is heterogeneous and includes point mutations, small deletions, and insertions distributed along the entire coding sequence[8-10]. In *CDH1* germline variation carriers, the lifetime penetrance is estimated to be approximately 70%[11]. The identification of *CDH1* mutations offers the opportunity for the development of cancer risk-reduction strategies for unaffected at-risk individuals. About 90% of gastric carcinoma presents a sporadic setting and only 10% shows a familial cluster; among this group, about 15% are considered as hereditary syndromes, such as the HDGC. For sporadic GC, germline *CDH1* mutations are seldom reported.

In this study, we carried out a comprehensive screen of *CDH1* germline mutations in 236 Chinese GC patients (175 sporadic cases and 61 cases with hereditary predisposition) (Table 1) and identified four novel germline *CDH1* mutations in sporadic GC patients. In addition, the *CDH1* polymorphism frequencies in Chinese GC patients and controls were determined. Furthermore, functional assays were carried out to evaluate the impact of the novel mutations identified.

MATERIALS AND METHODS

Subjects

Gastric cancer patients from the East District of China having disease onset between January 1 and December 31, 2008, in whom tumors had been confirmed using histology, were investigated. The samples consisted of 178 men and 58 women with a mean age of 62.3 ± 9.4 years and an age range of 30-84 years. A total of 240 cancer-free controls were recruited (mean age of 61.8 ± 10.1 years, age range of 26-82 years) (Table 1). Details regarding the following information are summarized in Table 1: gastric cancer family history, age of onset and histological and tumor-node-metastasis (TNM) staging classifications. Informed consent, according to the Ethics Committee of the Medical School of Nanjing University, was obtained from all subjects who underwent genetic testing.

Table 1 Frequency distributions of variables in gastric cancer cases and controls *n* (%)

Variables	Cases	Controls	*P* value
Number	236	240	
Age (yr)			0.997
≤ 49	19 (8.1)	19 (7.9)	
50-59	65 (27.5)	65 (27.1)	
60-69	94 (39.8)	98 (40.8)	
≥ 70	58 (24.6)	58 (24.2)	
Gender			0.915
Male	178 (75.4)	180 (75.0)	
Female	58 (24.6)	60 (25.0)	
Family history			
Familial recurrence for gastric cancer[1]	6 (2.5)		
Low familial recurrence for gastric cancer[2]	39 (16.5)		
Young age (< 50 yr) of sporadic disease	16 (6.8)		
Old age (≥ 50 yr) of sporadic disease	175 (74.2)		
Histologic grade[3]			
Poorly differentiated adenocarcinoma	64 (39.5)		
Moderately differentiated adenocarcinoma	69 (42.6)		
Well differentiated adenocarcinoma	29 (17.9)		
Depth invasion (pT)[3,4]			
pT1	12 (7.4)		
pT2	32 (19.8)		
pT3	109 (67.3)		
pT4	9 (5.5)		
Lymph node involvement (pN)[3,4]			
pN0	35 (21.6)		
pN1	3 (1.9)		
pN2	65 (40.1)		
pN3	59 (36.4)		
Distant metastasis (M)[3,4]			
M0	161 (99.4)		
M1	1 (0.6)		
TNM stage[3,4]			
Stage Ⅰ	8 (4.9)		
Stage Ⅱ	39 (24.1)		
Stage Ⅲ	115 (71.0)		

[1]Individuals with gastric cancer and two or more first-degree relatives with gastric cancer or related cancers; [2]Individuals with gastric cancer and one first-degree relative with gastric cancer or related cancers; [3]Available for 162 cases; [4]According to the National Comprehensive Cancer Network guidelines on tumor-node-metastasis (TNM) Staging Classification for Carcinoma of the Stomach (7th ed., 2010) by the American Joint Committee on cancer.

Genotyping analysis

Genomic DNA was extracted from peripheral blood leukocytes using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Mutation screening of *CDH1* exons 2-16 and neighboring intronic sequences was performed using

polymerase chain reaction (PCR) and high-resolution melting analysis using a LightScanner system (Idaho technology, Salt Lake City, UT, United States). The samples that presented abnormal profiles were sequenced on an ABI 3130-Avant automated sequencer (Applied Biosystems, Foster City, CA, United States). The region around the transcription start site (TSS) of the *CDH1* gene (From promoter region to intron 1 of *CDH1* gene) was genotyped using PCR and directly sequenced on the ABI 3130-Avant automated sequencer. Primer sequences and PCR conditions are available upon request.

Promoter luciferase activity assay

A dual-luciferase reporter assay system (http://www.promega.com) was used to examine the effects of novel sequence variation in the promoter region on the transcriptional activity of *CDH1*. Briefly, DNA fragments around the TSS (-345 to 271 bp) were amplified by PCR using genomic DNA containing either the particular variant sequence or *CDH1* wild type as a template. The amplified fragments were designed to contain the region possessing basal promoter activity. The following primer sequences were used: 5'-ATGCCTCGAGCCATCTCCAAAACGAACAAAC-3' (forward) and 5'-ATGCAAGCTTGAAGGGAAGCGGTGACGAC-3' (reverse), which include the restriction sites (underlined) for Xho I and Hind Ⅲ, respectively. The PCR products were digested with Xho I and Hind Ⅲ and subsequently cloned into the pGL3-basic vector carrying the firefly luciferase gene (Promega). The nucleotide sequence of the fragment inserted into each plasmid was confirmed by DNA sequencing. Plasmids were then transiently transfected into Hela cells using the Lipofect transfection reagent (Tiangen Biotech, Beijing, Co., Ltd., China). All plasmids were co-transfected with the renilla luciferase gene containing the pRL-CMV plasmid (Promega) as an internal standard. Cell extracts were prepared, and luciferase activity was measured by a luminometer instrument (Promega) using the dual-luciferase reporter assay system (Promega). The transcriptional activity in each cell extract was determined from the level of firefly luciferase after normalization to renilla luciferase activity. Four independent experiments were performed using DNA from plasmid preparations.

RNA splicing analysis on clinical samples

Because it has been recognized that DNA sequence variants localized in exon-intron boundaries could be pathogenic by affecting exon definition and the splicing of pre-mRNA[12,13], we used RNA splicing assay to evaluate the variant located at the 5' terminal of intron 9. Total RNA from frozen tumor tissue and paired normal tissue was extracted using RNAiso Plus (TaKaRa Biotechnology [(Dalian) Co., Ltd.]. Reverse transcription (RT)-PCR was performed in 2 steps. First strand cDNA synthesis was performed using PrimerScript RT reagent Kit (TaKaRa) with random DNA hexamers and oligo-dT primer according to the manufacturer's protocol. cDNA was amplified in the region of exons 7-10. Primer sequences were 5'-GGACCGAGAGAGTTTCCCTACG-3' (sense) and 5'-GTTATTTTCTGTTCCATAAATG-3' (antisense). PCR conditions were as follows: 35 cycles at 94 ℃ for 30 s, 58 ℃ for 30 s and 72 ℃ for 30 s, followed by a final extension at 72 ℃ for 5 min. Agarose gel electrophoresis was carried out using 2% gels run at 100 V for 40 min. The purified amplification products were sequenced on the ABI 3130-Avant automated sequencer.

Bioinformatics analysis of CDH1 variants

The impact of amino acid allelic variants on protein structure/function can be predicted via analysis of multiple sequence alignments and protein 3D-structures. The sorting intolerant from tolerant (SIFT) algorithm and *Poly*morphism *Phen*otyping (PolyPhen) were adopted.

SIFT is a program that predicts the effect of amino acid substitutions on protein function based on sequence conservation during evolution and the nature of the amino acids substituted in a gene of interest[14]. The SIFT score was calculated online (http://sift.jcvi.org/). If the value is less than 0.05, the amino acid substitution is predicted as intolerant, while those with a value greater than or equal to 0.05 are classified as tolerated.

PolyPhen is an automatic tool for prediction of the possible impact of an amino acid substitution on the structure and function of a human protein based on straightforward physical and comparative considerations[15] (http://genetics.bwh.harvard.edu/pph/). Each of the two amino acid residues [the original residue and the single-nucleotide polymorphism (SNP)] was entered and the difference between the position-specific independent counts (PSIC) scores of the two residues was computed. The higher a PSIC score difference is, the higher the functional impact a particular amino acid substitution is likely to have. A PSIC score difference of 1.5 and above is considered to be damaging.

Statistical analysis

χ^2 tests or Fisher's exact tests were used to compare the distribution of variables between cases and controls. Luciferase activities were compared using Student's unpaired *t* test. All statistical tests were two-sided, with a *P* value of 0.05 considered to be significant, using SPSS software (version 16).

RESULTS

Characteristics of the study population

The study was comprised of 236 gastric cancer cases and 240 cancer-free controls. There were no significant differences in the distributions of age or gender between the cases and controls ($P = 0.997$ and 0.915, respectively) (Table 1). The majority of studied cases were sporadic; approximately 20% had a family history of cancer. The tumor type was assessed in 162 cases, and more than 80% of the cases had poorly differentiated or moderately differentiated adenocarcinoma; more than 70% of the cases were in TNM Stage Ⅲ (Table 1).

Table 2 Novel *CDH1* mutations and polymorphism identified in Chinese gastric cancer patients *n* (%)

Gene location	Sequence variant	Consequence	Genotype		
			Gastric cancer patients (*n* = 236)	Control (*n* = 240)	*P* value
5′UTR	c.-49 G>T	Substitution at the 5′UTR	4 (1.7)	3 (1.3)	0.723
Exon 1	c.44_46delTGC	Loss of the 15th code (Leu)	2 (0.85)	0 (0.0)	0.245
Exon 5	c.604G>A	Missense V202I	1 (0.4)	0 (0.0)	0.496
Intron 9	c.1320+7 A>G	Substitution of invariant A	1 (0.4)	0 (0.0)	0.496

Two-sided χ^2 test or Fisher's exact test for genotype distribution

Table 3 Polymorphisms identified in *CDH1* in Chinese gastric cancer patients and controls

Gene location	Sequence variant	Condon	MAF in 236 GC patients	MAF in 240 controls	Reported MAF[1]
Promoter	-164del T(g.4837delT)	-	0.004del T	0.000del T	rs5030658: NA
Promoter	-161C>A(g.4840C>A)	-	0.242A	0.227A	rs16260: 0.227A
Promoter	-73A>C (g.4928A>C)	-	0.129C	0.146C	rs28372783: 0.040C
Intron 1	c.48+6C>T	-	0.280C	0.271C	rs3743674: 0.214C
Intron1	c.48+62_48+63delinsCGTGCCCCAGCCC	-	0.280del[2]	0.271del[2]	rs3833051: NA
Exon 7	c.894C>T	A298A	0.002T	0.000T	rs139110184: NA
Exon 9	c.1224G>A	A408A	0.002A	0.000A	rs200161607: 0.001A
Exon 12	c.1888C>G	L630V	0.004G	0.004G	rs2276331: 0.002G
Exon 13	c.2076T>C	A692A	0.377T	0.404T	rs1801552: 0.307T
Exon 14	c.2253C>T	N751N	0.089T	0.102T	rs33964119: 0.058T

[1]From http://www.ncbi.nlm.nih.gov/projects/SNP/; [2]Coincident with MAF of c.48+6C>T variant. GC: Gastric cancer; MAF: Minor allele count; NA: Not available.

Table 4 Available clinical-pathologic characteristics of the gastric cancer cases with novel *CDH1* mutations

ID code	Age onset (yr)	Gender	Family history	Histologic grade	Depth invasion (pT)	Lymph node involvement (pN)	Distant metastasis (M)	CDH1 variants
G45	66	Female	Sporadic	Poorly differentiated adenocarcinoma	pT3	pN3	M0	c.44_46delTGC
G68	51	Male	Sporadic	NA	NA	NA	NA	c.44_46delTGC
G150[1]	58	Male	Sporadic	Moderately differentiated adenocarcinoma	pT2	pN3	M0	c.604G>A (V202I)
G26	56	Male	Sporadic	Moderately differentiated adenocarcinoma	pT3	pN2	M0	c.1320+7 A>G

[1]Also harbors the MLH1 c.2101C>A (Q701K) mutation[16]. NA: Not available.

CDH1 genetic screening revealed four novel germline sequence variants

Four novel *CDH1* germline variations were identified in gastric cancer patients. One of the variants was located in the *CDH1* 5'UTR (c.-49 G>T) and is seemed to be a polymorphism since it is found in both the cases and controls. The other three were only detected in the GC cases and not seen in the controls. One was a missense mutation in the coding region [c.604G>A (V202I)], one was a three-nucleotide deletion in exon 1 (c.44_46del TGC) and the other was an intronic variation (c.1320+7A>G) (Figure 1 and Table 2). In addition, ten *CDH1* polymorphisms (and their frequencies) were observed. The polymorphisms frequencies are similar to the data available at the SNP website (http://www.ncbi.nlm.nih.gov/projects/SNP/) (Table 3).

Clinical-pathologic characteristics of the GC cases with novel CDH1 mutations

The cases carrying novel *CDH1* mutations were all sporadic GC patients, with poorly differentiated or moderately differentiated adenocarcinoma (Table 4). The case with *CDH1* c.604G>A (V202I) mutation harbors the *MLH1* c.2101C>A (Q701K) mutation as well[16].

Functional characterization of the novel CDH1 variants

c.-49T variation contribute a slightly higher promoter activity of the *CDH1* gene than the wild type: The c.-49T alteration was near the TSS of the *CDH1* gene (49 bp before the start codon, and +76 bp relative to the TSS). To examine the potential effect of the c.-49T variation on E-cadherin gene transcription, a 616-bp promoter of the E-cadherin gene (-345 to 271) carrying either the G or T allele was inserted upstream of the luciferase gene in the pGL3 promoterless enhancer plasmid vector. The activity of the E-cadherin G/T promoter-luciferase reporter gene constructs was assessed using transient transfection assays in Hela cells. As shown in Figure 2, slightly higher luciferase activities were observed for the pGL-T construct compared with the pGL-G (wild type)

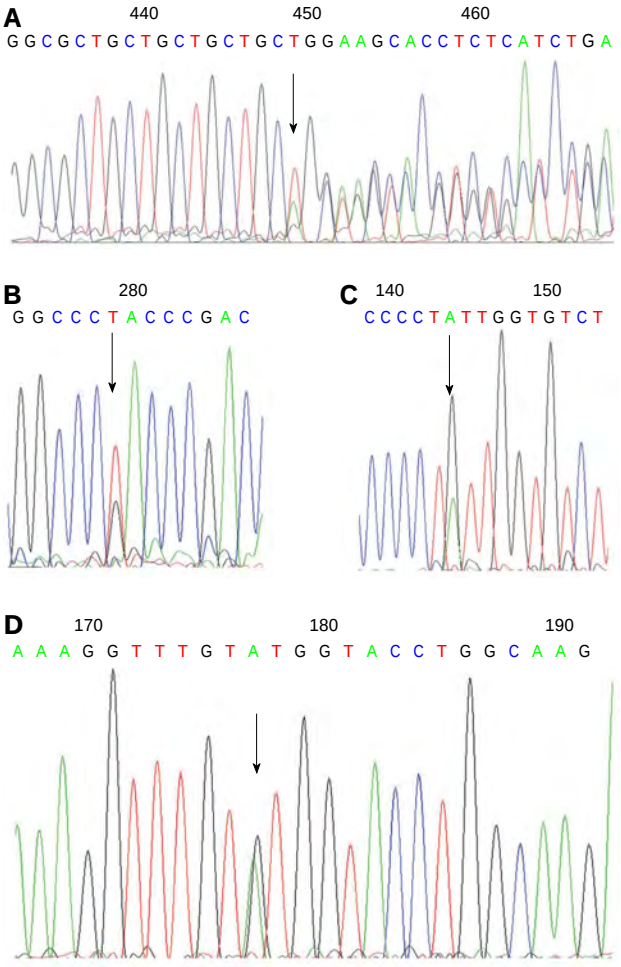


Figure 1 Novel variations detected in this study. A: c.44_46del TGC; B: c.-49G>T; C: c.604G>A (V202I); D: c.1320+7A>G.

construct. The average activity of the promoter having the c.-49T variation was 133.7% (P = 0.002) relative to the *CDH1* wild type promoter.

c.44_46del TGC variant causes the loss of one leucine in the signal peptide region of the E-cadherin protein: Codon sequence analysis demonstrated that the three-nucleotide deletion c.44_46del TGC causes the loss of a single amino acid [the 15th codon (Leucine)] in exon 1 of CDH1, which is in the signal peptide region of the E-cadherin protein.

Intron variation (c.1320+7A>G) and the silent mutation [c.1224G>A (A408A)] do not induce CDH1 splicing defects: To evaluate functional consequence of those novel mutations detected on pre-mRNA splicing, we analyzed cDNA produced *in vivo* from tissues retrieved from the patients harboring the novel mutations. The PCR fragment generated using primers flanking the silent mutation [c.1224G>A (A408A)] and the one intron variation (c.1320+7A>G) indicated normal-sized mRNA. This result demonstrated that a splicing defect was not likely to occur as a consequence of these mutations (Figure 3).

Table 5 *CDH1* missense mutations analyzed by sorting intolerant from tolerant and PolyPhen

Sequence variant	Structural alteration	SIFT scores	PSIC score difference	Prediction
c.604G>A	V202I	0.20	0.381	Benign
c.1888C>G	L630V	0.02	1.748	Probably damaging

SIFT: Sorting intolerant from tolerant; PSIC: Position-specific independent counts.

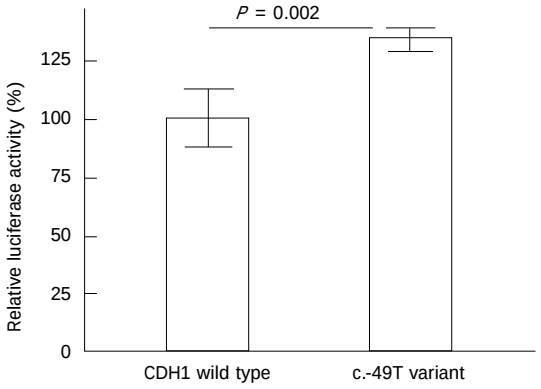


Figure 2 Luciferase reporter assay for the *CDH1* c.-49 G>T variant. The average relative luciferase activity (with standard deviation) is shown. The activity of *CDH1* wild type was defined as 100%.

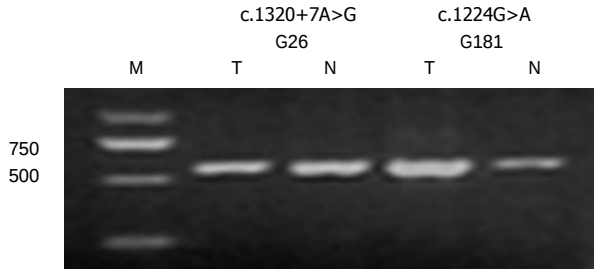


Figure 3 Results of reverse transcription polymerase chain reaction for *CDH1* in patient G26 (c.1320+7A>G, intron 9) and patient G181 [c.1224G>A(A408A), exon 9] using primers in exons 7 and 10. Both patients had an expected band of 567 bp. M: Molecular size markers; T: Tumor tissue; N: Paired normal tissue.

c.1888C>G (L630V) missense mutation might impair E-cadherin protein function while the V202I mutation does not: Two *CDH1* missense mutations were detected. Both the SIFT score and the PolyPhen analysis demonstrated that the L630V variant was sorted as being intolerant, suggesting that this amino acid substitution is predicted to damage protein function. The other variant, V202I, of CDH1 was sorted as tolerant (Table 5).

DISCUSSION

The discovery of genetic variants responsible for the pathogenesis of gastric cancer is important in understanding this disease. Although screening of *CDH1* germline mutations in hereditary GC has been fairly well established, the report of *CDH1* germline mutations in

sporadic GC is limited. Bacani *et al*[17] identified a germline deletion (nt41delT) in a 30-year-old sporadic GC patient and suggested that 2%-3% of cases of early-onset gastric cancer in North America may be owing to high-risk genetic mutations. Garziera *et al*[18] reported a germline missense mutation in *CDH1* exon 6, c. 820 G>A (G274S) in one sporadic Italian gastric cancer patient. Here, we report a population-based study of GC to determine the role of germline mutations in a population at a high risk for GC. The majority of studied cases were sporadic. We have studied all of the coding and promoter core regions of the most important gene implicated in GC and have identified four novel *CDH1* sequence variants distributed along the entire coding sequence and the non-coding regions in the *CDH1* gene. This is consistent with previously published reports[19], suggesting that *CDH1* mutations have arisen without mutational hotspots.

The c.-49 G>T transition was detected in 4/236 (1.7%) of GC patients and 3/240 (1.3%) of cancer-free controls; however, these differences did not achieve significance (P = 0.723, Table 2). One patient with this variant had low familial recurrence for gastric cancer, but IHC showed normal CDH1 protein expression. Previous studies have demonstrated that a fragment spanning -399 to +31 bp relative to the TSS of the *CDH1* gene possesses basal promoter activity[20]. As this variant is around the TSS of the *CDH1* gene, a luciferase reporter assay was carried out. This *in vitro* assay showed that the c.-49T promoter had 33% higher activity than the promoter containing the c.-49G (Figure 2). So this polymorphism might be helpful for E-cadherin transcription, though the increase in transcription activity is limited (only 33%).

The c.44_46del TGC variant was detected in two sporadic GC patients of 51 years and 66 years and was not detected in the 240 controls. One patient's pathological data was available, which showed a poorly differentiated adenocarcinoma (Table 4). To the best of our knowledge, this variant has never been reported in the open access mutation database and literatures. As the parents of the probands are not available for mutation analyzing, we are not certain whether it's a *de novo* mutation. This variant seems to be a rare variant with an allele frequency in GC patients of 0.85% and can even be considered as a kind of mutation hotspot, as it has been detected in two patients with no relationship. Recently rare variants have been reported in several diseases, include cancer. The identified rare variants often have functional effects on protein-protein interactions. Further, rare variants might confer a stronger increase in disease risk than common variants and may make a substantial contribution to the multifactorial inheritance of common chronic diseases[21-24]. Codon sequence analysis demonstrated that the c.44_46del TGC variant causes the loss of a single amino acid [the 15th codon (Leucine)] in exon 1 of CDH1 which is in the signal peptide region of the E-cadherin protein. This amino acid loss might have effect on E-cadherin protein, and further functional analysis should be carried out to investigate associations of the variant with phenotype.

Growing evidence has shown that rare single base substitutions localized in exon-intron boundaries can disrupt one of the cis-transcriptional elements known as exonic splicing enhancers and affect normal pre-mRNA splicing[12,13]. Therefore, it appears reasonable to verify the effect of variants at the mRNA level. The sequence alteration c.1320+7A>G, located in an exon-intron boundary, was detected in a 56-year-old GC patient and not in the 240 controls (Table 2). RNA splicing assay demonstrated that this variation did not affect exon splicing ability (Figure 3) and might be rare polymorphism.

The CDH1 molecule consists of five tandemly repeated extracellular domains (EC1-EC5, containing exons 4-13), each about 110 amino acids in length. This large extracellular domain is responsible for Ca^{2+} binding and is important for cell-cell adhesion. The NH2-terminal EC1 domain is required for lateral E-cadherin dimerization contributing to the intercellular junction[25-27]. A novel missense mutation, V202I, is located in the middle of EC1. While EC1 shows remarkably high conservation between various species[28], SIFT and PolyPhen analyses both showed that V202I might be a tolerant variation (Table 5). The GC patient with this variation carried the *MLH1* c.2101C>A (Q701K) mutation as well. IHC analysis in the index patient demonstrated a loss of MLH1 protein and normal expression of MSH2 and E-cadherin[16]; therefore, we suggest that the *MLH1* c.2101 C>A (Q701K) mutation, and not the *CDH1* c.604G>A (V202I) variation, might be the cause of GC in this patient.

In addition, frequencies of *CDH1* polymorphisms in Chinese GC patients and controls were reported which were similar to those reported at http://www.ncbi.nlm.nih.gov/projects/SNP/ (Table 3). It needs to say something about the rare polymorphism, c.1888C>G (L630V). The SIFT score of the CDH1 variants and the PolyPhen analysis both showed that the L630V variant probably damages protein function (Table 5). However, data from case-control analysis did not support an effect of this L630V variant (Table 3). Accordingly, the pathogenic role of this polymorphism remains elusive and *in vitro* approaches should be performed to elucidate its function.

In conclusion, this study reveals novel mutations in sporadic GC patients in China, a high-incidence country for GC. Though the pathogenic role of the variants remains still uncertain, our findings display the necessity to scan germline *CDH1* variants in sporadic gastric cancer population.

COMMENTS

Background

Although E-cadherin gene (*CDH1*) germline mutations are well implicated in hereditary diffuse gastric cancer, the report of *CDH1* germline mutations in sporadic gastric cancer (GC) is limited.

Research frontiers

Here, the authors report a population-based study of GC to determine the role

of *CDH1* germline mutations in a population at a high risk for GC. The majority of studied cases were sporadic.

Innovations and breakthroughs

The authors have studied all of the coding and promoter core regions of *CDH1* and identified four novel *CDH1* sequence variants, including one transition near the transcription start site, one three-nucleotide deletion in code region, one missense mutation, and one variation in exon-intron boundary. Three of the four variants were detected only in sporadic GC patients and not in the 240 cancer-free controls. Though the functional significance of the variants remains still uncertain, this study reveals novel mutations in sporadic GC patients which had been poorly investigated for susceptibility genes

Applications

The findings display the necessity to scan germline *CDH1* variants in sporadic gastric cancer population.

Terminology

High-resolution melting analysis, is a high-throughput single-nucleotide polymorphism genotyping technology based on the analysis of the melting profile of polymerase chain reaction products.

Peer review

In this study, the authors identified 4 novel *CDH1* germline mutations in different patients harbouring GC with a sporadic setting. The identification of mutations represents an important discovery, to assess the cancer risk for the novel generations.

REFERENCES

1 **Ushijima T**, Sasako M. Focus on gastric cancer. *Cancer Cell* 2004; **5**: 121-125 [PMID: 14998488]

2 **Nakamura K**, Sugano H, Takagi K. Carcinoma of the stomach in incipient phase: its histogenesis and histological appearances. *Gann* 1968; **59**: 251-258 [PMID: 5726267]

3 **Lauren P**. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. An attempt at a histo-clinical classification. *Acta Pathol Microbiol Scand* 1965; **64**: 31-49 [PMID: 14320675]

4 **Takeichi M**. Cadherin cell adhesion receptors as a morphogenetic regulator. *Science* 1991; **251**: 1451-1455 [PMID: 2006419]

5 **Ringwald M**, Schuh R, Vestweber D, Eistetter H, Lottspeich F, Engel J, Dölz R, Jähnig F, Epplen J, Mayer S. The structure of cell adhesion molecule uvomorulin. Insights into the molecular mechanism of Ca2+-dependent cell adhesion. *EMBO J* 1987; **6**: 3647-3653 [PMID: 3501370]

6 **Pedrazzani C**, Corso G, Marrelli D, Roviello F. E-cadherin and hereditary diffuse gastric cancer. *Surgery* 2007; **142**: 645-657 [PMID: 17981184]

7 **Corso G**, Marrelli D, Pascale V, Vindigni C, Roviello F. Frequency of CDH1 germline mutations in gastric carcinoma coming from high- and low-risk areas: metanalysis and systematic review of the literature. *BMC Cancer* 2012; **12**: 8 [PMID: 22225527]

8 **Brooks-Wilson AR**, Kaurah P, Suriano G, Leach S, Senz J, Grehan N, Butterfield YS, Jeyes J, Schinas J, Bacani J, Kelsey M, Ferreira P, MacGillivray B, MacLeod P, Micek M, Ford J, Foulkes W, Australie K, Greenberg C, LaPointe M, Gilpin C, Nikkel S, Gilchrist D, Hughes R, Jackson CE, Monaghan KG, Oliveira MJ, Seruca R, Gallinger S, Caldas C, Huntsman D. Germline E-cadherin mutations in hereditary diffuse gastric cancer: assessment of 42 new families and review of genetic screening criteria. *J Med Genet* 2004; **41**: 508-517 [PMID: 15235021]

9 **Kaurah P**, MacMillan A, Boyd N, Senz J, De Luca A, Chun N, Suriano G, Zaor S, Van Manen L, Gilpin C, Nikkel S, Connolly-Wilson M, Weissman S, Rubinstein WS, Sebold C, Greenstein R, Stroop J, Yim D, Panzini B, McKinnon W, Greenblatt M, Wirtzfeld D, Fontaine D, Coit D, Yoon S, Chung D, Lauwers G, Pizzuti A, Vaccaro C, Redal MA, Oliveira C, Tischkowitz M, Olschwang S, Gallinger S, Lynch H, Green J, Ford J, Pharoah P, Fernandez B, Huntsman D. Founder and recurrent CDH1 mutations in families with hereditary diffuse gastric cancer. *JAMA* 2007; **297**: 2360-2372 [PMID: 17545690 DOI: 10.1001/jama.297.21.2360]

10 **Shah MA**, Salo-Mullen E, Stadler Z, Ruggeri JM, Mirander M, Pristyazhnyuk Y, Zhang L. De novo CDH1 mutation in a family presenting with early-onset diffuse gastric cancer. *Clin Genet* 2012; **82**: 283-287 [PMID: 21696387 DOI: 10.1111/j.1399-0004.2011.01744.x]

11 **Pharoah PD**, Guilford P, Caldas C. Incidence of gastric cancer and breast cancer in CDH1 (E-cadherin) mutation carriers from hereditary diffuse gastric cancer families. *Gastroenterology* 2001; **121**: 1348-1353 [PMID: 11729114]

12 **Cartegni L**, Chew SL, Krainer AR. Listening to silence and understanding nonsense: exonic mutations that affect splicing. *Nat Rev Genet* 2002; **3**: 285-298 [PMID: 11967553 DOI: 10.1038/nrg775]

13 **Tournier I**, Vezain M, Martins A, Charbonnier F, Baert-Desurmont S, Olschwang S, Wang Q, Buisine MP, Soret J, Tazi J, Frébourg T, Tosi M. A large fraction of unclassified variants of the mismatch repair genes MLH1 and MSH2 is associated with splicing defects. *Hum Mutat* 2008; **29**: 1412-1424 [PMID: 18561205 DOI: 10.1002/humu.20796]

14 **Ng PC**, Henikoff S. SIFT: Predicting amino acid changes that affect protein function. *Nucleic Acids Res* 2003; **31**: 3812-3814 [PMID: 12824425]

15 **Ramensky V**, Bork P, Sunyaev S. Human non-synonymous SNPs: server and survey. *Nucleic Acids Res* 2002; **30**: 3894-3900 [PMID: 12202775]

16 **Zhi W**, Xue B, Wang L, Xiao N, He Q, Wang Y, Fan Y. The MLH1 2101C& gt; A (Q701K) variant increases the risk of gastric cancer in Chinese males. *BMC Gastroenterol* 2011; **11**: 133 [PMID: 22136435 DOI: 10.1186/1471-230X-11-133]

17 **Bacani JT**, Soares M, Zwingerman R, di Nicola N, Senz J, Riddell R, Huntsman DG, Gallinger S. CDH1/E-cadherin germline mutations in early-onset gastric cancer. *J Med Genet* 2006; **43**: 867-872 [PMID: 16801346 DOI: 10.1136/jmg.2006.043133]

18 **Garziera M**, De Re V, Geremia S, Seruca R, Figueiredo J, Melo S, Simões-Correia J, Caggiari L, De Zorzi M, Canzonieri V, Cannizzaro R, Toffoli G. A novel CDH1 germline missense mutation in a sporadic gastric cancer patient in north-east of Italy. *Clin Exp Med* 2012 Apr 29; Epub ahead of print [PMID: 22543498 DOI: 10.1007/s10238-012-0184-7]

19 **Blair V**, Martin I, Shaw D, Winship I, Kerr D, Arnold J, Harawira P, McLeod M, Parry S, Charlton A, Findlay M, Cox B, Humar B, More H, Guilford P. Hereditary diffuse gastric cancer: diagnosis and management. *Clin Gastroenterol Hepatol* 2006; **4**: 262-275 [PMID: 16527687]

20 **Giroldi LA**, Bringuier PP, de Weijert M, Jansen C, van Bokhoven A, Schalken JA. Role of E boxes in the repression of E-cadherin expression. *Biochem Biophys Res Commun* 1997; **241**: 453-458 [PMID: 9425291 DOI: 10.1006/bbrc.1997.7831]

21 **Bodmer W**, Bonilla C. Common and rare variants in multifactorial susceptibility to common diseases. *Nat Genet* 2008; **40**: 695-701 [PMID: 18509313 DOI: 10.1038/ng.f.136]

22 **Manolio TA**, Collins FS, Cox NJ, Goldstein DB, Hindorff LA, Hunter DJ, McCarthy MI, Ramos EM, Cardon LR, Chakravarti A, Cho JH, Guttmacher AE, Kong A, Kruglyak L, Mardis E, Rotimi CN, Slatkin M, Valle D, Whittemore AS, Boehnke M, Clark AG, Eichler EE, Gibson G, Haines JL, Mackay TF, McCarroll SA, Visscher PM. Finding the missing heritability of complex diseases. *Nature* 2009; **461**: 747-753 [PMID: 19812666 DOI: 10.1038/nature08494]

23 **Gorlov IP**, Gorlova OY, Sunyaev SR, Spitz MR, Amos CI. Shifting paradigm of association studies: value of rare single-nucleotide polymorphisms. *Am J Hum Genet* 2008; **82**: 100-112 [PMID: 18179889 DOI: 10.1016/j.ajhg.2007.09.006]

24 **Fearnhead NS**, Winney B, Bodmer WF. Rare variant hy-

pothesis for multifactorial inheritance: susceptibility to colorectal adenomas as a model. *Cell Cycle* 2005; **4**: 521-525 [PMID: 15753653]

25 **Parisini E**, Higgins JM, Liu JH, Brenner MB, Wang JH. The crystal structure of human E-cadherin domains 1 and 2, and comparison with other cadherins in the context of adhesion mechanism. *J Mol Biol* 2007; **373**: 401-411 [PMID: 17850815 DOI: 10.1016/j.jmb.2007.08.011]

26 **Nelson WJ**. Regulation of cell-cell adhesion by the cadherin-catenin complex. *Biochem Soc Trans* 2008; **36**: 149-155 [PMID: 18363555 DOI: 10.1042/BST0360149]

27 **Shi Q**, Maruthamuthu V, Li F, Leckband D. Allosteric cross talk between cadherin extracellular domains. *Biophys J* 2010; **99**: 95-104 [PMID: 20655837 DOI: 10.1016/j.bpj.2010.03.062]

28 **Berx G**, Staes K, van Hengel J, Molemans F, Bussemakers MJ, van Bokhoven A, van Roy F. Cloning and characterization of the human invasion suppressor gene E-cadherin (CDH1). *Genomics* 1995; **26**: 281-289 [PMID: 7601454]

P- Reviewer Marrelli D **S- Editor** Zhai HH **L- Editor** A **E- Editor** Li JY



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i6.917

World J Gastroenterol 2013 February 14; 19(6): 917-922
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Effects of early enteral nutrition on immune function of severe acute pancreatitis patients

Jia-Kui Sun, Xin-Wei Mu, Wei-Qin Li, Zhi-Hui Tong, Jing Li, Shu-Yun Zheng

Jia-Kui Sun, Xin-Wei Mu, Jing Li, Shu-Yun Zheng, Department of Intensive Care Unit, Nanjing First Hospital, Nanjing Medical University, Nanjing 210006, Jiangsu Province, China
Jia-Kui Sun, Wei-Qin Li, Zhi-Hui Tong, Department of General Surgery, Jinling Hospital, Nanjing University School of Medicine, Nanjing 210002, Jiangsu Province, China
Author contributions: Sun JK, Li WQ and Tong ZH designed the research; Sun JK, Mu XW, Li J and Zheng SY performed the research; Sun JK and Li WQ analyzed the data; and Sun JK wrote the paper.
Supported by Grants from the Key Project of the Eleventh Five-Year Plan of People's Liberation Army, No. 06G041
Correspondence to: Wei-Qin Li, Professor, Department of General Surgery, Jinling Hospital, Nanjing University School of Medicine, No. 305, Zhongshan East Road, Nanjing 210002, Jiangsu Province, China. sjk0935119@163.com
Telephone: +86-25-80860066 Fax: +86-25-84803956
Received: September 28, 2012 Revised: November 22, 2012
Accepted: December 15, 2012
Published online: February 14, 2013

Abstract

AIM: To investigate the effects of early enteral nutrition (EEN) on the immune function and clinical outcome of patients with severe acute pancreatitis (SAP).

METHODS: Patients were randomly allocated to receive EEN or delayed enteral nutrition (DEN). Enteral nutrition was started within 48 h after admission in EEN group, whereas from the 8th day in DEN group. All the immunologic parameters and C-reactive protein (CRP) levels were collected on days 1, 3, 7 and 14 after admission. The clinical outcome variables were also recorded.

RESULTS: Sixty SAP patients were enrolled to this study. The CD4+ T-lymphocyte percentage, CD4+/CD8+ ratio, and the CRP levels in EEN group became significantly lower than in DEN group from the 7th day after admission. In contrast, the immunoglobulin G (IgG) levels and human leukocyte antigen-DR expression in EEN group became significantly higher than in DEN group from the 7th day after admission. No difference of CD8+ T-lymphocyte percentage, IgM and IgA levels was found between the two groups. The incidences of multiple organ dysfunction syndrome, systemic inflammatory response syndrome, and pancreatic infection as well as the duration of intensive care unit stay were significantly lower in EEN group than in DEN group. However, there was no difference of hospital mortality between the two groups.

CONCLUSION: EEN moderates the excessive immune response during the early stage of SAP without leading to subsequent immunosuppression. EEN can improve the clinical outcome, but not decrease the hospital mortality of SAP patients.

© 2013 Baishideng. All rights reserved.

Key words: Early enteral nutrition; Immune; Severe acute pancreatitis

Sun JK, Mu XW, Li WQ, Tong ZH, Li J, Zheng SY. Effects of early enteral nutrition on immune function of severe acute pancreatitis patients. *World J Gastroenterol* 2013; 19(6): 917-922 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i6/917.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i6.917

INTRODUCTION

Severe acute pancreatitis (SAP) is a systemic disease characterized by a high mortality because of multiple organ dysfunction syndrome (MODS) and infectious complications[1,2]. During recent years, numerous studies concluded that immune dysregulation might play an important role in the development of MODS and infectious complications in SAP patients[3-5]. In the early stage of SAP, immune imbalance mainly appears as excessive immune

response, which is associated with systemic inflammatory response syndrome (SIRS) and MODS[3-6]. Therefore, some clinicians advocate using immunomodulatory therapy in the acute phase of SAP[4,7,8]. However, no consensus on the efficacy of immunotherapy has been reached due to the conflicting results of relevant experiments.

As an essential therapeutic modality for SAP, early enteral nutrition (EEN) could increase antioxidant activity, modulate inflammatory response, and decrease the incidence of SIRS and subsequent MODS[9]. Hence, we infer that EEN might exert some underlying influence on the immune function of SAP patients. However, there have been few trials investigating the effects of EEN on the immune function of the patients during the initial stage of SAP. Zou *et al*[10] reported that enteral immunonutrition enhanced the immune function in pigs with SAP. Belabed *et al*[11] found that the immune enhancing diets was efficient in preserving lymphocyte function in head-injured rats. Nevertheless, the impact of standard enteral nutrition on the immunologic function of SAP patients remains unclear.

Therefore, this study aimed to investigate the influence of EEN on the immune function and clinical outcome of early-stage SAP patients.

MATERIALS AND METHODS

Study design

This is a single-center, prospective, and randomized controlled clinical trial. Patients were randomly allocated to receive either EEN or delayed enteral nutrition (DEN) on admission. The study protocol was approved by the Ethics Committee of the hospital, and informed consent was obtained from each patient or his/her first-degree relatives. This study was also registered at Clinical Trials.gov (Identifier: NCT01507766).

Patients

All adult SAP patients (aged 18-70 years) admitted within 3 d of symptom onset to the Surgical Intensive Care Unit (SICU), the Department of General Surgery, Jinling Hospital from September 2010 to September 2011 were enrolled into this study. SAP was defined as presence of one or more local complications (*e.g.*, pseudocyst, necrosis or abscess) or/and organ failure and acute physiology and chronic health evaluation Ⅱ (APACHE Ⅱ) score > 8 according to the widely used Atlanta criteria formulated in 1992[12]. Patients with chronic organs dysfunction or immunodeficiency or malnutrition, patients who had received artificial nutrition (either enteral nutrition or parenteral nutrition) before admission, and patients with ileus or pancreatitis in pregnancy were all excluded. All the SAP patients received specialized medical therapy for SAP[2,13] such as intensive monitoring, oxygen administration, fluid resuscitation, stopping oral feeding, exocrine pancreatic suppression, and antibiotic prophylaxis.

Patients in EEN group received enteral nutrition within 48 h after admission, whereas patients in the DEN group received enteral nutrition beginning from the 8th day after admission.

Nutrition protocols

A nasojejunal tube (size 10F, Flocare, Nutricia Ltd, Wuxi, China) was placed beyond the Treitz' ligament endoscopically or radiologically, and the position of tip was confirmed by fluoroscopy. The tube was placed within 24 h after admission in EEN group, and enteral nutrition was started from the next 24 h. Patients in DEN group received feeding on the 8th day after admission, and a nasojejunal tube was placed on the 7th day. Peptide-based formula (Peptisorb, Nutricia Ltd) was used in the first 24-48 h of feeding, and if patients were tolerant, whole protein formula (Nutrison Fibre, Nutricia Ltd) would be established subsequently. The goal intake of enteral nutrition was calculated as 20-25 kcal/kg per day and protein need was calculated as 1.5 g/kg per day[14,15]. The feeding rate was initiated at 15-20 mL/h and increased gradually by 15-20 mL every 6-8 h, using a pump. If the patients were intolerant due to abdominal distension and so on, we would slow down the feeding rate, dilute the feedings concentration, and use prokinetic agents to improve the intestinal motility.

Total parenteral nutrition was given during the first week of admission in DEN group. The caloric intake of parenteral nutrition was determined as 20-25 kcal/kg per day and the calorie: nitrogen ratio was calculated as 120-150:1[16,17]. Fifty to seventy percentages of total energy need were supplied by glucose, and the use of lipids was on the basis of serum triglyceride levels[16]. Furthermore, sufficient vitamins, electrolytes, trace elements, and insulin were also added into the intravenous solution.

Data collection

The baseline characteristics of patients including age, sex, body mass index, and etiology were recorded on admission. The APACHE Ⅱ score, sequential organ failure assessment score, and C-reactive protein (CRP) levels were also collected. The CRP levels and the immunologic parameters including CD4+, CD8+ T-lymphocyte percentage, CD4+/CD8+ ratio, human leukocyte antigen-DR (HLA-DR), immunoglobulin A (IgA), IgG and IgM in peripheral blood were collected on days 1, 3, 7 and 14 after admission[4,6,18]. T-lymphocyte subset percentage and HLA-DR expression were detected by flow cytometry (BD FACS Aria, United States). IgA, IgG and IgM were measured by enzyme-linked immunosorbent assay. The incidences of SIRS and MODS during the first 2 wk after admission, the incidence of surgical operation, the development of pancreatic infection, the duration of ICU stay, and the hospital mortality were also recorded.

Statistical analysis

All the data were presented as median (interquartile range), if not stated otherwise. Categorical variables were expressed as absolute numbers or in percentages, and were analyzed using χ^2 test. Continuous variables were com-

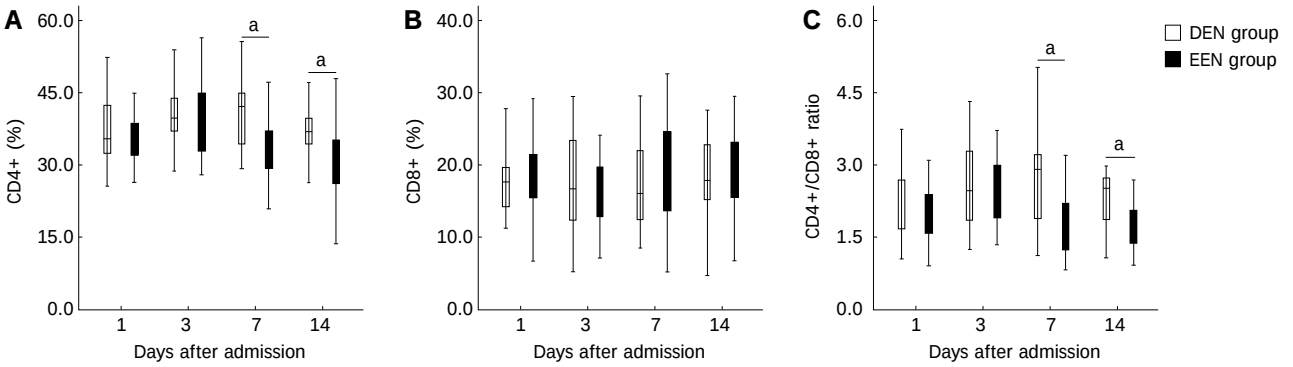


Figure 1 Comparisons of T-lymphocyte subsets percentage between early enteral nutrition and delayed enteral nutrition groups. Results are expressed as median (interquartile range). A: Comparison of CD4+ T-lymphocyte percentage between early enteral nutrition (EEN) and delayed enteral nutrition (DEN) groups; B: Comparison of CD8+ T-lymphocyte percentage between EEN and DEN groups; C: Comparison of CD4+/CD8+ ratio between EEN and DEN groups. $^{a}P < 0.05$, EEN groups *vs* DEN groups.

Table 1 Demographic data and clinical parameters on admission

	DEN group (n = 30)	EEN group (n = 30)	P value
Age (yr)	43 (34.5-51)	45 (35-52)	0.594
Sex (male:female)	18:12	20:10	0.287
Etiology n (%)			
Biliary origin	17 (57)	19 (63)	0.278
Hyperlipidemia	8 (27)	6 (20)	0.373
Alcohol abuse	3 (10)	4 (13)	1.000
Idiopathic	2 (7)	1 (3)	1.000
BMI	24.6 (23.5-26.8)	25.8 (23.9-28.8)	0.158
APACHE Ⅱ score	9.5 (8.5-11)	10 (8-11.5)	0.994
SOFA score	4.5 (3.5-5.5)	4 (3-5)	0.880
CRP (mg/L)	203.5 (188-253)	195 (161-247.5)	0.214

DEN: Delayed enteral nutrition; EEN; Early enteral nutrition; BMI: Body mass index; APACHE Ⅱ: Acute physiology and chronic health evaluation Ⅱ; SOFA: Sequential organ failure assessment; CRP: C-reactive protein.

pared by the Mann-Whitney *U* test or Wilcoxon signed-rank test as appropriate. Statistical Package for the Social Sciences (SPSS, version 17.0, Chicago, IL, United States) software was used for statistical analysis. $P < 0.05$ was considered statistically significant.

RESULTS

A total of 60 eligible patients with SAP (30 in each group) were enrolled into this clinical trial during the study period. The principal etiological factors of SAP were biliary origin (60%, 36/60) and hyperlipidemia (23%, 14/60). The demographic data and clinical parameters of the patients on admission are presented in Table 1. Three patients (5%, 3/60) died of MODS and septic shock during hospital stay.

Comparison of immune parameters and CRP levels between two groups

Figure 1 shows the differences of T-lymphocyte subsets percentage between DEN and EEN groups. As shown in Figure 1A, patients in EEN group had significantly lower CD4+ T-lymphocyte percentage from the 7th day ($P < 0.05$) after admission. Similar results were detected in the CD4+/CD8+ ratio (Figure 1C). However, there was no difference of CD8+ T-lymphocyte percentage between the two groups during the two weeks after admission (Figure 1B).

Figure 2 presents the disparities of immunoglobulin subtypes between the two groups. As shown in Figure 2A, patients in EEN group had significantly higher IgG levels from the 7th day ($P < 0.05$) after admission. Nevertheless, no difference of IgM and IgA between the two groups was found during the two weeks after admission (Figure 2B and C).

As shown in Figure 3A, patients in EEN group had significantly higher HLA-DR expression from the 7th day ($P < 0.05$) after admission. In contrast, the CRP levels of patients in EEN group were significantly lower than in patients of the DEN group from the 7th day ($P < 0.05$) after admission (Figure 3B).

Table 2 Clinical outcome variables n (%)

	DEN group (n = 30)	EEN group (n = 30)	P value
Hospital mortality	1 (3)	2 (7)	1.000
ICU stay (d)	12 (8-21)	9 (5-14)	0.033
Pancreatic infection	10 (33)	3 (10)	0.028
MODS	13 (43)	5 (17)	0.024
SIRS	22 (73)	12 (40)	0.009
Surgical operation	4 (13)	2 (7)	0.671

DEN: Delayed enteral nutrition; EEN: Early enteral nutrition; ICU: Intensive care unit; MODS: Multiple organ dysfunction syndrome; SIRS: Systemic inflammatory response syndrome.

Comparison of outcome variables between two groups

As presented in Table 2, patients in EEN group had significantly lower incidences of MODS, SIRS and pancreatic infection as well as shorter ICU stay during hospital stay. However, no difference of hospital mortality and operation incidence was found between the two groups.

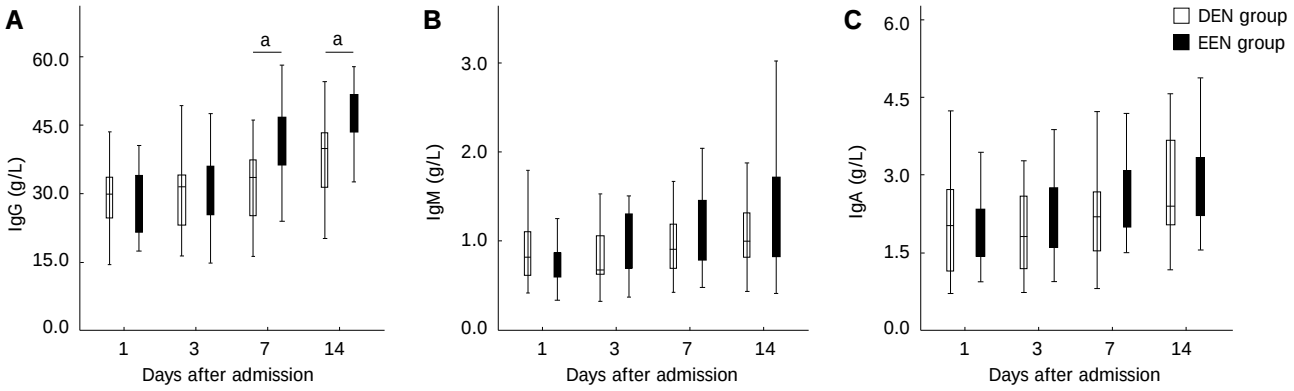


Figure 2 Comparisons of immunoglobulin subtypes between early enteral nutrition and delayed enteral nutrition groups. Results are expressed as median (interquartile range). A: Comparison of immunoglobulin G (IgG) levels between early enteral nutrition (EEN) and delayed enteral nutrition (DEN) groups; B: Comparison of IgM levels between EEN and DEN groups; C: Comparison of IgA levels between EEN and DEN groups. [a]*P* < 0.05, EEN groups *vs* DEN groups.

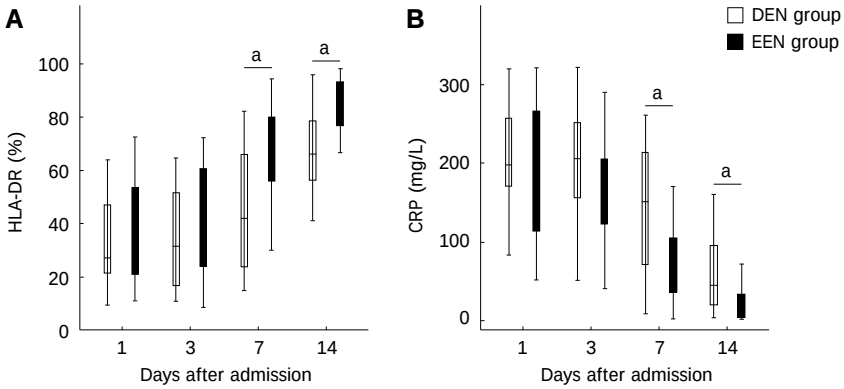


Figure 3 Comparisons of human leukocyte antigen-DR expression and C-reactive protein levels between early enteral nutrition and delayed enteral nutrition groups. Results are expressed as median (interquartile range). A: Comparison of human leukocyte antigen-DR (HLA-DR) expression between early enteral nutrition (EEN) and delayed enteral nutrition (DEN) groups; B: Comparison of C-reactive protein (CRP) levels between EEN and DEN groups. [a]*P* < 0.05, EEN groups *vs* DEN groups.

DISCUSSION

This clinical study investigated the effects of EEN on the immune function as well as on the clinical outcome of SAP patients. We found that EEN could moderate the excessive immune response during the early stage of SAP without leading to subsequent immunosuppression. Moreover, EEN administration could improve the clinical outcome, but not decrease the hospital mortality of SAP patients.

Immune dysregulation is one of the main causes responsible for a high mortality of SAP. Recent studies have shown that excessive immune response might play an important role in the development of SIRS and MODS in the early stage of SAP[3-6,19]. The imbalance of Th1/Th2 cells (differentiated from the Th0 cells which were derived from CD4+ T-lymphocytes) is the principal mechanism of excessive immune response[4,19]. Th1 cells mainly produce some anti-inflammatory factors, such as interleukin 10 (IL-10) and IL-4, whereas Th2 cells mainly release the pro-inflammatory factors, such as IL-6, and tumor necrosis factor-α (TNF-α)[3,4,19]. Pietruczuk *et al*[19] found that Th1 cells were suppressed more strongly than Th2 cells in the acute phase of SAP, thus the pro-inflammatory factors produced by Th2 cells were over-activated and then triggered the strong inflammatory reaction. For this reason, some researchers considered using immunosuppressive therapy for the patients with early-stage SAP. Up till now, a number of immunosuppressive agents (*e.g.*, dexamethasone, IL-10, and anti-tumor necrosis factor monoclonal antibodies) have been studied in SAP animal experiments[4,8]. Unfortunately, the findings were inconsistent or even conflicting, and very few of these studies involved SAP patients. Moreover, inappropriate immunosuppression might be associated with subsequent infectious complications during the late stage of SAP[3,4,6]. Therefore, the effects of immunomodulatory therapy in SAP were still unclear, and further clinical trails are extremely needed.

Gut is the primary immune organ providing an initial barrier against extraneous antigens and microbes[20,21]. The previous studies reported that gut immune response was highly associated with enteral nutrition, and deficiency of enteral stimulation might induce immune suppression[20,21]. In addition, enteral nutrition (especially EEN) had been confirmed to modulate inflammatory response, maintain gut integrity, and release immunomodulating agents[9,22]. Based on the above findings, we postulated that EEN might have some underlying impacts on the immune function of SAP patients, however, few clinical trials about it have been conducted. Therefore, the principal purpose of this clinical study was to investigate the influence of EEN on the immune function of early-stage SAP patients.

CD4+, CD8+ T-lymphocyte percentage and CD4+/CD8+ ratio were closely related to the cellular immune

function of SAP[23,24]. Uehara *et al*[5] observed that both CD4+ percentage and CD4+/CD8+ ratio were significantly reduced in patients with acute pancreatitis, whereas Liu *et al*[6] reported that the CD4+ percentage was increased significantly on the 7th day in SAP patients after admission. In the present study, we found that EEN could reduce CD4+ T-lymphocyte percentage as well as CD4+/CD8+ ratio from the 7th day after admission. And patients in EEN group also had significantly lower CRP levels from the same day. Furthermore, the initial incidences of SIRS and MODS in EEN group were significantly lower than in DEN group. These results indicated that EEN might be able to suppress the excessive immune response during the early phase of SAP. It has been demonstrated that EEN can prevent the release of pro-inflammatory mediators (*e.g.*, IL-6 and TNF-α) and promote the release of anti-inflammatory factors (*e.g.*, IL-10)[8,9]. In other words, EEN might suppress the overactivation of Th2 cells and promote the production of Th1 cells, and then moderate the imbalance of Th1/Th2 cells. This may be the underlying mechanisms of our conclusion.

In addition, since excessive or prolonged immunosuppressive therapy might induce the development of infectious complications, whether EEN could lead to subsequent immunosuppression still need to be further investigated. Immunoglobulin concentrations and HLA-DR expression levels were also closely related to the immune function of SAP patients. Zou *et al*[10] reported that the serum IgG levels of SAP pigs were significantly decreased on day 2 after induction of SAP, and then were increased significantly on day 8. Yu *et al*[25] found that continuous HLA-DR suppression was highly associated with infectious complications and poor outcome in SAP patients, and the HLA-DR suppression was inversely correlated with CRP levels. In our study as shown in Figures 2A and 3A, patients in EEN group had significantly higher IgG levels and HLA-DR expression from the 7th day after admission. And the IgM and IgA levels of patients in EEN group were also not lower than that of patients in DEN group. Furthermore, the incidence of pancreatic infection in EEN group was significantly lower than in DEN group, and this result is also consistent with the findings of previous studies[9,22]. These phenomena suggested that EEN did not lead to immunosuppression during the late stage of SAP.

There are several limitations in this study. Due to the small sample size and the single-center design, our results might be uncertain for a definite conclusion, and the accuracy should be tested by further large-sized studies. Moreover, since this study did not base on a pathophysiological model, the precise mechanisms of EEN in SAP patients should be verified by more basic experiments.

In conclusion, EEN could moderate the excessive immune response during the early stage of SAP without leading to subsequent immunosuppression. Moreover, EEN could improve the clinical outcome, but not decrease the hospital mortality of SAP patients. However, the precise mechanisms of EEN for SAP are still not clear, and further studies are required to verify our conclusions.

COMMENTS

Background

The immune dysregulation might play an important role in the development of multiple organ dysfunction syndrome (MODS) and infectious complications in severe acute pancreatitis (SAP) patients. In the early stage of SAP, immune imbalance mainly appears as excessive immune response, therefore, some clinicians advocate using immunomodulatory therapy for SAP. However, no consensus on the efficacy of immunotherapy has been reached due to the conflicting results of relevant experiments.

Research frontiers

Early enteral nutrition (EEN) could increase antioxidant activity, modulate inflammatory response, and decrease the incidence of systemic inflammatory response syndrome (SIRS) and subsequent MODS. Hence, the authors infer that EEN might have some underlying influence on the immune function of SAP patients. However, there have been few trials investigating the effects of EEN on the immune function of the patients with SAP at the initial stage.

Innovations and breakthroughs

The study found that EEN could moderate the excessive immune response during the early stage of SAP without leading to subsequent immunosuppression; and EEN could improve the clinical outcome, but not decrease the hospital mortality of SAP patients.

Applications

The results of this study indicate that enteral feedings should be provided as early as possible to correct the immune dysregulation of SAP patients.

Terminology

MODS, is a progressive condition usually characterized by combined dysfunction of multiple organs or systems due to various etiological factors in critically ill patients; SIRS, is diagnosed when two or more of the following criteria are met: body temperature < 36 ℃ or > 38 ℃; heart rate > 90 beats/min; tachypnea > 20 breaths/min, or an arterial partial pressure of carbon dioxide < 32 mmHg; white blood cell count less than 4 × 10^9/L or greater than 12 × 10^9/L, or the presence of > 10% immature neutrophils (band forms).

Peer review

Overall, presented paper is of a very good quality. Study design is appropriate and clear, helps to answer a clinical relevant question. Data analysis is sufficient. Manuscript is well structured, language is of sufficient quality.

REFERENCES

1 **Frossard JL**, Steer ML, Pastor CM. Acute pancreatitis. *Lancet* 2008; **371**: 143-152 [PMID: 18191686 DOI: 10.1016/S0140-6736(08)60107-5]

2 **Brisinda G**, Vanella S, Crocco A, Mazzari A, Tomaiuolo P, Santullo F, Grossi U, Crucitti A. Severe acute pancreatitis: advances and insights in assessment of severity and management. *Eur J Gastroenterol Hepatol* 2011; **23**: 541-551 [PMID: 21659951 DOI: 10.1097/MEG.0b013e328346e21e]

3 **Shen Y**, Cui N, Miao B, Zhao E. Immune dysregulation in patients with severe acute pancreatitis. *Inflammation* 2011; **34**: 36-42 [PMID: 20405190 DOI: 10.1007/s10753-010-9205-4]

4 **Zhang XP**, Chen HQ, Liu F, Zhang J. Advances in researches on the immune dysregulation and therapy of severe acute pancreatitis. *J Zhejiang Univ Sci B* 2009; **10**: 493-498 [PMID: 19585666 DOI: 10.1631/jzus.B0820265]

5 **Uehara S**, Gothoh K, Handa H, Tomita H, Tomita Y. Immune function in patients with acute pancreatitis. *J Gastroenterol Hepatol* 2003; **18**: 363-370 [PMID: 12653883 DOI: 10.1046/j.1440-1746.2003.02979.x]

6 **Liu Z**, Shen Y, Cui N, Yang J. Clinical observation of immunity for severe acute pancreatitis. *Inflammation* 2011; **34**: 426-431 [PMID: 20842417 DOI: 10.1007/s10753-010-9249-5]

7 **Rau BM**, Krüger CM, Hasel C, Oliveira V, Rubie C, Beger HG, Schilling MK. Effects of immunosuppressive and immunostimulative treatment on pancreatic injury and

mortality in severe acute experimental pancreatitis. *Pancreas* 2006; **33**: 174-183 [PMID: 16868484 DOI: 10.1097/01.mpa.0000226895.16817.a1]
8 **Kylänpää ML**, Repo H, Puolakkainen PA. Inflammation and immunosuppression in severe acute pancreatitis. *World J Gastroenterol* 2010; **16**: 2867-2872 [PMID: 20556831 DOI: 10.3748/wjg.v16.i23.2867]
9 **Oláh A**, Romics L. Early enteral nutrition in acute pancreatitis--benefits and limitations. *Langenbecks Arch Surg* 2008; **393**: 261-269 [PMID: 18266002 DOI: 10.1007/s00423-008-0291-9]
10 **Zou XP**, Chen M, Wei W, Cao J, Chen L, Tian M. Effects of enteral immunonutrition on the maintenance of gut barrier function and immune function in pigs with severe acute pancreatitis. *JPEN J Parenter Enteral Nutr* 2010; **34**: 554-566 [PMID: 20852186 DOI: 10.1177/0148607110362691]
11 **Belabed L**, Charrueau C, Besson V, Gupta S, Walrand S, Marchand-Verrecchia C, Richon S, Nafziger J, Plotkine M, Chaumeil JC, Cynober L, Moinard C. Impairment of lymphocyte function in head-injured rats: effects of standard and immune-enhancing diets for enteral nutrition. *Clin Nutr* 2006; **25**: 832-841 [PMID: 16678308 DOI: 10.1016/j.clnu.2006.02.003]
12 **Bradley EL**. A clinically based classification system for acute pancreatitis. Summary of the International Symposium on Acute Pancreatitis, Atlanta, Ga, September 11 through 13, 1992. *Arch Surg* 1993; **128**: 586-590 [PMID: 8489394 DOI: 10.1001/archsurg.1993.01420170122019]
13 **De Campos T**, Braga CF, Kuryura L, Hebara D, Assef JC, Rasslan S. Changes in the management of patients with severe acute pancreatitis. *Arq Gastroenterol* 2008; **45**: 181-185 [PMID: 18852942 DOI: 10.1590/S0004-28032008000300002]
14 **Meier R**, Ockenga J, Pertkiewicz M, Pap A, Milinic N, Macfie J, Löser C, Keim V. ESPEN Guidelines on Enteral Nutrition: Pancreas. *Clin Nutr* 2006; **25**: 275-284 [PMID: 16678943 DOI: 10.1016/j.clnu.2006.01.019]
15 **Kreymann KG**, Berger MM, Deutz NE, Hiesmayr M, Jolliet P, Kazandjiev G, Nitenberg G, van den Berghe G, Wernerman J, Ebner C, Hartl W, Heymann C, Spies C. ESPEN Guidelines on Enteral Nutrition: Intensive care. *Clin Nutr* 2006; **25**: 210-223 [PMID: 16697087 DOI: 10.1016/j.clnu.2006.01.021]
16 **Gianotti L**, Meier R, Lobo DN, Bassi C, Dejong CH, Ockenga J, Irtun O, MacFie J. ESPEN Guidelines on Parenteral Nutrition: pancreas. *Clin Nutr* 2009; **28**: 428-435 [PMID: 19464771 DOI: 10.1016/j.clnu.2009.04.003]
17 **McClave SA**, Martindale RG, Vanek VW, McCarthy M, Roberts P, Taylor B, Ochoa JB, Napolitano L, Cresci G. Guidelines for the Provision and Assessment of Nutrition Support Therapy in the Adult Critically Ill Patient: Society of Critical Care Medicine (SCCM) and American Society for Parenteral and Enteral Nutrition (A.S.P.E.N.). *JPEN J Parenter Enteral Nutr* 2009; **33**: 277-316 [PMID: 19398613 DOI: 10.1177/0148607109335234]
18 **Wang X**, Li W, Zhang F, Pan L, Li N, Li J. Fish oil-supplemented parenteral nutrition in severe acute pancreatitis patients and effects on immune function and infectious risk: a randomized controlled trial. *Inflammation* 2009; **32**: 304-309 [PMID: 19568921 DOI: 10.1007/s10753-009-9136-0]
19 **Pietruczuk M**, Dabrowska MI, Wereszczynska-Siemiatkowska U, Dabrowski A. Alteration of peripheral blood lymphocyte subsets in acute pancreatitis. *World J Gastroenterol* 2006; **12**: 5344-5351 [PMID: 16981265]
20 **MacDonald TT**. The mucosal immune system. *Parasite Immunol* 2003; **25**: 235-246 [PMID: 12969442 DOI: 10.1046/j.1365-3024.2003.00632.x]
21 **Cunningham-Rundles S**. Nutrition and the mucosal immune system. *Curr Opin Gastroenterol* 2001; **17**: 171-176 [PMID: 11224675 DOI: 10.1097/00001574-200103000-00013]
22 **Hegazi R**, Raina A, Graham T, Rolniak S, Centa P, Kandil H, O'Keefe SJ. Early jejunal feeding initiation and clinical outcomes in patients with severe acute pancreatitis. *JPEN J Parenter Enteral Nutr* 2011; **35**: 91-96 [PMID: 21224435 DOI: 10.1177/0148607110376196]
23 **Vaccari M**, Boasso A, Fenizia C, Fuchs D, Hryniewicz A, Morgan T, Weiss D, Doster MN, Heraud JM, Shearer GM, Franchini G. Fatal pancreatitis in simian immunodeficiency virus SIV(mac251)-infected macaques treated with 2',3'-dideoxyinosine and stavudine following cytotoxic-T-lymphocyte-associated antigen 4 and indoleamine 2,3-dioxygenase blockade. *J Virol* 2012; **86**: 108-113 [PMID: 22013040 DOI: 10.1128/JVI.05609-11]
24 **Uchida K**, Kusuda T, Koyabu M, Miyoshi H, Fukata N, Sumimoto K, Fukui Y, Sakaguchi Y, Ikeura T, Shimatani M, Fukui T, Matsushita M, Takaoka M, Nishio A, Okazaki K. Regulatory T cells in type 1 autoimmune pancreatitis. *Int J Rheumatol* 2012; **2012**: 795026 [PMID: 22536257 DOI: 10.1155/2012/795026]
25 **Yu WK**, Li WQ, Li N, Li JS. Mononuclear histocompatibility leukocyte antigen-DR expression in the early phase of acute pancreatitis. *Pancreatology* 2004; **4**: 233-243 [PMID: 15166475 DOI: 10.1159/000078748]

P- Reviewers Bradley EL, Dambrauskas Z, Pan WS, Pezzilli R, Twomey PJ **S- Editor** Wen LL **L- Editor** A **E- Editor** Li JY



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i6.923

World J Gastroenterol 2013 February 14; 19(6): 923-930
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Testing for hepatitis B infection in prospective chemotherapy patients: A retrospective study

Ying Wang, Xin-Mei Luo, Dan Yang, Jie Zhang, Hong-Yu Zhuo, Jian Zhang, Yu Jiang

Ying Wang, Xin-Mei Luo, Dan Yang, Jie Zhang, Hong-Yu Zhuo, Jian Zhang, Yu Jiang, Department of Medical Oncology, Cancer Center, State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China

Author contributions: Wang Y and Luo XM performed the majority of this study and contributed equally to this work; Yang D, Zhang J, Zhuo HY and Zhang J collected vital clinical data and were involved in revising the manuscript; Wang Y, Luo XM and Jiang Y wrote the manuscript; and Jiang Y designed the study and provided financial support for this work.
Supported by The National Natural Science Foundation of China, No. 30801373
Correspondence to: Yu Jiang, MD, Department of Medical Oncology, Cancer Center, State Key Laboratory of Biotherapy, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China. jiangyu1973@hotmail.com
Telephone: +86-28-85422683 Fax: +86-28-85423278
Received: August 8, 2012 Revised: November 21, 2012
Accepted: December 5, 2012
Published online: February 14, 2013

Abstract

AIM: To estimate hepatitis B virus (HBV) infection testing rate in cancer patients before chemotherapy with a focus on HBV reactivation.

METHODS: A retrospective study was conducted from January 1, 2009 to June 30, 2010. Inclusion required that patients be naïve to cancer chemotherapy but have indications for it. Patients who did not receive chemotherapy for any reason were excluded. Important clinical information, such as the levels of HBV DNA and serological markers were collected. HBV reactivation was defined as an increase in serum HBV DNA to > 1 log higher than that of the pre-exacerbation baseline, or serum HBV DNA conversion from negative to positive. HBV DNA levels > 1000 copies/mL were defined as HBV DNA positive. The χ^2 or Fisher's exact test was used for analysis of categorized data. Multiple logistic regression analysis was used to estimate the odd ratio and 95%CI of the HBV screening rate.

RESULTS: Of 6646 patients, 5616 (84.5%) received chemotherapy. Only 17.1% of the cancer patients received pre-chemotherapy HBV testing (43.2% for hematological malignancies and 14.9% for solid tumors). Patients who had received rituximab therapy, had elevated aminotransferase levels, or had hematological malignancies were more likely to receive HBV testing. The prevalence of hepatitis B surface antigen (HBsAg) positivity was 13.4%. HBV reactivation (appearance of HBV DNA or an increase in HBV DNA levels by 1 $\log_{10}$) was observed in 33.1% (53/160) of the patients after chemotherapy. Among patients without prophylactic antiviral therapy, the reactivation rate was 43.9% (43/98) in the solid tumor group. Two reactivation cases occurred in patients who were HBsAg negative, but positive for hepatitis B core antibody. HBV reactivation was more likely to occur in patients with lymphoma, high levels of HBV DNA, or hepatitis B e antigen, and in men.

CONCLUSION: Less than 20% of patients received HBV testing before chemotherapy. HBV reactivation would have occurred in about 50% of infected patients with solid tumors without antiviral prophylaxis.

© 2013 Baishideng. All rights reserved.

Key words: Chemotherapy; Hematologic malignancy; Hepatitis B virus; Hepatitis B virus reactivation; Solid tumor

Wang Y, Luo XM, Yang D, Zhang J, Zhuo HY, Zhang J, Jiang Y. Testing for hepatitis B infection in prospective chemotherapy patients: A retrospective study. *World J Gastroenterol* 2013; 19(6): 923-930 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i6/923.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i6.923

INTRODUCTION

Hepatitis B virus (HBV) infection is a global health problem. According to estimates of the World Health Organization, nearly 2 billion people worldwide have been infected with HBV, and > 350 million have chronic infection[1]. Hepatitis B is endemic in China, and the weighted prevalence of hepatitis B surface antigen (HBsAg) for the Chinese population is 7.2%[2]. Cancer patients receiving intensive chemotherapy may be at risk for HBV reactivation[3-8]. A previous study has reported that the reactivation rate of HBV infection after chemotherapy was as high as 73%[8]. It has been observed in a meta-analysis that the average reactivation rate of HBV infection was nearly 34% (162/475 patients), and the mortality rate was about 7% (27/394 patients)[9]. HBV reactivation might lead to a delay in chemotherapy, therefore, antiviral therapy should be administered immediately[3]. However, this might affect the efficacy of chemotherapy.

Corticosteroids, monoclonal antibodies (such as rituximab and alemtuzumab), and nearly all types of chemotherapeutic agents have been involved in HBV reactivation[5]. A diagnosis of lymphoma is another risk factor for HBV reactivation[4]. A multivariate analysis has revealed that patients with lymphoma or breast cancer are more likely to develop HBV reactivation after chemotherapy[10].

Recently, more attention has been paid to HBV testing before chemotherapy. Many clinical practice guidelines, such as the associated guidelines of the European Association for the Study of Liver Disease (EASL) and the American Association for the Study of Liver Diseases, advise HBV testing before starting chemotherapy[11,12]. The United States Centers for Disease Control and Prevention (CDC) also recommend testing for chronic HBV infection in cancer patients receiving chemotherapy[13]. It has been reported in recent years that the HBV infection testing rates before chemotherapy in Canada and the United States were 14%[14] and 16.7%[15], respectively. However, to the best of our knowledge, few studies have focused on this issue in a high endemic region of HBV infection such as China.

The aim of this study was to assess the rate of HBV serological testing prior to initiation of chemotherapy and the rate of HBV reactivation after chemotherapy in cancer patients in China.

MATERIALS AND METHODS

Patients

From January 1, 2009 to June 30, 2010, all cancer patients who were seen in the Cancer Center of West China Hospital were analyzed. Inclusion in this study required that patients be naïve to cancer chemotherapy but have indications for it. Patients who did not receive chemotherapy for any reason were excluded. This study was approved by the Institutional Review Board of West China Hospital and carried out according to the provisions of the Helsinki Declaration.

Methods and definitions

A retrospective study of the chart information on cancer patients was undertaken. HBV reactivation was defined as an increase in serum HBV DNA to > 1 log higher than that of the pre-exacerbation baseline, or serum HBV DNA conversion from negative to positive[16]. In our center, HBV DNA levels > 1000 copies/mL were defined as HBV DNA positive. Important clinical information, such as the levels of HBV DNA and serological markers [including HBsAg, hepatitis B surface antibody (anti-HBs), hepatitis B e antigen (HBeAg), HBeAg antibody and hepatitis B core antibody (anti-HBc)], were collected and measured by a microparticle enzyme-linked immunosorbent assay (Santa Cruz Biotechnology, CA, United States). All hepatitis B serological marker results were reported as positive or negative. EASL guidelines recommend that all patients requiring chemotherapy be tested for HBsAg and anti-HBc prior to initiation of treatment[11], therefore, the presence of either HBsAg-positive or isolated anti-HBc-positive results was defined as positivity for an HBV serological marker (HBV-sm). HBV DNA levels were tested using a real-time polymerase chain reaction detection system (Applied Biosystems, Foster City, CA, United States). Individuals with chronically elevated alanine aminotransferase (ALT) or aspartate aminotransferase (AST) should be routinely tested for HBV[12], and ALT and AST levels were analyzed using an automatic biochemistry analyzer (Olympus AU5400, Olympus Corporation, Tokyo, Japan). Values for ALT or AST above the upper limit of normal (58 IU/L in our hospital) were defined as elevated.

Statistical analysis

Frequencies and percentages were used for statistical descriptions. The χ^2 or Fisher's exact test was used for analysis of categorized data. A nonparametric Mann-Whitney/Wilcoxon test or *t* test was used for analysis of quantitative data. Multiple logistic regression analysis was used to estimate the odd ratio (OR) and 95%CI of the HBV screening rate. All statistical analyses were carried out using SPSS version 18.0 statistical software, and statistical significance was defined as $P < 0.05$.

RESULTS

Patient characteristics

From the beginning of January 2009 to the end of June 2010, a total of 6646 cancer patients were reviewed. Among these patients, 5616 (84.5%) received chemotherapy (Figure 1). Combined with other chemotherapeutic agents, rituximab was given to 49 patients (17.4%) and/or steroids were given to 203 (72.2%) patients with hematological malignancies. Patient characteristics are shown in Table 1.

Testing for HBV infection

As shown in Figure 1, 49.3% (2770/5616) patients were screened for HBV before initiation of chemotherapy.

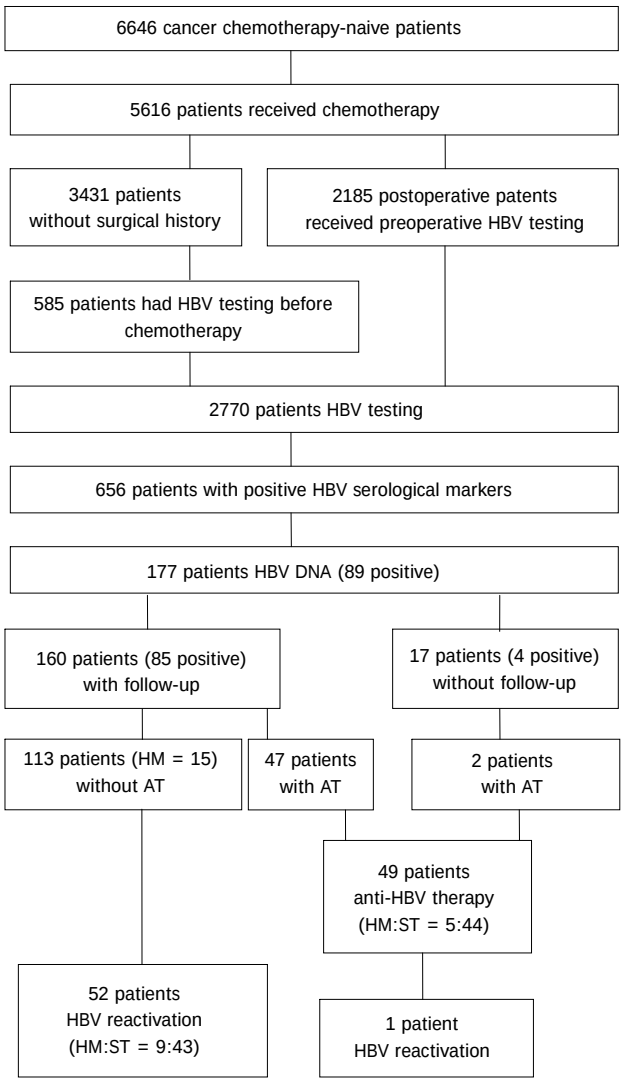


Figure 1 Study flow diagram. HM: Hematological malignancy; ST: Solid tumor; AT: Anti-HBV therapy; HBV: Hepatitis B virus.

The testing rate for patients with hematologic malignancies and solid tumors was 47.7% (134/281) and 49.4% (2636/5335), respectively. However, the reason for HBV serological testing in 2185 patients was not in preparation for chemotherapy, but as a routine check before surgery. Only 17.1% (585/3431) of the cancer patients received pre-chemotherapy HBV testing. The exact pre-chemotherapy testing rate for hematological malignancies and solid tumors was 43.2% (112/259) and 14.9% (473/3172), respectively. As shown in Table 2, in univariate analysis, the characteristics of age > 50 years, male sex, history of HBV infection, rituximab use, elevated aminotransferases, and hematological malignancies were associated with HBV testing. In multiple logistic regression analysis, patients with rituximab therapy (OR: 1.96, 95%CI: 1.04-3.67, $P < 0.05$), elevated aminotransferase levels (OR: 10.88, 95%CI: 8.01-14.78, $P < 0.001$), and hematological malignancies (OR: 4.17, 95%CI: 3.10-5.61, $P < 0.001$) were more likely to have received pre-chemotherapy HBV serological testing.

Table 1 Characteristics of 5616 cancer patients who received chemotherapy *n* (%)

Characteristics	Hematological malignancy	Solid tumor		Total
	(*n* = 281)	HCC (*n* = 237)	Others (*n* = 5098)	(*n* = 5616)
Age (yr), mean (range)	50.5 (18-83)	53.1 (39-67)	54.4 (41-67)	54.1 (29-79)
Sex				
Men	176 (62.6)	201 (84.8)	2567 (50.4)	2944 (52.4)
Women	105 (37.4)	36 (17.9)	2531 (49.6)	2672 (47.6)
Surgical history	22 (7.8)	6 (2.5)	2157 (42.3)	2185 (38.9)
History of HBV infection[1]	27 (9.6)	126 (53.2)	179 (3.5)	332 (5.9)
Aminotransferase				
Normal (< 58 IU/mL)	251 (89.3)	144 (60.8)	4816 (94.5)	5211 (92.8)
Elevated	30 (10.7)	93 (39.2)	282 (5.5)	405 (7.2)

[1]History of hepatitis B surface antigen positivity. HCC: Hepatocellular carcinoma; HBV: Hepatitis B virus.

Table 2 Univariate logistic regression analysis of factors associated with testing for hepatitis B virus serological markers prior to chemotherapy, but excluding those tested preoperatively (*n* = 3431)

	No.	Screening *n* (%)	*P* value
Age (yr)			< 0.01
≤ 50	1215	240 (19.8)	
> 50	2216	345 (15.6)	
Sex			< 0.05
Men	2031	374 (18.4)	
Women	1400	211 (15.1)	
Rituximab therapy[a]			< 0.01
Yes	49	25 (51.0)	
No	3382	560 (16.6)	
History of HBV infection			< 0.01
Yes	141	45 (31.9)	
No	3290	540 (16.4)	
ALT and/or AST[b]			< 0.01
Abnormal	205	128 (62.4)	
Normal	3226	457 (14.2)	
Types of tumor[c]			< 0.01
Hematological	259	112 (43.2)	
Solid	3172	473 (14.9)	< 0.05
Liver cancer	78	18 (23.1)	
Others	3094	455 (14.7)	

[a]$P = 0.038$ (OR: 1.96, 95%CI: 1.04-3.67); [b]$P < 0.001$ (OR: 10.88, 95%CI: 8.01-14.78); [c]$P < 0.001$ (OR: 4.17, 95%CI: 3.10-5.61). OR: Odds ratio; HBV: Hepatitis B virus; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

HBV serological markers

The prevalence of HBsAg positivity was 13.4%. The prevalence of HBV-sm positivity was 23.7%. The prevalence of HBsAg positivity ($P < 0.001$) and HBsAg and/or anti-HBc positivity ($P < 0.001$) in the male patients were significantly higher than in the female patients. In patients < 20 years old, the rate of HBV-sm positivity was significantly lower than in patients in the older age group ($P < 0.001$) (Table 3).

Table 3 Prevalence of hepatitis B virus serum markers and DNA in 2770 patients *n* (%)

	No.	HBsAg+	HBsAb+	HBeAg+	HBeAb+	Isolated HBcAb+	HBV-sm positive[1]	Screening HBV DNA[2]
Total	2770	372 (13.4)	1367 (49.4)	43 (1.6)	600 (21.7)	284 (10.3)	656 (23.7)	177 (27.0)
Age (yr)								
≤ 20	27	2 (7.4)	14 (51.9)	1 (3.7)	2 (7.4)	1 (3.7)	3 (11.1)	1 (33.3)
>20	2743	370 (13.5)	1353 (49.3)	42 (1.5)	598 (21.8)	283 (10.3)	653 (23.8)	176 (27.0)
Sex								
Women	1495	144 (9.6)	780 (52.2)	15 (1.0)	280 (18.7)	146 (9.8)	290 (19.4)	52 (17.9)
Men	1275	228 (17.9)	587 (46.7)	28 (2.2)	320 (25.5)	138 (10.8)	366 (28.7)	125 (34.2)
Types of tumor								
Hematological	134	21 (15.7)	51 (38.1)	3 (2.2)	26 (19.4)	7 (5.2)	28 (20.9)	23 (82.1)
Solid	2636	351 (13.3)	1316 (49.9)	40 (1.5)	574 (21.8)	277 (10.5)	628 (23.8)	154 (24.5)
Liver cancer	177	114 (64.4)	45 (25.4)	17 (9.6)	114 (64.4)	27 (15.3)	141 (79.7)	68 (48.2)
Others	2459	207 (8.4)	1271 (41.7)	23 (0.9)	114 (64.4)	250 (10.2)	487 (19.8)	84 (3.5)

[1]HBV-sm positive: HBV serological marker positive (HBsAg positive and/or isolated HBcAb positive); [2]Percentage equals the screening number of HBV DNA/number of HBV-sm positive. HBV: Hepatitis B virus; HBsAg: Hepatitis B surface antigen; HBsAb: Hepatitis B surface antibody; HBeAg: Hepatitis B e antigen; HBeAb: Hepatitis B e antibody; HBcAb: Hepatitis B core antibody.

Table 4 Characteristics of 160 hepatitis B surface antigen-seropositive cancer patients undergoing cytotoxic chemotherapy *n* (%)

	Patients developed HBV reactivation (*n* = 53)	Patients who did not develop HBV reactivation (*n* = 107)	*P* value
Sex[a]			< 0.05
Men	43 (38.4)	69 (61.6)	
Women	10 (20.8)	38 (79.2)	
Age (yr), median (range)	51.5 (24.8-78.2)	52.9 (27.8-77.9)	
Tumor type[b]			< 0.05
Lymphomas	10 (55.6)	8 (44.4)	
Solid tumor	43 (30.3)	99 (69.7)	
Liver cancer	25 (35.7)	45 (64.3)	
Others	18 (25.0)	54 (75.0)	
ALT levels, median (range) (normal < 58 IU/mL)	69.1 (21.9-116.3)	55.7 (18.2-93.2)	
HBeAg status[c]			< 0.01
Positive	17 (73.9)	6 (26.1)	
Negative	36 (26.3)	101 (73.7)	
HBV DNA status[d]			< 0.01
Positive	47 (45.2)	57 (54.8)	
Negative	6 (10.7)	50 (89.3)	
Use of rituximab			< 0.05
Yes	6 (60)	4 (40)	
No	47 (31.3)	103 (68.7)	
Use of corticosteroids			< 0.05
Yes	8 (61.5)	5 (38.5)	
No	45 (30.6)	102 (69.4)	
Receiving antiviral therapy			< 0.01
Yes	1 (2.1)	46 (97.9)	
No	52 (46.0)	61 (54.0)	

[a]*P* = 0.040 (OR: 2.634, 95%CI: 1.046-6.63); [b]*P* = 0.002 (OR: 5.700, 95%CI: 1.869-17.380); [c]*P* = 0.000 (OR: 6.064, 95%CI: 2.213-16.621); [d]*P* = 0.006 (OR: 5.982, 95%CI: 1.689-21.194). OR: Odds ratio; HBV: Hepatitis B virus; ALT: Alanine aminotransferase; HBeAg: Hepatitis B e antigen.

HBV DNA and antiviral therapy

Among the 656 patients who were positive for HBV serological markers, 177 (27.0%) received HBV DNA testing and 160 (24.4%) underwent re-examination. The HBV DNA testing rate was significantly higher in the hematological cancer group ($P < 0.001$). Antiviral therapy (lamivudine) was given to 55.1% (49/89) of the patients who were positive for HBV DNA. All five HBV-DNA-positive patients with hematological malignancies received antiviral therapy, but only 52.4% (44/84) of the HBV-DNA-positive patients with solid tumors were given antiviral therapy (Figure 1 and Table 3).

HBV reactivation

Two cases of reactivation occurred in HBsAg-negative but anti-HBc-positive patients. One patient had diffuse large B-cell lymphoma while the other had breast cancer. As shown in Figure 1, HBV reactivation was observed in 33.1% (53/160) of patients after chemotherapy. Within the virus-untreated cohort ($n = 113$), the reactivation rate was 46.0% (52/113 patients). Among the 47 patients who received antiviral therapy, only one case of reactivation (2.1%) occurred. However, HBV reactivation was observed in 60.0% (9/15 patients) in the hematological malignancy group and 43.9% (43/98) in the solid tumor group who had not received any antiviral therapy. The median onset time of HBV reactivation was 11 wk (range: 3-25 wk) after initiation of chemotherapy. HBV reactivation developed earlier ($P < 0.001$) in patients with lymphoma (9 wk, range: 4-14 wk) than in those with other cancers (15 wk, range: 9-21 wk). HBV reactivation also developed earlier ($P < 0.001$) in patients receiving CHOP (cyclophosphamide, doxorubicin, vincristine and prednisolone)-like regimens (8 wk, range: 3-15 wk) compared to non-CHOP protocols. Baseline HBV status (positive or negative) was not significantly associated with the time interval between initiation of chemotherapy and onset of HBV reactivation. In multivariate analysis (Table 4), HBV reactivation was more likely to occur in patients with lymphoma, high levels of HBV DNA, HBeAg positivity, and male patients. It was also found that antiviral prophylaxis for patients who were positive for HBV DNA significantly reduced the incidence of chemotherapy-induced HBV reactivation (OR: 0.009,

95%CI: 0.001-0.073, $P < 0.001$).

Chemotherapy drugs and HBV reactivation

Of the 160 patients who were re-tested for HBV, 13 had received corticosteroids (all patients had lymphoma) and 61.5% (8/13) developed HBV reactivation. In univariate analysis, HBV reactivation was more likely to occur in patients who had received corticosteroids ($P = 0.023$) and botanical chemotherapeutic drugs ($P = 0.048$). However, the components of the chemotherapy regimens (including corticosteroids) seemed not to be associated with HBV reactivation in multivariate analysis.

Hepatitis C virus co-infection

Of 5616 patients, 2679 (47.7%) were tested for hepatitis C virus (HCV). In this group, 305 and 12 patients were HBsAg positive and HCV RNA positive, respectively. Only four out of 305 patients (1.3%) were both HBsAg and HCV-RNA positive, and none of these patients developed HBV reactivation.

DISCUSSION

In recent years, many organizations have advocated that all chemotherapy candidates should receive HBV serological testing before starting treatment[11-13]. In the present study, it was observed that 49.3% of patients had received HBV serological testing prior to chemotherapy, which was much higher than reports from Canada (14%)[14] and the United States (16.7%)[15]. However, most of the patients were not for HBV infection because of impending chemotherapy, but as part of a preoperative routine (Figure 1). Therefore, only 17.1% of the cancer patients received pre-chemotherapy HBV testing, which is close to the testing rates in other published studies[14,15].

Patients with elevated aminotransferase levels prior to chemotherapy were more likely to be screened for HBV infection (OR: 10.88, $P < 0.001$). This is reasonable because elevated aminotransferase levels usually indicate liver inflammation, which may lead physicians to screen for HBV as a possible etiology. Guidelines have recommended that individuals with chronically elevated ALT or AST should be routinely screened for HBV infection[12]. However, not all patients with elevated aminotransferases receive HBV testing. It has been reported that only 30%-70% of oncologists screen for HBV infection before chemotherapy in patients with abnormal liver-associated enzymes[17,18]. The testing rate of only 62.4% in the current study is consistent with the data from other studies. The lack of HBV testing might be because some patients without a history of HBV infection had only slightly elevated aminotransferase levels that were insufficiently high to be a contraindication for chemotherapy.

In the current study, a relationship was found between the type of tumor and the rate of HBV serological testing. Patients with hematological malignancies (OR: 4.17, $P < 0.001$) were more likely to receive HBV testing before chemotherapy. The same phenomenon was also observed in an American study[19], which might be partly because lymphoma is a known risk factor for HBV reactivation[4,10]. Rituximab is a standard treatment for diffuse large B-cell lymphoma. Rituximab treatment was found to be an important indicator associated with HBV testing prior to chemotherapy (OR: 1.96, $P = 0.038$) in the present study. Testing for HBV infection before anticancer therapy is likely because of the known risk of HBV reactivation after rituximab administration[20].

In the current study, the HBV reactivation rate was found to be 33.1%, which was very close to the average reactivation rate (34%) observed in a prior meta-analysis[9].

The median onset time of HBV reactivation after initiation of chemotherapy was 11 wk (range: 3-25 wk), which was similar to that reported previously (16 wk, range: 4-36 wk)[21]. HBV reactivation was observed to occur earlier in patients with lymphoma and those receiving CHOP-like regimens in the current study. Previous studies have identified many risk factors related to HBV reactivation during anticancer therapy, such as male sex, younger age, absence of anti-HBs, HBeAg seropositivity, diagnosis of lymphoma or breast cancer, high pre-chemotherapy HBV DNA levels, and the use of steroids or rituximab[4,10,20,22]. In addition, it has also been reported that HBV DNA contains a glucocorticoid responsive element that stimulates HBV replication[23,24]. Multivariate analysis in the present study identified high baseline levels of HBV DNA, male patients, HBeAg positivity, and lymphoma to be predictive of HBV reactivation (Table 4). Further study is needed to determine the relationship between HBV reactivation and steroids (including their dose).

HBV reactivation rates were significantly higher in the non-lamivudine group compared with the lamivudine group (46.0% *vs* 2.1%). Moreover, multivariate analysis identified that antiviral prophylaxis significantly reduced the incidence of chemotherapy-induced HBV reactivation, which has also been reported previously[25-28]. Therefore, for patients with high baseline levels of HBV DNA, prophylactic lamivudine might be considered before chemotherapy. This is already recommended by EASL and CDC[11,13]. However, high baseline HBV viral loads are associated with the development of resistance to lamivudine (an antiviral agent with a low genetic barrier)[29]. After prophylactic administration of lamivudine, the rate of HBV reactivation was 5%-13.3% in previous studies[25,27,28]. It was observed in some small studies that none of the HBsAg-positive patients receiving chemotherapy or immunosuppressive therapy developed HBV reactivation after prophylactic entecavir[30,31]. Therefore, prophylactic administration of other antiviral agents with high genetic barriers to resistance, such as entecavir or tenofovir, might be considered suitable substitutes for lamivudine for HBV prophylaxis prior to chemotherapy.

In the current study, it was observed that 372 patients (13.4%) were HBsAg seropositive (Table 3). According to an epidemiological serological survey of hepatitis B[2], the

weighted prevalence of HBsAg for the Chinese population aged 1-59 years was 7.2%. Therefore, the prevalence of HBsAg in the current study seems to be higher. This might be because most of the patients in our study were > 20 years old (Table 3), and therefore, born before the introduction of the universal vaccination program[32]. Another reason might be that our patients with a history of HBV or elevated aminotransferases were more likely to be tested for HBV infection (Table 2).

Previous studies have reported that 0.72%-3.3% of patients who were negative for HBsAg developed *de novo* HBV-related hepatitis after chemotherapy[33-35]. Therefore, it is not sufficient to test for the presence of HBsAg alone as a marker of HBV infection. This is consistent with the EASL recommendations that cancer patients should be tested for both HBsAg and anti-HBc before chemotherapy[11]. In the current study, 284 patients (10.5%) were HBsAg negative but anti-HBc positive (Table 3), and two of these had HBV reactivation; one with diffuse large-B lymphoma and the other with breast cancer.

As mentioned before, high baseline HBV DNA levels are an independent risk factor for HBV reactivation[10,22]. EASL has recommended that cancer patients who are HBsAg or anti-HBc positive should have their HBV DNA levels measured[11]. In the current study, of the 656 patients who were HBsAg positive and/or anti-HBc positive, only 177 patients (27.0%) had been tested for HBV DNA (Table 3). Oncology physicians should pay more attention to this problem.

A high prevalence of occult HBV infection has been observed in HCV-infected patients[36,37]. The HCV co-infection rate among HBsAg carriers ranged from 3% to 18%[38,39], depending on the geographic location and selection of patients. In the current study, 4 patients (1.3%) were positive for both HBsAg and HCV RNA, and none developed HBV reactivation. This might have been because HCV replication is suspected to suppress HBV replication strongly[40].

The limitations of this study include the fact that it was a single-institution, retrospective study. Some important information was not recorded, such as the reasons why physicians made decisions regarding testing for HBV infection. This limitation may be resolved by a prospective study in the future.

In conclusion, only 17.1% of cancer patients received complete HBV testing prior to chemotherapy. Patients who were to receive rituximab therapy, had elevated aminotransferase levels, or had hematological malignancies were more likely to receive HBV testing. Only 27% of the patients who were positive for HBsAg and/or anti-HBc received testing for HBV DNA. HBV reactivation was observed in 33.1% of the patients, and was more likely to occur in patients with lymphoma, high levels of HBV DNA, HBeAg positivity, and male patients. Prophylactic antiviral therapy for patients with positive HBV DNA can significantly reduce the incidence of chemotherapy-induced HBV reactivation.

COMMENTS

Background

Cancer patients receiving intensive chemotherapy may be at risk for hepatitis B virus (HBV) reactivation. Recently, more attention has been paid to HBV testing before chemotherapy. Many clinical practice guidelines advise HBV testing before starting chemotherapy. However, few studies have focused on this issue in regions of high HBV endemicity, such as China.

Research frontiers

Authors demonstrated that only 17.1% of cancer patients received pre-chemotherapy HBV testing (43.2% for hematological malignancies and 14.9% for solid tumors). Patients who had received rituximab therapy, had elevated aminotransferase levels, or had hematological malignancies were more likely to receive HBV testing. HBV reactivation was more likely to occur in patients with lymphoma, high levels of HBV DNA, hepatitis B e antigen positivity, and male patients.

Innovations and breakthroughs

In recent years, more organizations have advocated that all chemotherapy candidates should receive HBV serological testing before starting treatment. This is believed to be the first study to report the exact pre-chemotherapy HBV testing rate in China; a region of high endemicity for HBV infection. The rate of HBV reactivation after chemotherapy and the median onset time are also reported.

Applications

Many clinical practice guidelines advise HBV testing before starting chemotherapy. However, the results in this study showed that only 17.1% of cancer patients received pre-chemotherapy HBV testing.

Terminology

HBV reactivation was defined as an increase in serum HBV DNA to > 1 log higher than that of the pre-exacerbation baseline, or serum HBV DNA conversion from negative to positive.

Peer review

In this retrospective analysis, the authors assessed the rate of HBV serological testing prior to the initiation of chemotherapy and the rate of HBV reactivation after chemotherapy in Chinese patients with cancer. The authors concluded that < 20% of patients received HBV testing before chemotherapy, and HBV reactivation would have occurred after chemotherapy in nearly half of these infected patients who did not receive pre-emptive antiviral therapy.

REFERENCES

1 **World Health Organization**. Hepatitis B: World Health Organization Fact Sheet 204. Available from: URL: http://www.who.int/mediacentre/factsheets/fs204/en/

2 **Liang X**, Bi S, Yang W, Wang L, Cui G, Cui F, Zhang Y, Liu J, Gong X, Chen Y, Wang F, Zheng H, Wang F, Guo J, Jia Z, Ma J, Wang H, Luo H, Li L, Jin S, Hadler SC, Wang Y. Epidemiological serosurvey of hepatitis B in China--declining HBV prevalence due to hepatitis B vaccination. *Vaccine* 2009; **27**: 6550-6557 [PMID: 19729084 DOI: 10.1016/j.vaccine.2009.08.048]

3 **Liu CJ**, Chen PJ, Chen DS, Kao JH. Hepatitis B virus reactivation in patients receiving cancer chemotherapy: natural history, pathogenesis, and management. *Hepatol Int* 2011 Jun 14; Epub ahead of print [PMID: 21670970 DOI: 10.1007/s12072-011-9279-6]

4 **Yeo W**, Chan PK, Zhong S, Ho WM, Steinberg JL, Tam JS, Hui P, Leung NW, Zee B, Johnson PJ. Frequency of hepatitis B virus reactivation in cancer patients undergoing cytotoxic chemotherapy: a prospective study of 626 patients with identification of risk factors. *J Med Virol* 2000; **62**: 299-307 [PMID: 11055239 DOI: 10.1002/1096-9071(200011)]

5 **Manzano-Alonso ML**, Castellano-Tortajada G. Reactivation of hepatitis B virus infection after cytotoxic chemotherapy or immunosuppressive therapy. *World J Gastroenterol* 2011; **17**: 1531-1537 [PMID: 21472116 DOI: 10.3748/wjg.v17.i12.]

6 **Niitsu N**, Hagiwara Y, Tanae K, Kohri M, Takahashi N. Prospective analysis of hepatitis B virus reactivation in patients with diffuse large B-cell lymphoma after rituximab combination chemotherapy. *J Clin Oncol* 2010; **28**: 5097-5100 [PMID:

20837949 DOI: 10.1200/JCO.2010.29.7531]
7 **Yeo W**, Lam KC, Zee B, Chan PS, Mo FK, Ho WM, Wong WL, Leung TW, Chan AT, Ma B, Mok TS, Johnson PJ. Hepatitis B reactivation in patients with hepatocellular carcinoma undergoing systemic chemotherapy. *Ann Oncol* 2004; **15**: 1661-1666 [PMID: 15520068 DOI: 10.1093/annonc/mdh430]
8 **Cheng AL**, Hsiung CA, Su IJ, Chen PJ, Chang MC, Tsao CJ, Kao WY, Uen WC, Hsu CH, Tien HF, Chao TY, Chen LT, Whang-Peng J. Steroid-free chemotherapy decreases risk of hepatitis B virus (HBV) reactivation in HBV-carriers with lymphoma. *Hepatology* 2003; **37**: 1320-1328 [PMID: 12774010 DOI: 10.1053/jhep.2003.50220]
9 **Loomba R**, Rowley A, Wesley R, Liang TJ, Hoofnagle JH, Pucino F, Csako G. Systematic review: the effect of preventive lamivudine on hepatitis B reactivation during chemotherapy. *Ann Intern Med* 2008; **148**: 519-528 [PMID: 18378948]
10 **Yeo W**, Zee B, Zhong S, Chan PK, Wong WL, Ho WM, Lam KC, Johnson PJ. Comprehensive analysis of risk factors associating with Hepatitis B virus (HBV) reactivation in cancer patients undergoing cytotoxic chemotherapy. *Br J Cancer* 2004; **90**: 1306-1311 [PMID: 15054446 DOI: 10.1038/sj.bjc.6601699]
11 **European Association For The Study Of The Liver**. EASL clinical practice guidelines. Management of chronic hepatitis B. *Gastroenterol Clin Biol* 2009; **33**: 539-554 [PMID: 19481389 DOI: 10.1016/j.gcb.2009.03.002]
12 **Lok AS**, McMahon BJ. Chronic hepatitis B. *Hepatology* 2007; **45**: 507-539 [PMID: 17256718 DOI: 10.1002/hep.21513]
13 **Weinbaum CM**, Williams I, Mast EE, Wang SA, Finelli L, Wasley A, Neitzel SM, Ward JW. Recommendations for identification and public health management of persons with chronic hepatitis B virus infection. *MMWR Recomm Rep* 2008; **57**: 1-20 [PMID: 18802412]
14 **Lee R**, Vu K, Bell CM, Hicks LK. Screening for hepatitis B surface antigen before chemotherapy: current practice and opportunities for improvement. *Curr Oncol* 2010; **17**: 32-38 [PMID: 21151407]
15 **Hwang J**, Fisch M, Zhang H, Kallen M, Routbort M, Lal L. S, Vierling J and Suarez-Almazor M. Reactivation of hepatitis B infection among patients with cancer. *J Clin Oncol* 2011; **29** Suppl 15: 9056
16 **Lau GK**. Hepatitis B reactivation after chemotherapy: two decades of clinical research. *Hepatol Int* 2008; **2**: 152-162 [PMID: 19669300 DOI: 10.1007/s12072-008-9056-3]
17 **Tran TT**, Rakoski MO, Martin P, Poordad F. Screening for hepatitis B in chemotherapy patients: survey of current oncology practices. *Aliment Pharmacol Ther* 2010; **31**: 240-246 [PMID: 19814747 DOI: 10.1111/j.1365-2036.2009.04158.x]
18 **Khokhar OS**, Farhadi A, McGrail L, Lewis JH. Oncologists and hepatitis B: a survey to determine current level of awareness and practice of antiviral prophylaxis to prevent reactivation. *Chemotherapy* 2009; **55**: 69-75 [PMID: 19077421]
19 **Hwang J**, Fisch M, Zhang H Kallen, M. Routbort, L. Lal, J. Vierling, M. Suarez-Almazor. Hepatitis B screening and positivity prior to chemotherapy. *J Clin Oncol* 2010; **28** Suppl 15: 9008
20 **Yeo W**, Chan TC, Leung NW, Lam WY, Mo FK, Chu MT, Chan HL, Hui EP, Lei KI, Mok TS, Chan PK. Hepatitis B virus reactivation in lymphoma patients with prior resolved hepatitis B undergoing anticancer therapy with or without rituximab. *J Clin Oncol* 2009; **27**: 605-611 [PMID: 19075267 DOI: 10.1200/JCO.2008.18.0182]
21 **Lau GK**, Yiu HH, Fong DY, Cheng HC, Au WY, Lai LS, Cheung M, Zhang HY, Lie A, Ngan R, Liang R. Early is superior to deferred preemptive lamivudine therapy for hepatitis B patients undergoing chemotherapy. *Gastroenterology* 2003; **125**: 1742-1749 [PMID: 14724827 DOI: 10.1053/j.gastro.2003.09.026]
22 **Lau GK**, Leung YH, Fong DY, Au WY, Kwong YL, Lie A, Hou JL, Wen YM, Nanj A, Liang R. High hepatitis B virus (HBV) DNA viral load as the most important risk factor for HBV reactivation in patients positive for HBV surface antigen undergoing autologous hematopoietic cell transplantation. *Blood* 2002; **99**: 2324-2330 [PMID: 11895763 DOI: 10.1182/blood.V99.7.2324]
23 **Tur-Kaspa R**, Burk RD, Shaul Y, Shafritz DA. Hepatitis B virus DNA contains a glucocorticoid-responsive element. *Proc Natl Acad Sci USA* 1986; **83**: 1627-1631 [PMID: 3006059]
24 **Liaw YF**. Hepatitis viruses under immunosuppressive agents. *J Gastroenterol Hepatol* 1998; **13**: 14-20 [PMID: 9737566 DOI: 10.1111/j.1440-1746.1998.tb00539.x]
25 **Lau GK**, He ML, Fong DY, Bartholomeusz A, Au WY, Lie AK, Locarnini S, Liang R. Preemptive use of lamivudine reduces hepatitis B exacerbation after allogeneic hematopoietic cell transplantation. *Hepatology* 2002; **36**: 702-709 [PMID: 12198664]
26 **Keam B**, Lee JH, Im SA, Yoon JH. Why, when, and how to prevent hepatitis B virus reactivation in cancer patients undergoing chemotherapy. *J Natl Compr Canc Netw* 2011; **9**: 465-477 [PMID: 21550967]
27 **Chen XQ**, Peng JW, Lin GN, Li M, Xia ZJ. The effect of prophylactic lamivudine on hepatitis B virus reactivation in HBsAg-positive patients with diffuse large B-cell lymphoma undergoing prolonged rituximab therapy. *Med Oncol* 2012; **29**: 1237-1241 [PMID: 21556931 DOI: 10.1007/s12032-011-9974-0]
28 **Yeo W**, Ho WM, Hui P, Chan PK, Lam KC, Lee JJ, Johnson PJ. Use of lamivudine to prevent hepatitis B virus reactivation during chemotherapy in breast cancer patients. *Breast Cancer Res Treat* 2004; **88**: 209-215 [PMID: 15609123]
29 **Zoulim F**, Poynard T, Degos F, Slama A, El Hasnaoui A, Blin P, Mercier F, Deny P, Landais P, Parvaz P, Trepo C. A prospective study of the evolution of lamivudine resistance mutations in patients with chronic hepatitis B treated with lamivudine. *J Viral Hepat* 2006; **13**: 278-288 [PMID: 16611195 DOI: 10.1111/j.1365-2893.2005.00712.x]
30 **Watanabe M**, Shibuya A, Takada J, Tanaka Y, Okuwaki Y, Minamino T, Hidaka H, Nakazawa T, Koizumi W. Entecavir is an optional agent to prevent hepatitis B virus (HBV) reactivation: a review of 16 patients. *Eur J Intern Med* 2010; **21**: 333-337 [PMID: 20603047 DOI: 10.1016/j.ejim.2010.04.010]
31 **Mimura N**, Kojima H, Tsujimura H, Ise M, Sakai C, Fukai K, Yokosuka O, Kumagai K. [Prevention of hepatitis B virus reactivation in B-cell lymphoma patients receiving chemotherapy with rituximab]. *Rinsho Ketsueki* 2010; **51**: 213-215 [PMID: 20379117]
32 **Liang X**, Bi S, Yang W, Wang L, Cui G, Cui F, Zhang Y, Liu J, Gong X, Chen Y, Wang F, Zheng H, Wang F, Guo J, Jia Z, Ma J, Wang H, Luo H, Li L, Jin S, Hadler SC, Wang Y. Evaluation of the impact of hepatitis B vaccination among children born during 1992-2005 in China. *J Infect Dis* 2009; **200**: 39-47 [PMID: 19469708 DOI: 10.1086/599332]
33 **Hui CK**, Cheung WW, Zhang HY, Au WY, Yueng YH, Leung AY, Leung N, Luk JM, Lie AK, Kwong YL, Liang R, Lau GK. Kinetics and risk of de novo hepatitis B infection in HBsAg-negative patients undergoing cytotoxic chemotherapy. *Gastroenterology* 2006; **131**: 59-68 [PMID: 16831590]
34 **Koo YX**, Tay M, Teh YE, Teng D, Tan DS, Tan IB, Tai DW, Quek R, Tao M, Lim ST. Risk of hepatitis B virus (HBV) reactivation in hepatitis B surface antigen negative/hepatitis B core antibody positive patients receiving rituximab-containing combination chemotherapy without routine antiviral prophylaxis. *Ann Hematol* 2011; **90**: 1219-1223 [PMID: 21520001]
35 **Sugauchi F**, Tanaka Y, Kusumoto S, Matsuura K, Sugiyama M, Kurbanov F, Ueda R, Mizokami M. Virological and clinical characteristics on reactivation of occult hepatitis B in patients with hematological malignancy. *J Med Virol* 2011; **83**: 412-418 [PMID: 21264861]
36 **Torbenson M**, Thomas DL. Occult hepatitis B. *Lancet Infect*

Dis 2002; **2**: 479-486 [PMID: 12150847]
37 **Raimondo G**, Pollicino T, Cacciola I, Squadrito G. Occult hepatitis B virus infection. *J Hepatol* 2007; **46**: 160-170 [PMID: 17112622 DOI: 10.1016/j.jhep.2006.10.007]
38 **Semnani S**, Roshandel G, Abdolahi N, Besharat S, Keshtkar AA, Joshaghani H, Moradi A, Kalavi K, Jabbari A, Kabir MJ, Hosseini SA, Sedaqat SM, Danesh A, Roshandel D. Hepatitis B/C virus co-infection in Iran: a seroepidemiological study. *Turk J Gastroenterol* 2007; **18**: 20-21 [PMID: 17450490]
39 **Gaeta GB**, Stornaiuolo G, Precone DF, Lobello S, Chiaramonte M, Stroffolini T, Colucci G, Rizzetto M. Epidemiological and clinical burden of chronic hepatitis B virus/hepatitis C virus infection. A multicenter Italian study. *J Hepatol* 2003; **39**: 1036-1041 [PMID: 14642623 DOI: 10.1016/S0168-8278(03)00470-7]
40 **Raimondo G**, Cacciamo G, Saitta C. Hepatitis B virus and hepatitis C virus co-infection: additive players in chronic liver disease? *Ann Hepatol* 2005; **4**: 100-106 [PMID: 16010242]

P- Reviewer Chen DY **S- Editor** Wen LL **L- Editor** A **E- Editor** Li JY



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i6.931

World J Gastroenterol 2013 February 14; 19(6): 931-935
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Quadruple therapy for eradication of *Helicobacter pylori*

Hai-Jun Ma, Jin-Liang Wang

Hai-Jun Ma, Jin-Liang Wang, Department of Gastroenterology, the First Affiliated Hospital of Henan University of Science and Technology, Luoyang 471003, Henan Province, China
Author contributions: Ma HJ collected data on the subject and prepare the manuscript; Wang JL conceived and designed the topic, reviewed and analyzed the literature.
Correspondence to: Jin-Liang Wang, Professor, Department of Gastroenterology, the First Affiliated Hospital of Henan University of Science and Technology, No. 24 Jinghua Road, Luoyang 471003, Henan Province, China. wangjinliang3008@163.com
Telephone: +86-379-64830750 Fax: +86-379-64907159
Received: September 18, 2012 Revised: November 11, 2012
Accepted: December 22, 2012
Published online: February 14, 2013

Abstract

AIM: To investigate quadruple therapy with rabeprazole, amoxicillin, levofloxacin and furazolidone for the eradication of *Helicobacter pylori* (*H. pylori*) infection.

METHODS: A total of 147 patients were divided into the experimental treatment group (n = 78) and the standard triple treatment group (n = 69). The experimental treatment group received rabeprazole 20 mg, amoxicillin 1.0 g, levofloxacin 0.2 g and furazolidone 0.1 g, twice daily. The standard triple treatment group received omeprazole 20 mg, amoxicillin 1.0 g and clarithromycin 0.5 g, twice daily.

RESULTS: One month after treatment, the ^{13}C urea breath test was carried out to detect *H. pylori*. The eradication rate using per-protocol analysis was 94.3% in the experimental treatment group and 73% in the standard triple treatment group (P < 0.05), and using intention to test analysis, these figures were 86% and 67% in the two groups, respectively. Side effects were observed in 34 patients, and included mild dizziness, nausea, diarrhea and increased bowel movement. Eleven of the 34 patients needed no treatment for their side effects.

CONCLUSION: Rabeprazole, amoxicillin, levofloxacin and furazolidone quadruple therapy is a safe method for the eradication of *H. pylori* with high efficacy and good tolerability.

© 2013 Baishideng. All rights reserved.

Key words: Rabeprazole; Amoxicillin; Levofloxacin; Furazolidone; *Helicobacter pylori*

Ma HJ, Wang JL. Quadruple therapy for eradication of *Helicobacter pylori*. *World J Gastroenterol* 2013; 19(6): 931-935 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i6/931.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i6.931

INTRODUCTION

Helicobacter pylori (*H. pylori*) is closely related to a number of gastrointestinal diseases. It is the main pathogenic factor in chronic gastritis and peptic ulcer disease, and is also the initiating factor in gastric cancer and gastric mucosa-associated lymphoid tissue lymphoma[1]. The ability to reliably eradicate this pathogen is important in the management of these diseases[2-5].

The eradication of *H. pylori* has been a research focus for the effective treatment of the above diseases. Currently, the worldwide standard triple therapy consists of a proton pump inhibitor (PPI) or bismuth, amoxicillin and metronidazole or clarithromycin. However, with the extensive use of antibiotics and the irregularity of treatment, there is increasingly high *H. pylori* resistance, especially resistance to metronidazole and clarithromycin. The effectiveness of "legacy triple therapy", which was recommended by the Maastricht Ⅲ Consensus Report, has demonstrated disappointingly low treatment success (*i.e.*, below 80%) worldwide[6]. The difficulty of *H. pylori* eradication is increasing. The consensus of the Third National Lushan Conference held at Lushan Mountain in Jiangxi in August 2007 states that there is high *H. pylori* resistance to metronidazole and clarithromycin, but low resistance to furazolidone, tetracycline and quinolones,

therefore, the effects of these antibiotics are relatively high, and may be used as the initial treatment choice. In our study, quadruple therapy with rabeprazole, amoxicillin, levofloxacin and furazolidone achieved good results.

MATERIALS AND METHODS

General information

All 147 patients underwent the treatment with either rabeprazole 20 mg, amoxicillin 1.0 g, levofloxacin 0.2 g and furazolidone 0.1 g, twice daily or omeprazole 20 mg, amoxicillin 1.0 g and clarithromycin 0.5 g, twice daily at the First Affiliated Hospital of Henan University of Science and Technology from January 2009 to December 2011 and were included in this study as sample cases. However, 78 patients who chose treatment with rabeprazole 20 mg, amoxicillin 1.0 g, levofloxacin 0.2 g and furazolidone 0.1 g, twice daily were included in the experimental treatment group, and 69 patients who chose treatment with omeprazole 20 mg, amoxicillin 1.0 g and clarithromycin 0.5 g, twice daily were included in the standard triple treatment group. All patients, who did not receive treatment before enrolling in this study, were diagnosed with chronic erosive gastritis or peptic ulcer disease by endoscopy. In addition, all patients had a positive rapid urease test and antrum mucosal tissue was obtained to detect the presence of *H. pylori*.

The patients by their own volition, were divided into the experimental treatment group (n = 78) and the standard triple treatment group (n = 69). The experimental treatment group consisted of 41 males and 37 females, aged 20 to 65 years, with an average age of 44.5 years; 34 patients had chronic erosive gastritis, 28 had a gastric ulcer and 16 had a duodenal ulcer. The standard triple treatment group consisted of 38 males and 31 females, aged 22 to 67 years, with an average age of 43.5 years; 31 patients had chronic erosive gastritis, 17 had a gastric ulcer and 21 had a duodenal ulcer. There were no statistically significant differences between the two groups regarding age and gender (Table 1).

Exclusion criteria

Patients with a history of gastrointestinal surgery, gastroesophageal reflux disease, non-steroidal anti-inflammatory drug-related stomach problems or ulcers, those taking PPIs or H_2 blockers, bismuth, antibiotics or other drugs which might affect gastric physiology two weeks before enrolling in the study and those who were allergic to the medicine tested were excluded.

Methods

The experimental treatment group received rabeprazole (Jiangsu Stockhausen Pharmaceuticals, Nanjing, China) 20 mg, amoxicillin (Shiyao Pharmaceutical Group, Shijiazhuang, China) 1.0 g, levofloxacin (Jiangsu Yabang Epson Pharmaceutical, Nanjng, China) 0.2 g and furazolidone (Wuhan Huawei Medicine, Wuhan, China) 0.1 g; the standard triple treatment group received omeprazole (Beijing Taiyang Pharmaceutical, Beijing, China) 20 mg, amoxicillin (Shiyao Pharmaceutical Group, Shijiazhuang, China) 1.0 g and clarithromycin (Guangzhou Baiyunshan Pharmaceutical Group, Guangzhou, China) 0.5 g. The drugs were given half an hour before meals in the morning and evening for 7 d.

Table 1 Baseline characteristics of the patients

Group	No. of patients	Age (yr)	Male[1]	Female[1]	Chronic erosive gastritis[2]	Gastric ulcer[2]	Duodenal ulcer[2]
Experimental treatment	78	44.5	41	37	34	28	16
Standard treatment	69	43.5	38	31	31	17	21

[1]χ^2 = 0.093, P > 0.05; [2]χ^2 = 2.96, P > 0.05.

Observed indicators

Patients were followed up four weeks after treatment, and the ^{13}C urea breath test (^{13}C-UBT) was carried out to detect the presence of *H. pylori*.

Ethics

This was a clinical study carried out in humans, and the protocol for this work was approved by the Institutional Ethics Committee of the First Affiliated Hospital of Henan University of Science and Technology. Before the study began, the patients signed an informed consent form which included name, age, gender, chief complaint, drug history and past medical history.

Statistical analysis

The *H. pylori* eradication rate was assessed based on intention-to-treat (ITT) and per-protocol (PP) analysis. The 95%CI were also calculated for both the ITT and PP analyses and the eradication rate. The patients who were lost to follow-up or could not complete the treatment course due to severe adverse reactions, were considered treatment failures and were excluded in the PP analysis. The χ^2 test and Fisher's exact test were used to compare the differences between the two groups in terms of baseline data, eradication rate and adverse reactions. $P < 0.05$ was considered significant.

RESULTS

Treatment results

In the experimental treatment group, 7 of the 78 patients were lost to follow up (9%), and of the 71 remaining patients, 67 were negative by the ^{13}C-UBT. In the standard triple treatment group, 6 of the 69 patients were lost to follow up (8.6%), and of the remaining 63 patients, 46 were negative by the ^{13}C-UBT.

The eradication rate following the PP analysis in the experimental treatment group was 94.3% (67/71), and was 73% (46/63) in the standard triple treatment group. The eradication rate following the ITT analysis was 86%

Table 2 ***Helicobacter pylori*** **eradication rate in the two groups**

Group	Eradication	No eradication	Follow-up lost	Eradication rate[1] (PP) (95%CI)	Eradication rate[2] (ITT) (95%CI)
Experimental treatment group	67	4	7	94% (88%-99%)	86% (78%-94%)
Standard triple treatment	46	17	6	74% (64%-84%)	67% (57%-77%)

[1]χ^2 = 12.10, P < 0.05; [2]χ^2 = 7.615, P < 0.05. PP: Per-protocol; ITT: Intention-to-treat.

Table 3 ***Helicobacter pylori*** **eradication rates in relation to disease *n* (%)**

Group	Chronic erosive gastritis	Gastric ulcer	Duodenal ulcer
Experimental treatment	29 (34)	24 (28)	14 (16)
Standard treatment	21 (31)	11 (17)	14 (21)

χ^2 = 2.29, P > 0.05

and 67% in the two groups, respectively. The eradication rates in the two groups were statistically significant (P < 0.05) (Table 2). The eradication rate in patients with different diseases in the two groups was not significantly different (Table 3).

Side effects

Major adverse reactions were abdominal pain, dry mouth, dizziness, nausea, vomiting and bloating symptoms, however, these reactions were mild and well tolerated. All patients were able to complete the treatment regimen (Table 4).

DISCUSSION

Warren and Marshall reported the isolation of Campylobacter unidentified (unidentified curved bacilli) from the stomach in 1983, which was officially named *H. pylori* in 1989. *H. pylori* is considered to be the etiologic cause of gastritis, peptic ulcer disease and is associated with the development of gastric cancer[7-9]. It is a spiral or curved gram-negative microaerophilic flagellated bacillus. The prevalence of *H. pylori* infection varies worldwide, and depends on socioeconomic status and sanitation conditions[10]. The diagnosis of *H. pylori* can be performed using invasive and noninvasive methods. Invasive methods include the urease test, which has a sensitivity ranging from 79.7% to 97.5% and a specificity from 97.2% to 100%[11-14], it is performed in the endoscopy unit and is a suitable rapid indirect test to confirm the presence of *H. pylori* in biopsy samples. The urea breath test is considered the gold standard for the diagnosis of *H. pylori* infection, and has a sensitivity and a specificity ranging from 90% to 100%[15-18]. Therefore, we suggest using the ^{13}C-UBT, or esophagogastroduodenoscopy for the assessment of *H. pylori* status.

With increased research on bacteria and drug treatment trials for *H. pylori* infection, it is now clear that antibiotics, antacids, metal preparations and other drugs are effective. Antibiotics used in the treatment of *H. pylori* infection include amoxicillin, tetracycline, clarithromycin, quinolones, furazolidone, metronidazoles and other drugs which have either bactericidal or bacteriostatic effects. Antacids include PPIs and histamine2-receptor antagonists. PPI-clarithromycin-amoxicillin or metronidazole treatment for 7 to 14 d is the first treatment choice for *H. pylori* infection[19]. However, several large clinical trials and meta-analyses have shown that the eradication rate with standard triple therapy has generally declined to unacceptable levels (*i.e.*, 80% or less) recently[20,21].

With increasing clinical use of antibiotics, *H. pylori* resistance appears to have significantly increased. Abuse and irrational drug use as well as repeated anti-*H. pylori* treatment failure are important causes of drug resistance[22].

According to the *H. pylori* Study Group of Digestive Diseases Division of the Chinese Medical Association, which investigated multiple regions of the country in 2007, the resistance rates of *H. pylori* to metronidazole, clarithromycin and amoxicillin were 75.6%, 27.6% and 2.7%, respectively[23]. *H. pylori* resistance to antibiotics is the most important factor in treatment failure. Therefore, the choice of antibiotics is the key to successful eradication of *H. pylori*, in the absence of drug susceptibility testing, and it is safe to use the less frequently used antibiotics which are less likely to induce antibiotic resistance.

The mechanism of action of PPIs involve: (1) direct inhibition of the growth of *H. pylori*; (2) inhibition of urease activity; and (3) increased intragastric pH and enhanced antibiotic activity[24]. Hepatic oxidation is mediated by the cytochrome P450 2C19 (CYP2C19) gene which is polymorphic and produces fast metabolizers and low metabolizers. This is another important factor in the failure of *H. pylori* eradication therapy, and one study suggested that this is the second leading cause of bacterial resistance[25]. The elimination of omeprazole, lansoprazole and pantoprazole involves hepatic oxidation mediated by cytochrome CYP2C19 and CYP3A4, while that of rabeprazole involves its reduction *via* a non-enzymatic pathway to rabeprazole-thioether and partial metabolism by CYP2C19 and CYP3A4[21]. Thus, rabeprazole is less susceptible to the influence of genetic polymorphisms of either CYP2C19 or CYP3A4, and was found to depend only on its pharmacokinetic and pharmacodynamic characteristics. The pKa of rabeprazole is 5.0 and those of other PPIs, such as omeprazole and lansoprazole, are around 4.0, rabeprazole accumulates in an acidic space up to a concentration ten-fold greater than do other PPIs. In addition, rabeprazole is known to transform into the acid-activated form much faster than other PPIs, which further contributes to the inhibition of proton pumps immediately after the arrival of rabeprazole at the target site[26].

Levofloxacin is a third-generation fluoroquinolone, its antibacterial mechanism involves inhibition of bacterial type Ⅱ topoisomerase. Topoisomerase controls DNA to-

Table 4 Side effect profile of patients

Group	No adverse reactions	Abdominal pain	Nausea	Vomiting	Dry throat	Bloating	Dizziness	Total
Experimental treatment	58	8	10	6	4	5	7	40
Standard triple	55	7	6	5	3	2	1	24
Total episodes	113	15	16	11	7	7	8	64

$\chi^2 = 2.13$, $P > 0.05$.

pology in DNA replication and repair, it is a key enzyme in transcription. Levofloxacin, blocks DNA replication by inhibiting bacterial DNA gyrase A subunit and inhibits topoisomerase Ⅳ activity, thus has a bactericidal effect[27]. In addition, levofloxacin has no cross-resistance to *B*-lactam and macrolide antibiotics.

The clinical use of furazolidone is not very extensive, and it is not a commonly used drug in the standard regimen. However, it has a significant effect on *H. pylori* and does not tend to produce drug resistance. The reported furazolidone sensitivity rate is generally 100%[28], and it is inexpensive, therefore, we chose this medication for our *H. pylori* infected patients, and the results showed that inclusion of this drug in the quadruple regimen for *H. pylori* infection resulted in a high eradication rate, thus, this agent has good efficacy. The most common side effects of furazolidone are nausea, vomiting and other gastrointestinal reactions. In this study, gastrointestinal reactions were seen in patients taking furazolidone, however, multiple neuritis was not noted, and the drug was well tolerated.

In summary, these experimental results demonstrated that rabeprazole - amoxicillin - levofloxacin - furazolidone quadruple combination therapy is a safe, highly effective and well tolerated regimen for eradication of *H. pylori*.

COMMENTS

Background

Helicobacter pylori (*H. pylori*) eradication rates with standard triple therapy are declining worldwide. The optimal management of *H. pylori* is evolving and new treatment combinations for antibiotic resistant *H. pylori* strains are required.

Research frontiers

Standard triple therapy represents the accepted standard therapy for *H. pylori* as the organism is known to be susceptible to clarithromycin, and local antimicrobial resistance rates are below 20%, while newer treatment regimens (sequential, quadruple, concomitant and hybrid therapies) and various combinations of new and old antibiotics aimed at eradicating the organism more effectively are increasing in popularity.

Innovations and breakthroughs

In this study, the authors provide further evidence of the efficacy and tolerability of quadruple eradication therapy for *H. pylori* infection.

Applications

Empirical therapy can be employed to treat *H. pylori* infection if antimicrobial sensitivity data are unavailable.

Terminology

A 7-d quadruple therapy consisting of rabeprazole (20 mg *bid*) amoxicillin (1.0 g, *bid*) levofloxacin (0.2 g, *bid*) and furazolidone (0.1 g, *bid*), has high efficacy in the treatment of *H. pylori* infection with an eradication rate of 86% by intention-to-treat analysis and 94% by per-protocol analysis.

Peer review

This is a nice study, particularly for populations who have a relative high prevalence of CYP2C19 polymorphism. The article represents an important contribution to the treatment of *H. pylori*.

REFERENCES

1 **Cheng H**, Hu FL, Li J. Influence of resistance of Helicobacter pylori to antibiotics on the Helicobacter pylori eradication regimens. *Zhonghua Yixue Zazhi* 2006; **86**: 2679-2682

2 **Fukase K**, Kato M, Kikuchi S, Inoue K, Uemura N, Okamoto S, Terao S, Amagai K, Hayashi S, Asaka M. Effect of eradication of Helicobacter pylori on incidence of metachronous gastric carcinoma after endoscopic resection of early gastric cancer: an open-label, randomised controlled trial. *Lancet* 2008; **372**: 392-397 [PMID: 18675689 DOI: 10.1016/S0140-6736(08)61158-9]

3 **Malfertheiner P**, Chan FK, McColl KE. Peptic ulcer disease. *Lancet* 2009; **374**: 1449-1461 [PMID: 19683340 DOI: 10.1016/S0140-6736(09)60938-7]

4 **Stathis A**, Bertoni F, Zucca E. Treatment of gastric marginal zone lymphoma of MALT type. *Expert Opin Pharmacother* 2010; **11**: 2141-2152 [PMID: 20586708 DOI: 10.1517/14656566.2010.497141]

5 **Malfertheiner P**, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D, Hunt R, Rokkas T, Vakil N, Kuipers EJ. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. *Gut* 2007; **56**: 772-781 [PMID: 17170018 DOI: 10.1036/Gut.2006.101634]

6 **Fuccio L**, Zagari RM, Eusebi LH, Laterza L, Cennamo V, Ceroni L, Grilli D, Bazzoli F. Meta-analysis: can Helicobacter pylori eradication treatment reduce the risk for gastric cancer? *Ann Intern Med* 2009; **151**: 121-128 [PMID: 19620164]

7 **Marshall BJ**, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984; **1**: 1311-1315 [PMID: 6145023 DOI: 10.1016/s0140-6736(84)9186-6]

8 **Mattar R**, Marques SB, Monteiro Mdo S, Dos Santos AF, Iriya K, Carrilho FJ. Helicobacter pylori cag pathogenicity island genes: clinical relevance for peptic ulcer disease development in Brazil. *J Med Microbiol* 2007; **56**: 9-14 [PMID: 17172510 DOI: 10.1099/jmm.0.46824-0]

9 **Mattar R**, Monteiro MS, Marques SB, Zilberstein B, Hashimoto CL, Carrilho FJ. Association of LEC and tnpA Helicobacter pylori genes with gastric cancer in a Brazilian population. *Infect Agent Cancer* 2010; **5**: 1 [PMID: 20205796 DOI: 10.1186/1750-9378-5-1]

10 **Ahmed KS**, Khan AA, Ahmed I, Tiwari SK, Habeeb A, Ahi JD, Abid Z, Ahmed N, Habibullah CM. Impact of household hygiene and water source on the prevalence and transmission of Helicobacter pylori: a South Indian perspective. *Singapore Med J* 2007; **48**: 543-549 [PMID: 17538754]

11 **Calvet X**, Sánchez-Delgado J, Montserrat A, Lario S, Ramírez-Lázaro MJ, Quesada M, Casalots A, Suárez D, Campo R, Brullet E, Junquera F, Sanfeliu I, Segura F. Accuracy of diagnostic tests for Helicobacter pylori: a reappraisal. *Clin Infect Dis* 2009; **48**: 1385-1391 [PMID: 19368506]

12 **Al-Humayed SM**, Ahmed ME, Bello CS, Tayyar MA. Comparison of 4 laboratory methods for detection of Helicobacter pylori. *Saudi Med J* 2008; **29**: 530-532 [PMID: 18382793]

13 **Kalem F**, Ozdemir M, Baysal B. [Investigation of the pres-

ence of Helicobacter pylori by different methods in patients with dyspeptic complaints]. *Mikrobiyol Bul* 2010; **44**: 29-34 [PMID: 20455396]
14 **Redéen S**, Petersson F, Törnkrantz E, Levander H, Mårdh E, Borch K. Reliability of Diagnostic Tests for Helicobacter pylori Infection. *Gastroenterol Res Pract* 2011; **2011**: 940650 [PMID: 21826138 DOI: 10.1155/2011/940650]
15 **Peng NJ**, Lai KH, Lo GH, Hsu PI. Comparison of noninvasive diagnostic tests for Helicobacter pylori infection. *Med Princ Pract* 2009; **18**: 57-61 [PMID: 19060493 DOI: 10.1159/000163048]
16 **Vaira D**, Gatta L, Ricci C, Miglioli M. Review article: diagnosis of Helicobacter pylori infection. *Aliment Pharmacol Ther* 2002; **16** Suppl 1: 16-23 [PMID: 11849123 DOI: 10.146/j/1365-2036.2002.0160s1016.x]
17 **Jonaitis LV**, Kiudelis G, Kupcinskas L. Evaluation of a novel 14C-urea breath test "Heliprobe" in diagnosis of Helicobacter pylori infection. *Medicina* (Kaunas) 2007; **43**: 32-35 [PMID: 17297281]
18 **Wu W**, Yang Y, Sun G. Recent Insights into Antibiotic Resistance in Helicobacter pylori Eradication. *Gastroenterol Res Pract* 2012; **2012**: 723183 [PMID: 22829809 DOI: 10.1155/2012/723183]
19 **Snyder J**. Multiple forms of exploitation in international research: the need for multiple standards of fairness. *Am J Bioeth* 2010; **10**: 40-41 [PMID: 20526969 DOI: 10.1080/15265161.2010.482631]
20 **Gisbert JP**, Pajares R, Pajares JM. Evolution of Helicobacter pylori therapy from a meta-analytical perspective. *Helicobacter* 2007; **12** Suppl 2: 50-58 [PMID: 17991177 DOI: 10.1111/j.1523-5378.2007.00576.x]
21 **Miura M**, Satoh S, Tada H, Habuchi T, Suzuki T. Stereoselective metabolism of rabeprazole-thioether to rabeprazole by human liver microsomes. *Eur J Clin Pharmacol* 2006; **62**: 113-117 [PMID: 16389533]
22 **Mégraud F**. H pylori antibiotic resistance: prevalence, importance, and advances in testing. *Gut* 2004; **53**: 1374-1384 [PMID: 15306603 DOI: 10.1136/gut.2003.022111]
23 **The Chinese Medical Association Helicobacter pylori Group of Digestive Diseases Branch/National Helicobacter pylori Research Group**. H. pylori drug situation in China as well as resistant to treatment. *Gastroenterology* 2007; **12**: 525-530
24 **Sheu BS**, Kao AW, Cheng HC, Hunag SF, Chen TW, Lu CC, Wu JJ. Esomeprazole 40 mg twice daily in triple therapy and the efficacy of Helicobacter pylori eradication related to CYP2C19 metabolism. *Aliment Pharmacol Ther* 2005; **21**: 283-288 [PMID: 15691303 DOI: 10.1111/j.1365-2036.2005.02281.x]
25 **Schwab M**, Schaeffeler E, Klotz U, Treiber G. CYP2C19 polymorphism is a major predictor of treatment failure in white patients by use of lansoprazole-based quadruple therapy for eradication of Helicobacter pylori. *Clin Pharmacol Ther* 2004; **76**: 201-209 [PMID: 15371981 DOI: 10.1016/j.clpt.2004.05.002]
26 **Besancon M**, Simon A, Sachs G, Shin JM. Sites of reaction of the gastric H,K-ATPase with extracytoplasmic thiol reagents. *J Biol Chem* 1997; **272**: 22438-22446 [PMID: 9278394 DOI: 10.1074/jbc.272.36.22438]
27 **Hang HH**. Comparative study of H.pylori eradication rate between moxifloxacin and amoxicillin. *Haixia Yaoxve* 2009; **21**: 115-116
28 **Branca G**, Spanu T, Cammarota G, Schito AM, Gasbarrini A, Gasbarrini GB, Fadda G. High levels of dual resistance to clarithromycin and metronidazole and in vitro activity of levofloxacin against Helicobacter pylori isolates from patients after failure of therapy. *Int J Antimicrob Agents* 2004; **24**: 433-438 [PMID: 15519473 DOI: 10.1016/j.ijantimicag.2004.02.032]

P- Reviewers Gardner JD, Matsushima M, McRorie JW
S- Editor Song XX **L- Editor** A **E- Editor** Li JY



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i6.936

World J Gastroenterol 2013 February 14; 19(6): 936-945
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

META-ANALYSIS

Pre-existing diabetes mellitus increases the risk of gastric cancer: A meta-analysis

Jae Moon Yoon, Ki Young Son, Chun Sick Eom, Daniel Durrance, Sang Min Park

Jae Moon Yoon, Sang Min Park, Department of Family Medicine, Seoul National University Hospital, Seoul National University College of Medicine, Seoul 110-744, South Korea
Ki Young Son, Center for Health Promotion, Seoul National University Hospital, Seoul 110-744, South Korea
Chun Sick Eom, Department of Family Medicine, Hallym University, Chuncheon Sacred Heart Hospital, ChunCheon 200-704, South Korea
Daniel Durrance, Family Medicine, Graduate School of Korea University College of Medicine, Seoul 110-799, South Korea
Author contributions: Yoon JM and Son KY have contributed equally to this article; Yoon JM and Son KY designed study, analyzed data and wrote manuscript; Eom CS contributed discussion and wrote manuscript; Durrance D edited manuscript; Park SM designed study, contributed discussion and edited manuscript.
Supported by Grant from the Seoul National University Hospital Research Fund, No. 04-2011-0540
Correspondence to: Dr. Sang Min Park, Department of Family Medicine, Seoul National University Hospital, Seoul National University College of Medicine, 101 Daehak-ro, Jongno-gu, Seoul 110-744, South Korea. sangmin.park.snuh@gmail.com
Telephone: +82-2-20723331 Fax: +82-2-7663276
Received: August 18, 2012 Revised: October 22, 2012
Accepted: October 30, 2012
Published online: February 14, 2013

Abstract

AIM: To systematically assess the association between diabetes and incidence of gastric cancer.

METHODS: We searched MedLine (PubMed), EMBASE, and the Cochrane Library without any limitations with respect to publication date or language, we also searched the references of qualifying articles. Case-control studies and cohort studies comparing the risk of gastric cancer between diabetic patients and control subjects were included. We excluded studies reporting only standardized incidence ratios without control groups and those that investigated only mortality but not incidence. Seventeen studies met our criteria, and the qualities of these studies were assessed using the Newcastle-Ottawa Quality Assessment Scale. We performed a meta-analysis of pre-existing diabetes and gastric cancer incidence using the DerSimonian-Laird method for random-effects. For subgroup analyses, we separated the studies by study type, region, sex and method to determine confounding factors and reliability. We also conducted subgroup analyses to examine the effects of smoking, *Helicobacter pylori* (*H. pylori*) infection, and cancer site. Publication bias was evaluated using Begg's test.

RESULTS: A random-effects model meta-analysis showed an increased gastric cancer risk in diabetic patients [relative risk (RR) = 1.19; 95%CI: 1.08-1.31]. Subgroup analyses indicated that this result persisted in cohort studies (RR = 1.20; 95%CI: 1.08-1.34), in studies on populations of both Western (RR = 1.18; 95%CI: 1.03-1.36) and Eastern countries (RR = 1.19; 95%CI: 1.02-1.38), in a female subgroup (RR=1.24; 95%CI: 1.01-1.52), and in highly qualified studies (RR = 1.17; 95%CI: 1.05-1.31). Moreover, these results persisted when the analysis was confined to studies adjusted for well-known gastric cancer risk factors such as smoking (RR = 1.17; 95%CI: 1.01-1.34) and *H. pylori* infection (RR = 2.35; 95%CI: 1.24-4.46).

CONCLUSION: Pre-existing diabetes mellitus may increase the risk of gastric cancer by approximately 19%. This effect seems to be unrelated to geographical region.

© 2013 Baishideng. All rights reserved.

Key words: Disease association; Diabetes mellitus; Gastric cancer; Incidence; Risk; Meta-analysis

Yoon JM, Son KY, Eom CS, Durrance D, Park SM. Pre-existing diabetes mellitus increases the risk of gastric cancer: A meta-analysis. *World J Gastroenterol* 2013; 19(6): 936-945 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i6/936.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i6.936

INTRODUCTION

Diabetes mellitus is currently an epidemic. Approximately 171 million individuals worldwide had diabetes mellitus in 2000, and it is estimated that 366 million people will have diabetes by 2030, which corresponds to nearly 4.5% of the global population[1]. The estimated prevalence of diabetes among adults in the United States currently ranges from 5.9% to 12.4% (median 8.3%)[2], and some cancers, such as colorectal, endometrial, breast, liver, and pancreatic cancer, are reportedly more prevalent in diabetes patients[3-7]. Diabetes mellitus has also been associated with an increased risk of long-term and all-cause mortality in cancer patients[8].

Although the incidence and associated mortality of gastric cancer is decreasing, it remains the fourth most common cancer and the second leading cause of cancer-related mortality worldwide[9]. Despite the success in gastric cancer control, the persistence of the public health burden of gastric cancer indicates the need to expand efforts to identify and address the individual and social determinants of the disease. The public health significance of a potential causal link between diabetes mellitus and gastric cancer highlights the need for a systematic assessment of the association between these 2 diseases.

Therefore, several systematic reviews have recently been performed to examine the above mentioned associations. One review suggested that diabetic individuals have an increased risk of gastric cancer[10]. However, this review had some limitations as it included studies without control groups[11-14] and excluded appropriate studies[15,16]. Two other reviews showing inconclusive results[17,18] did not include any recent studies[19,20], and did not differentiate between "incidence" and "mortality" in terms of describing the risk[17]. Such methodological weaknesses of the previous meta-analysis made it difficult to understand the association between preexisting diabetes mellitus and the risk of gastric cancer.

In the present study, we aimed to clarify the association between diabetes and gastric cancer through an extensive search of the literature, which we reviewed using strict criteria.

MATERIALS AND METHODS

Methods

The procedures performed in this meta-analysis are in accordance with recent guidelines for the reporting of meta-analysis (PRISMA guidelines).

Data sources and searches

We conducted a systematic search of electronic databases and the bibliographies of all eligible studies to identify all relevant studies. We initiated the search on February 7, 2012 without any limitations with respect to publication date or language. The electronic databases searched included MedLine (PubMed), EMBASE, and the Cochrane Library. The search strategy included terms for diabetes (glucose, diabetes, or hyperglycemia), gastric cancer (stomach cancer, gastric cancer, stomach malignant neoplasm, or gastric malignant neoplasm), and risk (incidence, prevalence, or risk). We also searched the references of included articles.

Study selection

Case-control studies, cohort studies, and randomized controlled trials comparing the risk of gastric cancer between diabetic patients and control subjects were eligible for inclusion. We included studies evaluating self-reported diabetes, registered diabetes, and high HbA1c and blood glucose levels. To be included in our meta-analysis, articles had to contain both of the following: (1) a risk estimate (hazard ratio, relative risk, or odds ratio relating preexisting diabetes to subsequent occurrences of gastric cancer); and (2) an estimate of precision (standard error or 95%CI). We also included articles that failed to report precision directly, but from which we could reconstruct a precision estimate using the data described[21,22]. We excluded studies reporting only standardized incidence ratios without control groups. We also excluded studies that investigated only mortality without incidence and studies in which the classification of the subjects' diabetes status (diabetic or non-diabetic) was not possible due to insufficient ranges in blood glucose levels. Studies were excluded if either the abstract[23] or the full text[24] was unavailable after author contact was made.

Data extraction and quality assessment

The titles, abstracts, and full articles were reviewed independently by two authors (Yoon JM and Son KY). Yoon JM performed a full abstraction of the data, and Son KY verified the accuracy. Disagreements were resolved by discussion, consensus, and arbitration by a third author (Park SM). Abstracted data included type of study, study population characteristics, criteria for diabetes mellitus or hyperglycemia, duration of follow-up, incidence of cancer, adjustment variables, and study quality. Quality was assessed using the Newcastle-Ottawa Quality Assessment Scale (NOS) for case-control or cohort studies.

Statistical analysis

To avoid overlapping patient populations, we compared data sources and geographic locations. If a patient population was found to overlap, we included the article with the most comprehensive population or the most adjusted risk estimate associated with preexisting diabetes. If an article reported estimates separately by sex[15,19,25-29] or by different cohorts[16], we regarded the study as 2 independent studies. If an article did not report the risk ratio and confidence interval, we estimated the risk using Fisher's exact test without adjustment[20-22], and the estimates were rounded off to the nearest hundredth. In case-control studies, odds ratios were regarded as relative risks because of the low prevalence of gastric cancer[9,30,31].

For the meta-analysis, we calculated pooled estimates for all the studies. For the subgroup analyses, we separated studies by study type, region, sex, and method to determine the confounding factors and reliability. We also

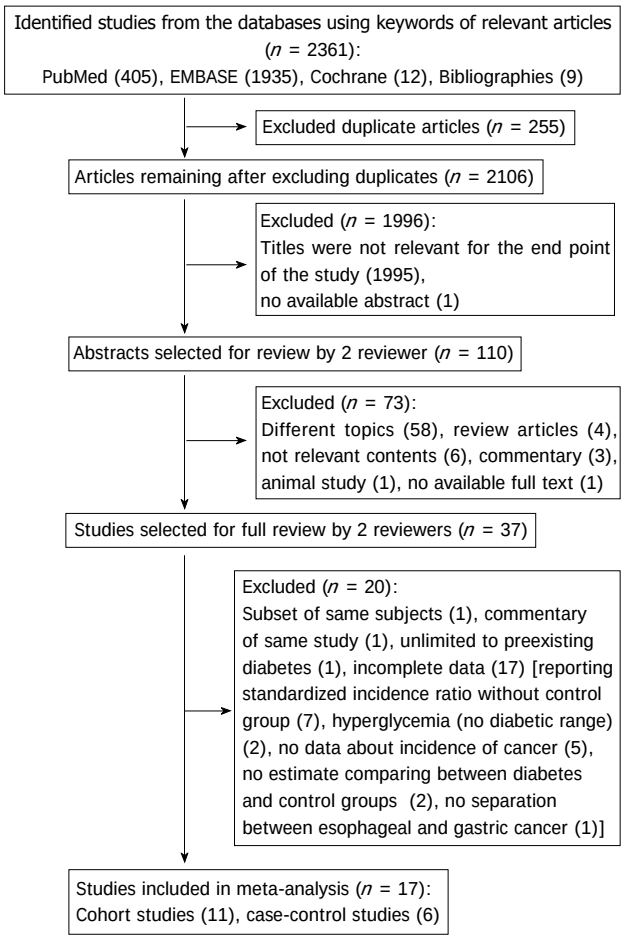


Figure 1 Flow diagram of studies identified and selected.

conducted subgroup analyses to examine the effects of smoking, *Helicobacter pylori* (*H. pylori*) infection, and cancer site, which are major factors influencing the risk of gastric cancer[32-34]. We calculated all the pooled estimates using the DerSimonian-Laird method for random effects. We also reported I^2 values for heterogeneity assessment. Publication bias was evaluated using Begg's test. All analyses were conducted using Stata software(version12.1, StataCorp, United States).

RESULTS

The systematic literature search identified 2361 relevant references (Figure 1). After screening the titles, we excluded 255 duplicated and 1996 non-relevant studies. By reviewing the abstracts, we further excluded 73 articles. The full texts of the 37 remaining articles were retrieved for formal review. After independent review, 20 studies were excluded; 2 studies[35,36] used the same populations as another trial[37], and 1 did not limit the exposure group to individuals with preexisting diabetes[38].

Seventeen studies reported only limited data (uncertain diabetes diagnosis[39,40], usage of a standardized incidence ratio[11,13,14,41-44], lack of an acceptable control group[45,46], reporting of only mortality data[47-51], and uncertain gastric cancer diagnosis[52]). Table 1 provides the details of the 17 studies that met our predefined inclusion and exclusion criteria. In total, 11 cohort[15,16,19,22,26-28,37,53-55] and 6 case-control[20,21,25,29,56,57] studies were used. We were unable to find any suitable randomized control trials.

Description of studies

The 17 articles included in our analysis were heterogeneous in many respects. Geographically, 6 studies were conducted in East Asia, 1 in West Asia, 5 in North America, and 5 in Europe. According to the cohort studies, the prevalence of diabetes varied from 3.0% to 13.2%. Four studies used measurement criteria to define diabetes mellitus, and 2 studies differentiated between gastric cardia and non-cardia cancer. The reporting of age and follow-up time varied widely. In 13 studies, a time interval of more than 1 year between the diagnoses of diabetes and cancer was required in order to minimize reverse causality, although 2 studies showed only the estimates without time intervals[16,54]. Quality varied from 4 to 9 stars according to the NOS, but 3 studies were downgraded because we used recalculated estimates without adjusting for pooled analysis[20-22]. After downgrading, the mean NOS score was 7.3. We therefore conducted subgroup analysis according to methodological quality (with "high-quality" defined as a score of $\geqslant 8$).

Fifteen studies included both sexes and 10 reported data separated by sex. In one of the latter, fewer than 5 female patients were diagnosed with both diabetes mellitus and subsequent gastric cancer[15]; the estimate was therefore not reported. In total, we found 28 independent groups separated by sex and cohort, and we yielded 27 eligible estimates. Only 2 studies described the specific risk ratios of cardia and non-cardia gastric cancers[20,22].

Overall

As shown in Figure 2, diabetes mellitus was associated with a significantly increased risk of gastric cancer in all included studies using random effects model analysis [relative risk (RR) = 1.19; 95%CI: 1.08-1.31; I^2 = 70.7%; 95%CI: 56.8%-80.2%].

Subgroup meta-analysis

We examined methodological differences when determining the quality of the studies. According to Figure 2, subgroup meta-analysis by study design revealed significant associations in cohort studies (RR = 1.20; 95%CI: 1.08-1.34), while the results of case-control studies were not statistically significant. Positive associations were observed in more reliable subgroup studies using the registration record or laboratory measurements as diabetes criteria and studies that were highly graded by NOS. A subgroup meta-analysis of studies designed to minimize reverse causality, including the shortest cancer-free interval, indicated that diabetes seemed to increase subsequent gastric cancer (Table 2).

Regional and gender-based differences are presented in Table 2. East Asian (RR = 1.19; 95%CI: 1.02-1.38) and Western studies (RR = 1.18; 95%CI: 1.03-1.36) showed

Table 1 Characteristics of 15 studies included in the meta-analysis of the gastric cancer risk of preexisting diabetes

Study	Study type	Country	DM criteria	Age (yr)	Follow up (yr)	Least interval[1] (yr)	Sex	DM prevalence	RR or OR (95%CI)	Adjustment variables/matched variable of selection	NOS
Atchison *et al*[53]	CS	United States (veterans)	Registration	18-100	27	1	Male	13.2%	0.95 (0.89-1.02)	Age, time, latency, race, number of visits, diagnoses of alcohol-related conditions, obesity and COPD	8
Carstensen *et al*[19]	CS	Denmark	Registration	No limit	15	Not mentioned	Male Female		1.28 (1.15-1.43) 1.34 (1.14-1.58)	Age, calendar time, date of birth	8
Jiang *et al*[20]	CC	United States	Self reported	30-74		1	Both		1.51 (1.10-2.07)[2]	Age, sex, race, education, birth place, smoking and BMI	7 (5)[5]
Lin *et al*[22]	CS	United States	Self reported	50-71	10	2	Male Female	9.9% 7.2%	1.52 (1.21-1.92)[2] 1.62 (0.98-2.68)[2]	Age, calories, alcohol consumption, smoking, fruit consumption, vegetable consumption, ethnicity, education, and physical activity	8 (6)[5]
Wotton *et al*[16] ORLS1 ORLS2	CS	England	Registration	⩾ 30	35 9	0	Both	5.5% 4.0%	1.11 (0.89-1.37) 2.05 (1.30-3.10)	Sex, age in 5-year bands, time and district of residence	8
Chodick *et al*[54]	CS	Israel	Registration	⩾ 21	8	0	Male Female		1.44 (0.98-2.11) 0.99 (0.55-1.80)	Age, region, SES level, use of healthcare, BMI, and history of CVD/ matched for age, sex	8
Ikeda *et al*[37]	CS	Japan	HbA1c (5.0%-5.9% *vs* 7.0%)	⩾ 40	14	Not mentioned	Both	3.9%	2.69 (1.24-5.85)	Age, sex, *H. pylori*, history of peptic ulcer, BMI, TC, alcohol intake, smoking, dietary factor[4]	8
Ogunleye *et al*[55]	CS	Scotland	Registration (only type 2 DM)	No limit	11	1	Both		0.77 (0.36-1.66)	Deprivation deciles of Carstairs/matched for age, sex and doctor practice	8
Kuriki *et al*[29]	CC	Japan	Self reported	40-80		Not mentioned	Male Female		1.16 (0.93-1.44) 1.70 (1.16-2.48)	Age, BMI, drinking, smoking, exercise, bowel movement, FHx of cancer and DM, dietary restriction, vegetable intake, greasy food, snack	6
Inoue *et al*[27]	CS	Japan	Self reported	40-69	13	5	Male Female	6.7% 3.0%	1.09 (0.79-1.5) 1.92 (1.06-3.47)	Age, study area, history of CVD and IHD, smoking, BMI, physical activity, alcohol, vegetable, coffee	9
Jun *et al*[57]	NCC	Korea	FBS (< 99 mg/dL *vs* ⩾ 126 mg/dL)	⩾ 35	Case 2.4/ control 6.5 h	2	Both		1.77 (0.57-5.45)	*H. pylori*, smoking, drinking, and education level/matched on age, sex, year and area of enrollment, and follow-up duration	9
Khan *et al*[28]	CS	Japan	Self reported	40-79	19	2	Male Female	7.5% 4.6%	0.72 (0.40-1.09) 0.26 (0.08-0.82)	Age, BMI, smoking, drinking	8
Rapp *et al*[15]	CS	Austria	FBS (4.2-5.2 mmol/ L *vs* 7.0 mmol/L)	> 19	14	1	Male Female	3.9% 3.0%	0.84 (0.38-1.87) -[3]	Age, smoking, occupation, BMI	8
Rousseau *et al*[56]	CC	Canada	Self reported	35-70		2	Male		1.0 (0.5-1.8)	Age, income, education, ethnicity, proxy status, BMI, smoking, beta-carotene, alcohol	7
Jee *et al*[26]	CS	South Korea	FBS (< 90 mg/dL *vs* ⩾ 126 mg/dL) or DM medication	30-95	10	1	Male Female	5.1% 4.5%	1.11 (1.04-1.2) 1.15 (0.99-1.34)	Age, age squared, smoking, alcohol	8
La Vecchia *et al*[25]	CC	Italy	Self reported	⩽ 75		Not mentioned	Male Female		0.6 (0.4-1.0) 0.7 (0.4-1.2)	Age	6
O'Mara *et al*[21]	CC	USA (only white)	Self reported	30-89		1	Male Female		0.91 (0.28-2.27)[2] 1.57 (0.48-3.94)	Age	4 (3)[5]

[1]Interval between diabetes mellitus (DM) and gastric cancer to be selected as population; [2]Calculated from table without adjustment by Fisher's exact test; [3]No estimate was reported due to less than 5 cases in DM patients; [4]Energy, fat, salt, vitamin, dietary fibers; [5]The first numbers are Newcastle-Ottawa Quality Assessment Scale (NOS) of original studies. The second numbers in brackets are downgraded NOS because we calculated new estimates without adjustment. Subgroup analysis were done according to the second numbers. CS: Cohort study; CC: Case control study; NCC: Nested case-control study; BMI: Body mass index; FBS: Fasting blood glucose; FHx: Family history; TC: Total cholesterol; CVD: Cardiovascular disease; IHD: Ischemic heart disease; ORLS: Oxford Record Linkage Study; *H. pylori*: *Helicobacter pylori*; COPD: Chronic obstructive pulmonary disease.

similar increased risks. In the sexual subgroup, only women exhibited statistical significance (RR = 1.24; 95%CI: 1.01-1.52).

Pooled estimates adjusted for well-known gastric cancer risk factors (cigarette smoking and *H. pylori* infection) were also significant. The pooled estimate adjusted for ciga-

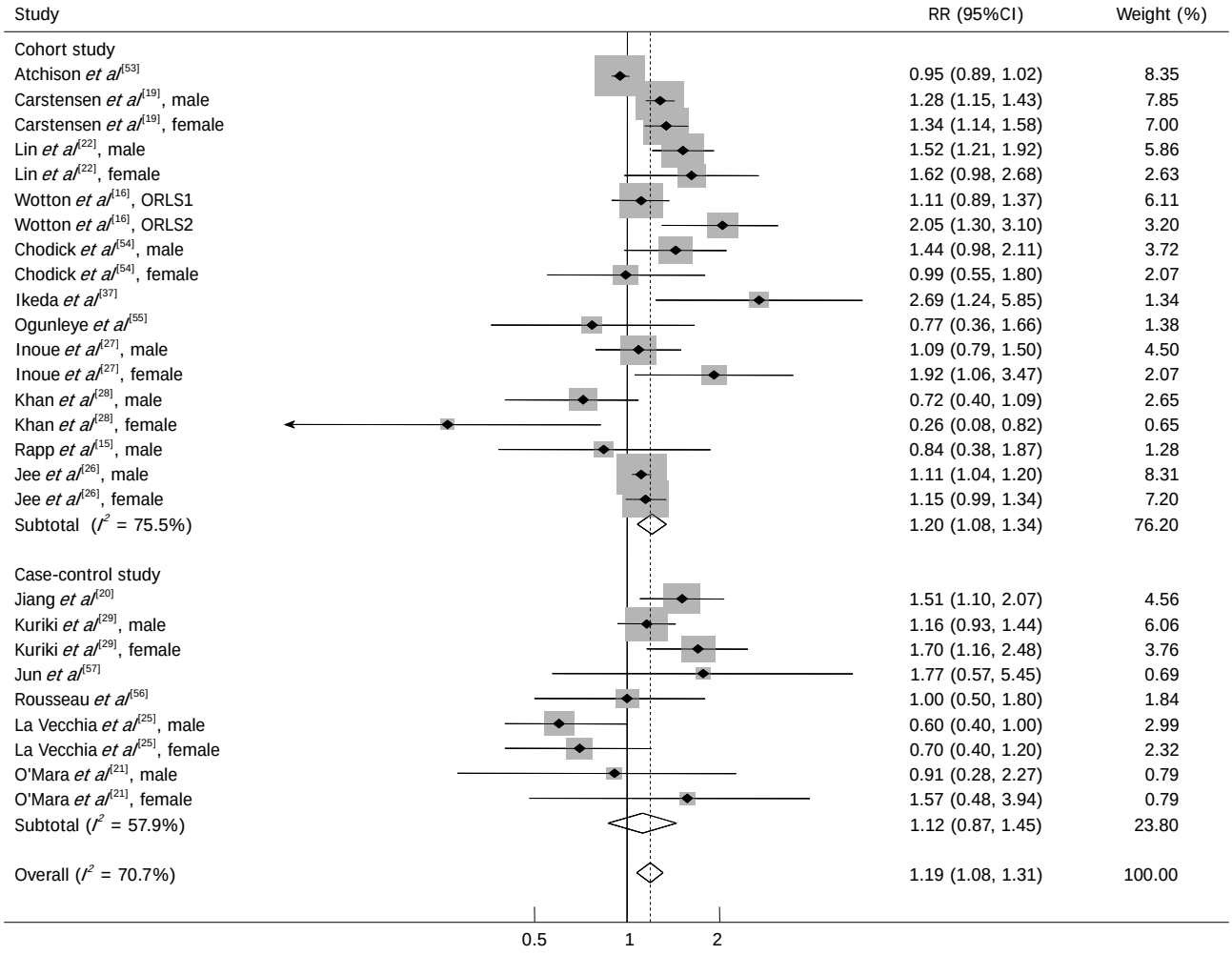


Figure 2 Meta-analysis and pooled relative risk of gastric cancer comparing non-cancer population with and without preexisting diabetes mellitus by random effect model. ORLS: Oxford Record Linkage Study.

rette smoking status was similar to the overall results (RR = 1.17; 95%CI: 1.01-1.34). The pooled estimate adjusted for *H. pylori* infection was greater than the overall estimate (RR = 2.35; 95%CI: 1.24-4.46). Nevertheless, subgroup analyses on either cardia or noncardia cancer did not produce significant results.

Publication bias

Begg's test indicated no publication bias in our analysis (Figure 3).

DISCUSSION

Summary of results

We found a significant association between preexisting diabetes and gastric cancer incidence. In diabetic patients, we found that the risk of subsequent gastric cancer incidence was increased by approximately 19%, and this effect was significant in women. The subgroups of cohort, Western and Eastern studies, showed similar results. These results persisted in studies using self-reported criteria and those adjusted for known risk factors such as cigarette smoking and *H. pylori* infection.

Explanations

We observed some discrepancy between our analysis and previous meta-analysis. Three previous meta-analysis have explored the association between diabetes and gastric cancer incidence. One of these, a meta-analysis of cohort studies[18], produced a misleading result due to the exclusion of appropriate cohort studies[16,19,54] and the inclusion of a study using a glucose level that was not clearly defined and an unrepresentative range of diabetes mellitus[40]. The study also presented an incomprehensible method for summarizing 2 estimates of cardia and noncardia cancer from the same control group. A different study[17] used the term "risk" without a distinction between incidence and mortality. This methodological weakness made the result heterogeneous and biologically implausible. Another meta-analysis[10] also excluded several recent studies[15,16,19,20] and included studies reporting a standardized incidence ratio without quality assessment.

To supplement the previous insufficient meta-analyses, we restricted inclusion in our analysis to studies reporting a risk ratio compared to a control group, and we assessed the quality of these studies using various criteria. As a result, we found that diabetes and the risk of gastric

Table 2 Preexisting diabetes and risk of gastric cancer by subgroup meta-analysis using random effect model

	Studies (*n*)	RR (95%CI)	I^2 % (95%CI)
Overall	27	1.19 (1.08-1.31)	70.7 (56.8-80.2)
Countries			
East Asian countries[1]	10	1.19 (1.02-1.38)	60.1 (20.0-80.1)
Western countries[2]	17	1.18 (1.03-1.36)	75.6 (61.0-84.8)
Study type			
Cohort study	18	1.20 (1.08-1.34)	75.5 (61.2-84.5)
Case control study	9	1.12 (0.87-1.45)	57.9 (11.8-79.9)
Sex			
Male	12	1.10 (0.97-1.24)	74.9 (55.7-85.8)
Female	9	1.24 (1.01-1.52)	58.9 (14.1-80.3)
DM criteria			
Registration	8	1.21 (1.02-1.44)	83.0 (67.8-91.0)
Measured[3]	5	1.15 (1.01-1.32)	35.5 (0.0-75.8)
Self report	14	1.13 (0.93-1.39)	65.2 (38.6-80.3)
Interval[4]			
One and more years	15	1.15 (1.02-1.31)	67.5 (44.3-81.0)
Quality (NOS)			
High quality (8 and more)	17	1.17 (1.05-1.31)	73.4 (57.0-83.6)
Low quality (7 and less)	10	1.20 (0.97-1.49)	61.2 (22.6-80.5)
Adjustment for risk factor			
Smoking[5]	12	1.17 (1.01-1.34)	52.6 (8.7-75.4)
H. pylori[5]	2	2.35 (1.24-4.46)	0.0 (UC)
Cancer site			
Cardia cancer	2	1.39 (0.72-2.69)	82.3 (UC)
Noncardia cancer	2	1.19 (0.80-1.77)	59.6 (UC)

[1]Japan, South Korea; [2]United States, Canada, Europe and Israel; [3]Using fasting blood glucose or HbA1c as criteria; [4]The least cancer-free time of subject according to study design; [5]The subgroup of studies designed to include the risk factor as adjustment variables. RR: Relative risk; UC: Unable to calculate due to low degree of freedom; NOS: Newcastle-Ottawa Quality Assessment Scale; *H. pylori*: *Helicobacter pylori*.

cancer were positively associated with each other. This positive association may be reliable based on consistent results from well-designed studies including cohort studies, studies of high quality based on assessment with the NOS, studies examining the time interval between onset of diabetes and subsequent gastric cancer, and studies using objective diabetic criteria (diabetes registry or laboratory measurements) that were adjusted based on important factors (smoking or *H. pylori* infection). Although a time interval of 1 year between diabetes and gastric cancer may be insufficient evidence of a causal relationship, we were able to minimize the reverse causality.

Regarding biological plausibility, the results of this study are consistent with previous studies in which preexisting diabetes was associated with an increased risk of a variety of cancers. The oncogenic properties of diabetes have been well documented. The increased insulin concentration in early diabetes can stimulate cell proliferation through activation of the insulin receptor or insulin-like growth factor-I receptor (IGF-IR) and the inhibition of IGF binding proteins, which may result in increased free and bioavailable IGF-I[58]. Additionally, a high level of serum IGF-I has been demonstrated to increase the risk for development of several carcinomas including gastrointestinal carcinomas[59]. Exogenous IGFs stimulate the proliferation of gastric cancer cells, while the blocking of

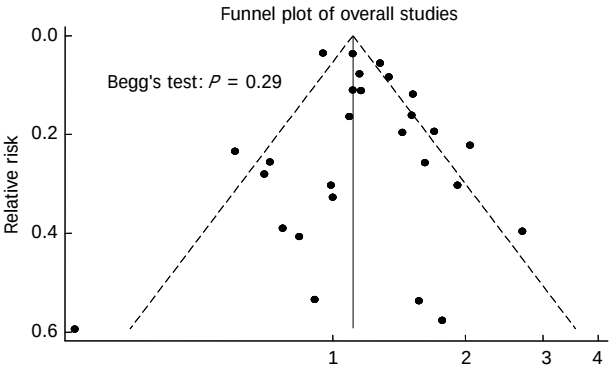


Figure 3 Funnel plots for publication bias.

IGF-IR inhibits tumor development[60,61].

Vascular endothelial growth factor (VEGF), the levels of which are increased in the blood of diabetic patients[62], is another cytokine that is related to the oncogenic properties of diabetes. VEGF is correlated with tumor vascularity and the frequency of hepatic metastasis, and it induces the proliferation and dilation of lymphatics culminating in node metastasis[63-65]. Moreover, the IGF/IGF-IR axis has also been shown to interact with the VEGF/VEGFR system in various tumors including gastrointestinal malignancies[66,67].

DNA damage in diabetic patients is another potential mechanism of oncogenesis. The increased production of reactive oxygen species could result in greater oxidative damage to DNA[68]. Furthermore, in an experimental study, high blood glucose levels were shown to directly induce DNA damage[69].

While we found that the association between diabetes and gastric cancer persisted irrespective of *H. pylori* infection, it nevertheless seems probable that diabetes and *H. pylori* infection, which is a known risk factor for gastric cancer, work synergistically to increase the risk of gastric cancer. Indeed, a previous cohort study in Japan demonstrated that a high HbA1c level and a concomitant *H. pylori* infection increased the gastric cancer risk synergistically[37]. It is possible that reactive oxygen-dependent DNA damage enhances the modifying effect of *H. pylori* on epithelial cell proliferation.

To confirm this synergistic effect, we also performed subgroup analyses by cancer site. Although noncardia gastric cancers were known to be strongly associated with *H. pylori* infection[70,71], we failed to show a similar relationship between diabetes mellitus and specific sites of gastric cancer. As further studies dealing with site-specific data are performed, a reassessment will be required.

Potential confounders

A positive association between diabetes and gastric cancer incidence was observed among women but not among men. These results must be interpreted carefully as there were only a few studies differentiating men and women. Sex hormones could represent one possible reason for this difference. In breast cancer, it is well established that

estrogen and IGF-1 interactively affect cell proliferation[72]. Gastrointestinal tissues, whether normal or cancerous, contain estrogen receptor β, and estrogen can bind to them[73,74]. Through an undefined mechanism, estrogen levels in diabetic women could influence or stimulate the proliferation of gastric cancer cells.

This gender-based difference could also be due to disparate methods of diabetes intervention. Metformin[46,75], aspirin[76,77], and statins[78] are known to protect against a number of cancers, while insulin is reported to increase the risk of several cancers[75]. Although gender differences in this matter were not explored in many previous studies, clues were obtained from the following studies: One study suggested that gender differences in adherence to diabetes management were minimal[79]. However, an Italian study reported that diabetic women were more likely to use insulin than men[80]. According to an American study, men use statins more frequently in type 2 diabetes mellitus[81]. These factors, however small, might contribute to the above-mentioned gender differences.

Limitations

Our study has several limitations. First, the studies we examined were highly heterogeneous. In particular, diabetes mellitus was defined by various methods and cut-off values. Diabetic prevalence and subject age also differed. Second, dietary food factors such as salt, nitrite[82-84], and fresh vegetable[85] intake could not be considered in the analysis despite being potential confounders. Finally, the selected studies contained no details regarding diabetes interventions that were sufficient to adjust for the effects of diabetes treatments.

In conclusion, despite the limitations, our meta-analysis suggests that preexisting diabetes mellitus may increase the risk of gastric cancer. Further prospective studies assessing confounders such as diabetes interventions are needed to specifically test the effects of diabetes mellitus on gastric cancer risk.

COMMENTS

Background

The burden of diabetes mellitus is increasing globally. Gastric cancer is the fourth most common cancer and the second leading cause of cancer-related mortality worldwide. Although the association between diabetes mellitus and a variety of cancers is well established, the association between diabetes mellitus and gastric cancer is currently unclear.

Research frontiers

Over the past 3 decades, many studies have been performed to understand the associations between preexisting diabetes mellitus and subsequent gastric cancer incidence. Moreover, several systematic reviews were recently performed to investigate these associations. However, these reviews were methodologically insufficient and thus could not achieve a comprehensive conclusion.

Innovations and breakthroughs

Based on this meta-analysis, preexisting diabetes mellitus may increase the risk of gastric cancer by approximately 19%. Similar associations were indicated in subgroup analyses of East Asian, Western, cohort, and high-quality studies. These findings were not presented clearly in previous systematic reviews.

Applications

Diabetes mellitus appears to be either directly or indirectly associated with the risk of gastric cancer. An exploration of the mechanism for this association may help them to reduce the gastric cancer risk.

Terminology

Insulin-like growth factors (IGFs) are proteins similar to insulin. IGF is a part of the cellular communication system. Recently, IGFs were proposed to play a role in aging. Vascular endothelial growth factor (VEGF) is a protein related to angiogenesis. In diabetes mellitus, the level of VEGF in the blood is high.

Peer review

This is a well-performed meta-analysis of currently available studies on the potential role of diabetes mellitus in the development of gastric cancer. The authors found that pre-existing diabetes mellitus may increase the risk of gastric cancer by approximately 19%. This finding was found to be unrelated to geographical region. This is a good meta-analysis and the authors have included many relevant issues missed by other research groups.

REFERENCES

1 **Wild S**, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. *Diabetes Care* 2004; **27**: 1047-1053 [PMID: 15111519 DOI: 10.2337/diacare.27.10.2569-a]

2 **Hughes E**, Kilmer G, Li Y, Valluru B, Brown J, Colclough G, Geathers S, Roberts H, Elam-Evans L, Balluz L. Surveillance for certain health behaviors among states and selected local areas - United States, 2008. *MMWR Surveill Summ* 2010; **59**: 1-221 [PMID: 21150869]

3 **Friberg E**, Orsini N, Mantzoros CS, Wolk A. Diabetes mellitus and risk of endometrial cancer: a meta-analysis. *Diabetologia* 2007; **50**: 1365-1374 [PMID: 17476474 DOI: 10.1007/s00125-007-0681-5]

4 **Larsson SC**, Mantzoros CS, Wolk A. Diabetes mellitus and risk of breast cancer: a meta-analysis. *Int J Cancer* 2007; **121**: 856-862 [PMID: 17397032 DOI: 10.1002/ijc.22717]

5 **Larsson SC**, Orsini N, Wolk A. Diabetes mellitus and risk of colorectal cancer: a meta-analysis. *J Natl Cancer Inst* 2005; **97**: 1679-1687 [PMID: 16288121 DOI: 10.1093/jnci/dji375]

6 **El-Serag HB**, Hampel H, Javadi F. The association between diabetes and hepatocellular carcinoma: a systematic review of epidemiologic evidence. *Clin Gastroenterol Hepatol* 2006; **4**: 369-380 [PMID: 16527702 DOI: 10.1016/j.cgh.2005.12.007]

7 **Huxley R**, Ansary-Moghaddam A, Berrington de González A, Barzi F, Woodward M. Type-II diabetes and pancreatic cancer: a meta-analysis of 36 studies. *Br J Cancer* 2005; **92**: 2076-2083 [PMID: 15886696 DOI: 10.1038/sj.bjc.6602619]

8 **Barone BB**, Yeh HC, Snyder CF, Peairs KS, Stein KB, Derr RL, Wolff AC, Brancati FL. Long-term all-cause mortality in cancer patients with preexisting diabetes mellitus: a systematic review and meta-analysis. *JAMA* 2008; **300**: 2754-2764 [PMID: 19088353 DOI: 10.1001/jama.2008.824]

9 **Crew KD**, Neugut AI. Epidemiology of gastric cancer. *World J Gastroenterol* 2006; **12**: 354-362 [PMID: 16489633]

10 **Tian T**, Zhang LQ, Ma XH, Zhou JN, Shen J. Diabetes mellitus and incidence and mortality of gastric cancer: a meta-analysis. *Exp Clin Endocrinol Diabetes* 2012; **120**: 217-223 [PMID: 22187293 DOI: 10.1055/s-0031-1297969]

11 **Adami HO**, McLaughlin J, Ekbom A, Berne C, Silverman D, Hacker D, Persson I. Cancer risk in patients with diabetes mellitus. *Cancer Causes Control* 1991; **2**: 307-314 [PMID: 1932543 DOI: 10.1007/BF00051670]

12 **Azfar RS**, Ogunleye T, Treat J. Annular blisters on the arm. *Arch Dermatol* 2009; **145**: 497 [PMID: 19380684 DOI: 10.1001/archdermatol.2009.27]

13 **Wideroff L**, Gridley G, Mellemkjaer L, Chow WH, Linet M, Keehn S, Borch-Johnsen K, Olsen JH. Cancer incidence in a population-based cohort of patients hospitalized with diabetes mellitus in Denmark. *J Natl Cancer Inst* 1997; **89**: 1360-1365 [PMID: 9308706 DOI: 10.1093/jnci/89.18.1360]

14 **Zendehdel K**, Nyrén O, Ostenson CG, Adami HO, Ekbom A, Ye W. Cancer incidence in patients with type 1 diabetes mellitus: a population-based cohort study in Sweden. *J*

Natl Cancer Inst 2003; **95**: 1797-1800 [PMID: 14652242 DOI: 10.1093/jnci/djg105]
15 **Rapp K**, Schroeder J, Klenk J, Ulmer H, Concin H, Diem G, Oberaigner W, Weiland SK. Fasting blood glucose and cancer risk in a cohort of more than 140,000 adults in Austria. *Diabetologia* 2006; **49**: 945-952 [PMID: 16557372 DOI: 10.1007/s00125-006-0207-6]
16 **Wotton CJ**, Yeates DG, Goldacre MJ. Cancer in patients admitted to hospital with diabetes mellitus aged 30 years and over: record linkage studies. *Diabetologia* 2011; **54**: 527-534 [PMID: 21116605 DOI: 10.1007/s00125-010-1987-2]
17 **Ge Z**, Ben Q, Qian J, Wang Y, Li Y. Diabetes mellitus and risk of gastric cancer: a systematic review and meta-analysis of observational studies. *Eur J Gastroenterol Hepatol* 2011; **23**: 1127-1135 [PMID: 21934509 DOI: 10.1097/MEG.0b013e32834b8d73]
18 **Marimuthu SP**, Vijayaragavan P, Moysich KB, Jayaprakash V. Diabetes mellitus and gastric carcinoma: Is there an association? *J Carcinog* 2011; **10**: 30 [PMID: 22190872 DOI: 10.4103/1477-3163.90481]
19 **Carstensen B**, Witte DR, Friis S. Cancer occurrence in Danish diabetic patients: duration and insulin effects. *Diabetologia* 2012; **55**: 948-958 [PMID: 22120574]
20 **Jiang X**, Bernstein L, Tseng CC, Wu AH. Diabetes and risk of esophageal and gastric adenocarcinomas. *Int J Cancer* 2012; **131**: 1417-1422 [PMID: 22161620 DOI: 10.1002/ijc.27390]
21 **O'Mara BA**, Byers T, Schoenfeld E. Diabetes mellitus and cancer risk: a multisite case-control study. *J Chronic Dis* 1985; **38**: 435-441 [PMID: 3998058 DOI: 10.1016/0021-9681(85)90139-0]
22 **Lin SW**, Freedman ND, Hollenbeck AR, Schatzkin A, Abnet CC. Prospective study of self-reported diabetes and risk of upper gastrointestinal cancers. *Cancer Epidemiol Biomarkers Prev* 2011; **20**: 954-961 [PMID: 21415356 DOI: 10.1158/1055-9965.EPI-10-1244]
23 **Ishida T**. So called "diabetic cancer risk" in the inpatient's health examination (Japanese). *Teishin Igaku* 1973; **25**: 107-115
24 **Svacina S**, Matoulek M, Svobodová S, Visokai V, Lipská L, Topolcan O, Zvárová J, Plavcová M. Gastrointestinal tract cancer and diabetes mellitus. *Vnitr Lek* 2004; **50**: 386-391 [PMID: 15305638]
25 **La Vecchia C**, Negri E, Franceschi S, D'Avanzo B, Boyle P. A case-control study of diabetes mellitus and cancer risk. *Br J Cancer* 1994; **70**: 950-953 [PMID: 7947103 DOI: 10.1038/bjc.1994.427]
26 **Jee SH**, Ohrr H, Sull JW, Yun JE, Ji M, Samet JM. Fasting serum glucose level and cancer risk in Korean men and women. *JAMA* 2005; **293**: 194-202 [PMID: 15644546 DOI: 10.1001/jama.293.2.194]
27 **Inoue M**, Iwasaki M, Otani T, Sasazuki S, Noda M, Tsugane S. Diabetes mellitus and the risk of cancer: results from a large-scale population-based cohort study in Japan. *Arch Intern Med* 2006; **166**: 1871-1877 [PMID: 17000944 DOI: 10.1001/archinte.166.17.1871]
28 **Khan M**, Mori M, Fujino Y, Shibata A, Sakauchi F, Washio M, Tamakoshi A. Site-specific cancer risk due to diabetes mellitus history: evidence from the Japan Collaborative Cohort (JACC) Study. *Asian Pac J Cancer Prev* 2006; **7**: 253-259 [PMID: 16839219]
29 **Kuriki K**, Hirose K, Tajima K. Diabetes and cancer risk for all and specific sites among Japanese men and women. *Eur J Cancer Prev* 2007; **16**: 83-89 [PMID: 17220709 DOI: 10.1097/01.cej.0000228404.37858.40]
30 **Boyle P**. World Cancer Report. International Agency for Research on Cancer Press, 2008: 344-349. Available from: URL: http: //www.iarc.fr/en/publications/pdfs-online/wcr/2008/index.php
31 **Forman D**, Burley VJ. Gastric cancer: global pattern of the disease and an overview of environmental risk factors. *Best Pract Res Clin Gastroenterol* 2006; **20**: 633-649 [PMID: 16997150 DOI: 10.1016/j.bpg.2006.04.008]
32 **Compare D**, Rocco A, Nardone G. Risk factors in gastric cancer. *Eur Rev Med Pharmacol Sci* 2010; **14**: 302-308 [PMID: 20496539]
33 **Trédaniel J**, Boffetta P, Buiatti E, Saracci R, Hirsch A. Tobacco smoking and gastric cancer: review and meta-analysis. *Int J Cancer* 1997; **72**: 565-573 [PMID: 9259392]
34 **González CA**, Pera G, Agudo A, Palli D, Krogh V, Vineis P, Tumino R, Panico S, Berglund G, Simán H, Nyrén O, Agren A, Martinez C, Dorronsoro M, Barricarte A, Tormo MJ, Quiros JR, Allen N, Bingham S, Day N, Miller A, Nagel G, Boeing H, Overvad K, Tjonneland A, Bueno-De-Mesquita HB, Boshuizen HC, Peeters P, Numans M, Clavel-Chapelon F, Helen I, Agapitos E, Lund E, Fahey M, Saracci R, Kaaks R, Riboli E. Smoking and the risk of gastric cancer in the European Prospective Investigation Into Cancer and Nutrition (EPIC). *Int J Cancer* 2003; **107**: 629-634 [PMID: 14520702 DOI: 10.1002/ijc.11426]
35 **Lippert E**, Endlicher E. Hyperglycemia: a risk factor for gastric cancer development?. *Z Gastroenterol* 2009; **47**: 850-853 [PMID: 19750434 DOI: 10.1055/s-0028-1109667]
36 **Yamagata H**, Kiyohara Y, Nakamura S, Kubo M, Tanizaki Y, Matsumoto T, Tanaka K, Kato I, Shirota T, Iida M. Impact of fasting plasma glucose levels on gastric cancer incidence in a general Japanese population: the Hisayama study. *Diabetes Care* 2005; **28**: 789-794 [PMID: 15793174 DOI: 10.2337/diacare.28.4.789]
37 **Ikeda F**, Doi Y, Yonemoto K, Ninomiya T, Kubo M, Shikata K, Hata J, Tanizaki Y, Matsumoto T, Iida M, Kiyohara Y. Hyperglycemia increases risk of gastric cancer posed by Helicobacter pylori infection: a population-based cohort study. *Gastroenterology* 2009; **136**: 1234-1241 [PMID: 19236964 DOI: 10.1053/j.gastro.2008.12.045]
38 **Koppert LB**, Janssen-Heijnen ML, Louwman MW, Lemmens VE, Wijnhoven BP, Tilanus HW, Coebergh JW. Comparison of comorbidity prevalence in oesophageal and gastric carcinoma patients: a population-based study. *Eur J Gastroenterol Hepatol* 2004; **16**: 681-688 [PMID: 15201582 DOI: 10.1097/01.meg.0000108331.52416.f1]
39 **Stocks T**, Rapp K, Bjørge T, Manjer J, Ulmer H, Selmer R, Lukanova A, Johansen D, Concin H, Tretli S, Hallmans G, Jonsson H, Stattin P. Blood glucose and risk of incident and fatal cancer in the metabolic syndrome and cancer project (me-can): analysis of six prospective cohorts. *PLoS Med* 2009; **6**: e1000201 [PMID: 20027213 DOI: 10.1371/journal.pmed.1000201]
40 **Stattin P**, Björ O, Ferrari P, Lukanova A, Lenner P, Lindahl B, Hallmans G, Kaaks R. Prospective study of hyperglycemia and cancer risk. *Diabetes Care* 2007; **30**: 561-567 [PMID: 17327321 DOI: 10.2337/dc06-0922]
41 **Shu X**, Ji J, Li X, Sundquist J, Sundquist K, Hemminki K. Cancer risk among patients hospitalized for Type 1 diabetes mellitus: a population-based cohort study in Sweden. *Diabet Med* 2010; **27**: 791-797 [PMID: 20636960 DOI: 10.1111/j.1464-5491.2010.03011.x]
42 **Ekbom A**, McLaughlin JK, Karlsson BM, Nyrén O, Gridley G, Adami HO, Fraumeni JF. Pancreatitis and pancreatic cancer: a population-based study. *J Natl Cancer Inst* 1994; **86**: 625-627 [PMID: 8145277]
43 **Ragozzino M**, Melton LJ, Chu CP, Palumbo PJ. Subsequent cancer risk in the incidence cohort of Rochester, Minnesota, residents with diabetes mellitus. *J Chronic Dis* 1982; **35**: 13-19 [PMID: 7068798 DOI: 10.1016/0021-9681(82)90025-X]
44 **Hjalgrim H**, Frisch M, Ekbom A, Kyvik KO, Melbye M, Green A. Cancer and diabetes--a follow-up study of two population-based cohorts of diabetic patients. *J Intern Med* 1997; **241**: 471-475 [PMID: 10497622 DOI: 10.1111/j.1365-2796.1997.tb00004.x]
45 **Bershteĭn LM**, Merabishvili VM, Semenova NV, Karpova

IA, Kovalevskiĭ AIu. Registry-based analysis of cancer and diabetes combination: prevalence and features. *Vopr Onkol* 2007; **53**: 285-290 [PMID: 18198608]
46 **Lee MS**, Hsu CC, Wahlqvist ML, Tsai HN, Chang YH, Huang YC. Type 2 diabetes increases and metformin reduces total, colorectal, liver and pancreatic cancer incidences in Taiwanese: a representative population prospective cohort study of 800,000 individuals. *BMC Cancer* 2011; **11**: 20 [PMID: 21241523 DOI: 10.1186/1471-2407-11-20]
47 **Tseng CH**. Diabetes conveys a higher risk of gastric cancer mortality despite an age-standardised decreasing trend in the general population in Taiwan. *Gut* 2011; **60**: 774-779 [PMID: 21193459 DOI: 10.1136/gut.2010.226522]
48 **Coughlin SS**, Calle EE, Teras LR, Petrelli J, Thun MJ. Diabetes mellitus as a predictor of cancer mortality in a large cohort of US adults. *Am J Epidemiol* 2004; **159**: 1160-1167 [PMID: 15191933 DOI: 10.1093/aje/kwh161159/12/1160]
49 **Batty GD**, Shipley MJ, Marmot M, Smith GD. Diabetes status and post-load plasma glucose concentration in relation to site-specific cancer mortality: findings from the original Whitehall study. *Cancer Causes Control* 2004; **15**: 873-881 [PMID: 15577289 DOI: 10.1007/s10552-004-1050-z]
50 **Levine W**, Dyer AR, Shekelle RB, Schoenberger JA, Stamler J. Post-load plasma glucose and cancer mortality in middle-aged men and women. 12-year follow-up findings of the Chicago Heart Association Detection Project in Industry. *Am J Epidemiol* 1990; **131**: 254-262 [PMID: 2296978]
51 **Smith GD**, Egger M, Shipley MJ, Marmot MG. Post-challenge glucose concentration, impaired glucose tolerance, diabetes, and cancer mortality in men. *Am J Epidemiol* 1992; **136**: 1110-1114 [PMID: 1462971]
52 **Rubenstein JH**, Davis J, Marrero JA, Inadomi JM. Relationship between diabetes mellitus and adenocarcinoma of the oesophagus and gastric cardia. *Aliment Pharmacol Ther* 2005; **22**: 267-271 [PMID: 16091065 DOI: 10.1111/j.1365-2036.2005.02544.x]
53 **Atchison EA**, Gridley G, Carreon JD, Leitzmann MF, McGlynn KA. Risk of cancer in a large cohort of U.S. veterans with diabetes. *Int J Cancer* 2011; **128**: 635-643 [PMID: 20473855 DOI: 10.1002/ijc.25362]
54 **Chodick G**, Heymann AD, Rosenmann L, Green MS, Flash S, Porath A, Kokia E, Shalev V. Diabetes and risk of incident cancer: a large population-based cohort study in Israel. *Cancer Causes Control* 2010; **21**: 879-887 [PMID: 20148361 DOI: 10.1007/s10552-010-9515-8]
55 **Ogunleye AA**, Ogston SA, Morris AD, Evans JM. A cohort study of the risk of cancer associated with type 2 diabetes. *Br J Cancer* 2009; **101**: 1199-1201 [PMID: 19690547 DOI: 10.1038/sj.bjc.6605240]
56 **Rousseau MC**, Parent ME, Pollak MN, Siemiatycki J. Diabetes mellitus and cancer risk in a population-based case-control study among men from Montreal, Canada. *Int J Cancer* 2006; **118**: 2105-2109 [PMID: 16284945 DOI: 10.1002/ijc.21600]
57 **Jun JK**, Gwack J, Park SK, Choi YH, Kim Y, Shin A, Chang SH, Shin HR, Yoo KY. Fasting serum glucose level and gastric cancer risk in a nested case-control study. *J Prev Med Public Health* 2006; **39**: 493-498 [PMID: 17168202]
58 **Giovannucci E**. Insulin, insulin-like growth factors and colon cancer: a review of the evidence. *J Nutr* 2001; **131**: 3109S-3120S [PMID: 11694656]
59 **Ma J**, Pollak MN, Giovannucci E, Chan JM, Tao Y, Hennekens CH, Stampfer MJ. Prospective study of colorectal cancer risk in men and plasma levels of insulin-like growth factor (IGF)-I and IGF-binding protein-3. *J Natl Cancer Inst* 1999; **91**: 620-625 [PMID: 10203281 DOI: 10.1093/jnci/91.7.620]
60 **Thompson MA**, Cox AJ, Whitehead RH, Jonas HA. Autocrine regulation of human tumor cell proliferation by insulin-like growth factor II: an in-vitro model. *Endocrinology* 1990; **126**: 3033-3042 [PMID: 1693565 DOI: 10.1210/endo-126-6-3033]
61 **Pavelić K**, Kolak T, Kapitanović S, Radosević S, Spaventi S, Kruslin B, Pavelić J. Gastric cancer: the role of insulin-like growth factor 2 (IGF 2) and its receptors (IGF 1R and M6-P/IGF 2R). *J Pathol* 2003; **201**: 430-438 [PMID: 14595755 DOI: 10.1002/path.1465]
62 **Mahdy RA**, Nada WM. Evaluation of the role of vascular endothelial growth factor in diabetic retinopathy. *Ophthalmic Res* 2011; **45**: 87-91 [PMID: 20720438 DOI: 10.1159/000317062]
63 **Tanigawa N**, Amaya H, Matsumura M, Shimomatsuya T. Correlation between expression of vascular endothelial growth factor and tumor vascularity, and patient outcome in human gastric carcinoma. *J Clin Oncol* 1997; **15**: 826-832 [PMID: 9053510]
64 **Maeda K**, Chung YS, Ogawa Y, Takatsuka S, Kang SM, Ogawa M, Sawada T, Sowa M. Prognostic value of vascular endothelial growth factor expression in gastric carcinoma. *Cancer* 1996; **77**: 858-863 [PMID: 8608475]
65 **Yonemura Y**, Endo Y, Fujita H, Fushida S, Ninomiya I, Bandou E, Taniguchi K, Miwa K, Ohoyama S, Sugiyama K, Sasaki T. Role of vascular endothelial growth factor C expression in the development of lymph node metastasis in gastric cancer. *Clin Cancer Res* 1999; **5**: 1823-1829 [PMID: 10430087]
66 **Warren RS**, Yuan H, Matli MR, Ferrara N, Donner DB. Induction of vascular endothelial growth factor by insulin-like growth factor 1 in colorectal carcinoma. *J Biol Chem* 1996; **271**: 29483-29488 [PMID: 8910616 DOI: 10.1074/jbc.271.46.29483]
67 **Akagi Y**, Liu W, Zebrowski B, Xie K, Ellis LM. Regulation of vascular endothelial growth factor expression in human colon cancer by insulin-like growth factor-I. *Cancer Res* 1998; **58**: 4008-4014 [PMID: 9731515]
68 **Dandona P**, Thusu K, Cook S, Snyder B, Makowski J, Armstrong D, Nicotera T. Oxidative damage to DNA in diabetes mellitus. *Lancet* 1996; **347**: 444-445 [PMID: 8618487 DOI: 10.1016/S0140-6736(96)90013-6]
69 **Lorenzi M**, Montisano DF, Toledo S, Barrieux A. High glucose induces DNA damage in cultured human endothelial cells. *J Clin Invest* 1986; **77**: 322-325 [PMID: 3944257 DOI: 10.1172/JCI112295]
70 **González CA**, Megraud F, Buissonniere A, Lujan Barroso L, Agudo A, Duell EJ, Boutron-Ruault MC, Clavel-Chapelon F, Palli D, Krogh V, Mattiello A, Tumino R, Sacerdote C, Quirós JR, Sanchez-Cantalejo E, Navarro C, Barricarte A, Dorronsoro M, Khaw KT, Wareham N, Allen NE, Tsilidis KK, Bas Bueno-de-Mesquita H, Jeurnink SM, Numans ME, Peeters PH, Lagiou P, Valanou E, Trichopoulou A, Kaaks R, Lukanova-McGregor A, Bergman MM, Boeing H, Manjer J, Lindkvist B, Stenling R, Hallmans G, Mortensen LM, Overvad K, Olsen A, Tjonneland A, Bakken K, Dumeaux V, Lund E, Jenab M, Romieu I, Michaud D, Mouw T, Carneiro F, Fenge C, Riboli E. Helicobacter pylori infection assessed by ELISA and by immunoblot and noncardia gastric cancer risk in a prospective study: the Eurgast-EPIC project. *Ann Oncol* 2012; **23**: 1320-1324 [PMID: 21917738 DOI: 10.1093/annonc/mdr384]
71 **Kamangar F**, Dawsey SM, Blaser MJ, Perez-Perez GI, Pietinen P, Newschaffer CJ, Abnet CC, Albanes D, Virtamo J, Taylor PR. Opposing risks of gastric cardia and noncardia gastric adenocarcinomas associated with Helicobacter pylori seropositivity. *J Natl Cancer Inst* 2006; **98**: 1445-1452 [PMID: 17047193 DOI: 10.1093/jnci/djj393]
72 **Lanzino M**, Morelli C, Garofalo C, Panno ML, Mauro L, Andò S, Sisci D. Interaction between estrogen receptor alpha and insulin/IGF signaling in breast cancer. *Curr Cancer Drug Targets* 2008; **8**: 597-610 [PMID: 18991569 DOI: 10.2174/156800908786241104]
73 **Matsuyama S**, Ohkura Y, Eguchi H, Kobayashi Y, Akagi K, Uchida K, Nakachi K, Gustafsson JA, Hayashi S. Estrogen

receptor beta is expressed in human stomach adenocarcinoma. *J Cancer Res Clin Oncol* 2002; **128**: 319-324 [PMID: 12073050 DOI: 10.1007/s00432-002-0336-3]

74 **Enmark E**, Pelto-Huikko M, Grandien K, Lagercrantz S, Lagercrantz J, Fried G, Nordenskjöld M, Gustafsson JA. Human estrogen receptor beta-gene structure, chromosomal localization, and expression pattern. *J Clin Endocrinol Metab* 1997; **82**: 4258-4265 [PMID: 9398750 DOI: 10.1210/jc.82.12.4258]

75 **Currie CJ**, Poole CD, Gale EA. The influence of glucose-lowering therapies on cancer risk in type 2 diabetes. *Diabetologia* 2009; **52**: 1766-1777 [PMID: 19572116 DOI: 10.1007/s00125-009-1440-6]

76 **Yang P**, Zhou Y, Chen B, Wan HW, Jia GQ, Bai HL, Wu XT. Aspirin use and the risk of gastric cancer: a meta-analysis. *Dig Dis Sci* 2010; **55**: 1533-1539 [PMID: 19672710 DOI: 10.1007/s10620-009-0915-0]

77 **Tian W**, Zhao Y, Liu S, Li X. Meta-analysis on the relationship between nonsteroidal anti-inflammatory drug use and gastric cancer. *Eur J Cancer Prev* 2010; **19**: 288-298 [PMID: 20386312 DOI: 10.1097/CEJ.0b013e328339648c]

78 **Kuoppala J**, Lamminpää A, Pukkala E. Statins and cancer: A systematic review and meta-analysis. *Eur J Cancer* 2008; **44**: 2122-2132 [PMID: 18707867 DOI: 10.1016/j.ejca.2008.06.025]

79 **Fitzgerald JT**, Anderson RM, Davis WK. Gender differences in diabetes attitudes and adherence. *Diabetes Educ* 1995; **21**: 523-529 [PMID: 8549255 DOI: 10.1177/014572179502100605]

80 **Franzini L**, Ardigò D, Cavalot F, Miccoli R, Rivellese AA, Trovati M, Zavaroni I, Vaccaro O. Women show worse control of type 2 diabetes and cardiovascular disease risk factors than men: Results from the MIND.IT Study Group of the Italian Society of Diabetology. *Nutr Metab Cardiovasc Dis* 2012 Mar 5; Epub ahead of print [PMID: 22397873 DOI: 10.1016/j.numecd.2011.12.003]

81 **Fu AZ**, Zhang Q, Davies MJ, Pentakota SR, Radican L, Seck T. Underutilization of statins in patients with type 2 diabetes in US clinical practice: a retrospective cohort study. *Curr Med Res Opin* 2011; **27**: 1035-1040 [PMID: 21410303 DOI: 10.1185/03007995.2011.567257]

82 **Huang XE**, Tajima K, Hamajima N, Xiang J, Inoue M, Hirose K, Tominaga S, Takezaki T, Kuroishi T, Tokudome S. Comparison of lifestyle and risk factors among Japanese with and without gastric cancer family history. *Int J Cancer* 2000; **86**: 421-424 [PMID: 10760832 DOI: 10.1002/(SICI)1097-0215(20000501)86:]

83 **Lee JK**, Park BJ, Yoo KY, Ahn YO. Dietary factors and stomach cancer: a case-control study in Korea. *Int J Epidemiol* 1995; **24**: 33-41 [PMID: 7797354 DOI: 10.1093/ije/24.1.33]

84 **Brenner H**, Rothenbacher D, Arndt V. Epidemiology of stomach cancer. *Methods Mol Biol* 2009; **472**: 467-477 [PMID: 19107449 DOI: 10.1007/978-1-60327-492-0_23]

85 **Liu C**, Russell RM. Nutrition and gastric cancer risk: an update. *Nutr Rev* 2008; **66**: 237-249 [PMID: 18454810 DOI: 10.1111/j.1753-4887.2008.00029.x]

P- Reviewer Lindberg G **S- Editor** Gou SX **L- Editor** A **E- Editor** Li JY



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i6.946

World J Gastroenterol 2013 February 14; 19(6): 946-950
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Dubin-Johnson syndrome coinciding with colon cancer and atherosclerosis

Eva Sticova, Milan Elleder, Helena Hulkova, Ondrej Luksan, Martin Sauer, Irena Wunschova-Moudra, Jan Novotny, Milan Jirsa

Eva Sticova, Ondrej Luksan, Milan Jirsa, Center for Experimental Medicine, Institute for Clinical and Experimental Medicine, 14021 Prague 4, Czech Republic
Eva Sticova, Third Faculty of Medicine, Charles University, 10000 Prague 10, Czech Republic
Milan Elleder, Helena Hulkova, Institute of Inherited Metabolic Disorders, First Faculty of Medicine, Charles University and VFN, 12808 Prague 2, Czech Republic
Martin Sauer, Department of Surgery IPVZ, Masaryk Hospital, 40113 Usti nad Labem, Czech Republic
Irena Wunschova-Moudra, Department of Thoracic Surgery, Masaryk Hospital, 40113 Usti nad Labem, Czech Republic
Jan Novotny, Gastroenterology Department, Masaryk Hospital, 40113 Usti nad Labem, Czech Republic
Author contributions: Sticova E and Jirsa M analyzed the mutations, the pathological data and were involved in writing and editing the manuscript; Elleder M and Hulkova H performed histochemical, immunohistological and ultrastructural analysis; Luksan O participated in mutation analysis; Sauer M, Wunschova-Moudra I and Novotny J were involved in collecting the clinical and laboratory data.
Supported by The Project (Ministry of Health, Czech Republic) for Development of Research Organization 00023001 (IKEM, Prague, Czech Republic) - Institutional support; and the grant SVV-2012-264502
Correspondence to: Eva Sticova, MD, Center for Experimental Medicine, Institute for Clinical and Experimental Medicine, Videnska 1958/9, 14021 Prague 4,
Czech Republic. eva.sticova@ikem.cz
Telephone: +420-236-055229 Fax: +420-241-721666
Received: July 12, 2012 Revised: October 10, 2012
Accepted: October 22, 2012
Published online: February 14, 2013

Abstract

Hyperbilirubinemia has been presumed to prevent the process of atherogenesis and cancerogenesis mainly by decreasing oxidative stress. Dubin-Johnson syndrome is a rare, autosomal recessive, inherited disorder characterized by biphasic, predominantly conjugated hyperbilirubinemia with no progression to end-stage liver disease. The molecular basis in Dubin-Johnson syndrome is absence or deficiency of human canalicular multispecific organic anion transporter MRP2/cMOAT caused by homozygous or compound heterozygous mutation(s) in *ABCC2* located on chromosome 10q24. Clinical onset of the syndrome is most often seen in the late teens or early adulthood. In this report, we describe a case of previously unrecognized Dubin-Johnson syndrome caused by two novel pathogenic mutations (c.2360_2366delCCCTGTC and c.3258+1G>A), coinciding with cholestatic liver disease in an 82-year-old male patient. The patient, suffering from advanced atherosclerosis with serious involvement of coronary arteries, developed colorectal cancer with nodal metastases. The subsequent findings do not support the protective role of Dubin-Johnson type hyperbilirubinemia.

© 2013 Baishideng. All rights reserved.

Key words: Dubin-Johnson syndrome; *ABCC2*; Hyperbilirubinemia; Oxidative stress; Atherosclerosis; Cancer

Sticova E, Elleder M, Hulkova H, Luksan O, Sauer M, Wunschova-Moudra I, Novotny J, Jirsa M. Dubin-Johnson syndrome coinciding with colon cancer and atherosclerosis. *World J Gastroenterol* 2013; 19(6): 946-950 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i6/946.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i6.946

INTRODUCTION

Recent data have indicated the potent antioxidant properties of mild or moderately elevated serum bilirubin levels with substantial positive clinical consequences and especially their protective effect on atherogenesis and cancerogenesis[1]. A direct link between low serum bilirubin levels and peripheral vascular disease and the protective

effect of mild or moderate unconjugated hyperbilirubinemia on atherosclerosis were confirmed in numerous clinical trials[2-5]. Antiproliferative, cytostatic and pro-apoptotic effects of bilirubin are well-known and have been demonstrated in both *in vitro* and *in vivo* studies[6-10]. However, conflicting data on the possible protective effect of elevated bilirubin levels against sporadic colorectal carcinoma have been reported in the literature[11-13].

Dubin-Johnson syndrome (DJS, OMIM 237500) is a rare, autosomal recessive disorder characterized by non-hemolytic hyperbilirubinemia with no progression to end-stage liver disease[14]. Both the conjugated and unconjugated form of bilirubin can be elevated in DJS subjects, with the former ranging from 17% to 88% of the total bilirubin with a mean value of 60%[14,15]. In addition to the fluctuating jaundice caused by biphasic hyperbilirubinemia due to absence or deficiency of human canalicular multispecific organic anion transporter MRP2/cMOAT[16-18], DJS subjects may suffer from non-specific symptoms such as weakness and abdominal discomfort. Urinary coproporphyrin output is normal; however, 80% of the coproporphyrin fraction is represented by coproporhyrin I (normally 25%)[19]. The biliary excretion of anionic dyes including bromosulfophthalein, indocyanine green and cholescintigraphy radiotracers is delayed. Liver histology in DJS shows an accumulation of distinctive melanin-like lysosomal pigment in an otherwise normal liver, which gives the organ a characteristic dark pink or even black colour[14,20].

In this report, we describe the case of unintentionally detected DJS complicating liver injury in an 82-year-old patient who was being followed for previously diagnosed colorectal cancer and ischemic heart disease.

CASE REPORT

An 82-year-old Caucasian male patient, a normotensive non-smoker with a history of right-sided hemicolectomy due to colorectal adenocarcinoma with nodal metastases five years ago and ischemic heart disease, was referred to the hospital due to clinical jaundice with elevated serum bilirubin (total bilirubin 220 μmol/L, direct bilirubin 171 μmol/L), gamma-glutamyltransferase (γGT) activity (4.7 μkat/L) and fluctuating alkaline phosphatase (ALP) activity (1.3-3.6 μkat/L) to exclude bile duct obstruction or metastatic liver disease. The patient complained of slight upper abdominal discomfort without pruritus or nausea. Complete blood count and serum electrolyte levels were normal, as was his serum lipid profile (serum cholesterol 3.5 mmol/L, high-density lipoprotein cholesterol 0.9 mmol/L, low-density lipoprotein cholesterol 2.5 mmol/L, triglycerides 1.3 mmol/L). Abdominal ultrasonography, endoscopic retrograde cholangiopancreatography and magnetic resonance cholangiopancreatography did not detect any obstacle or dilation of the biliary tree, and only slightly imperfect filling of the ductus choledochus was observed. Serological examinations for viral hepatitis (hepatitis A virus, hepatitis B virus and hepatitis C virus) and autoantibodies were completely negative. During the patient's hospitalization, the activity of γGT and ALP gradually decreased but, due to persistent marked hyperbilirubinemia, a percutaneous liver biopsy was performed.

The liver tissue obtained at biopsy was standardly processed. Sections cut at 4-6 μm were stained with hematoxylin and eosin, periodic acid Schiff, Schmorl's and van Gieson's method. Analysis of the liver biopsy specimen revealed preserved architecture of liver parenchyma with perivenular and perisinusoidal fibrosis, subtle steatosis and only discrete intracellular cholestasis in centrilobular hepatocytes. The most striking feature, however, was an intense parenchymal pigmentation, with centrilobular and midzonal accentuation, consisting of coarse brown black pigment (Figure 1, Panel A-C). Special stainings (Gömöri, silver ammonium complex-Masson's and Perls Prussian blue method) for pigment characterization were added. The pigment was negative in the Perls reaction and displayed rudimentary autofluorescence with gradually increasing intensity, especially in Shandon mounting medium and simultaneously reduced Masson's solution. A retrospective analysis of the patient's medical records back to 1994 revealed long-term, persistent, mixed, predominantly conjugated hyperbilirubinemia (total bilirubin fluctuating within the range 23.0-215.6 μmol/L, conjugated bilirubin 17.7-170 μmol/L). The conjugated-to-total bilirubin ratio was within the range of 40% (total bilirubin 202 μmol/L and conjugated bilirubin 80.6 μmol/L in 2009) to 89% (total bilirubin 41.8 μmol/L and conjugated bilirubin 37.2 μmol/L in 1994). Taking into account the normal activity of aminotransferases, γGT and ALP, DJS was suspected.

For immunohistochemical analysis, 4 to 6 μm-thick sections were incubated with the anti-MRP2 mouse monoclonal antibody (clone M2Ⅲ-6, Kamiya, Seattle, WA). The EnVision Peroxidase Kit (Dako, Glostrup, Denmark) was used for visualization and counterstaining with Harris's hematoxylin was performed. As a positive control, sections of an adult liver without cholestasis were stained, and liver sections incubated without primary antibody were used as negative controls. Immunohistochemical analysis for MRP2 protein was completely negative (Figure 1D).

Ultrastructural analysis was performed on the liver sample fixed with 4% buffered paraformaldehyde, osmicated, dehydrated in ascending ethanol solutions and embedded into Epon-Araldite mixture. Ultrathin sections were double-stained with uranyl acetate and lead nitrate and then examined under a JEM 1200 EX electron microscope. Intralysosomal localization of the pigment was demonstrated.

A mutation analysis of the *ABCC2* gene was indicated to confirm the diagnosis of DJS at the molecular level. Written informed consent was obtained from the patient before the genetic investigation. *ABCC2* was analyzed by direct sequencing of genomic DNA extracted from peripheral leucocytes. All 32 exons, with the adjacent parts of the intronic sequences, were amplified by polymerase chain reaction (PCR) using the intronic oligonucleotide primers listed in Table 1. Amplified fragments were gel-purified, extracted from the gel with QIA quick spin col-

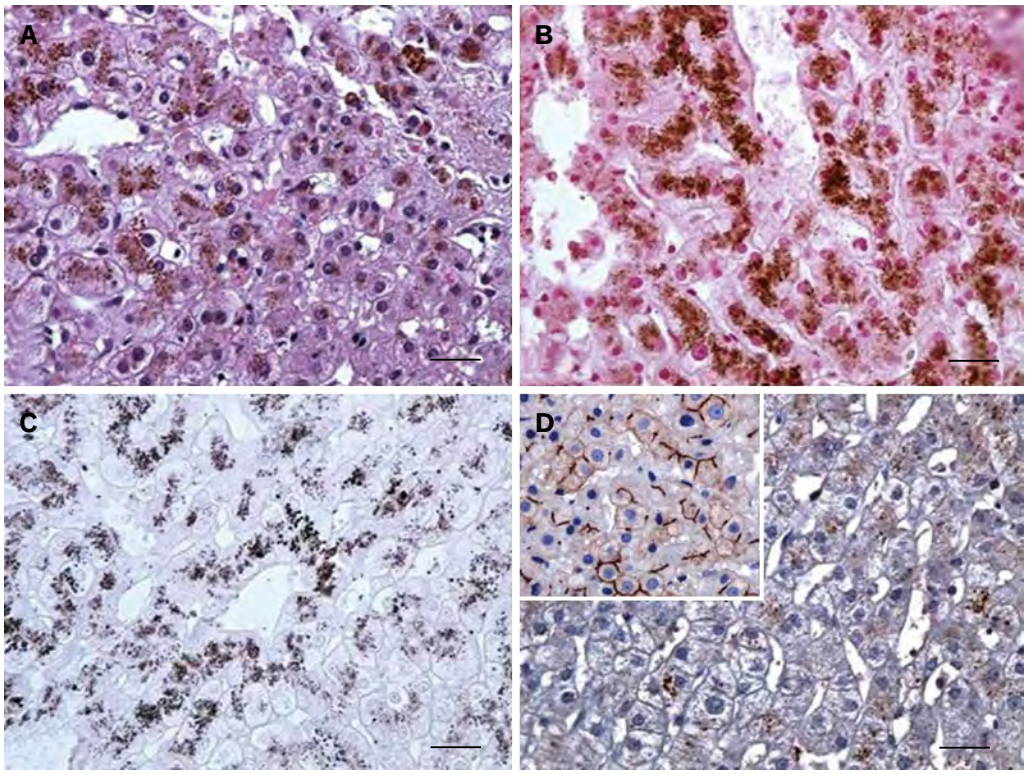


Figure 1 Histochemistry and immunohistology of the liver in Dubin-Johnson syndrome patient. A: Accumulation of distinctive dark brown pigment in hepatocytes was detected in hematoxylin and eosin staining; B: The pigment was negative in Perls reaction; C: The pigment reduced Masson's solution; D: Immunohistochemical analysis for ABCC2/MRP2 protein was negative compared to the positive control (inset). Original magnification ×400 (bars correspond to 100 μm).

Table 1 Primer pairs used for amplification of *ABCC2/MRP2* gene exons and promoters

Pair No.	Sequences of polymerase chain reaction primer pairs used to amplify the *ABCC2/MRP2* gene from genomic DNA		Exon No.
	Forward primer	Reverse primer	
1	5=-TTTACAATGCCTGGCAAAGG-3=	5=-CAGCATGATTCCTGGACTGC-3=	Promoter
2	5=-TCCCACATTCTGGATTTTGAC-3=	5=-ATAAAATAGCTTATCCAGTGTGAGC-3=	Promoter
3	5=-GGTCAGACCAATTTACATTTCCATC-3=	5=-CATAACCACCCATGCAGTATCC-3=	Promoter
4	5=-TACTTTGGGAACTGGTGAGTCT-3=	5=-AGAAGGCAATTTTGCGACTA-3=	1
5	5=-CACAAATAGGAAAATACGGATA-3=	5=-CCTGGGACAGCTGCTTA-3=	2
6	5=-CTGAATCACTGCATACCGCTTTT-3=	5=-CCAACCAAGCTTTGCCTCAC-3=	3
7	5=-CTGAATCACTGCATACCGCTTTT-3=	5=-AATTCGATCCTGGAGCTCAAC-3=	3_4
8	5=-TCATAGTAAATGGCATCAAGT-3=	5=-GGTGGAACATGAGCTTGAGT-3=	5_6
9	5=-AGTGGTGGAGATAGCCTC-3=	5=-GCTATAAAAATGTAAGGACA-3=	7
10	5=-GCCAGGGAGAGATGATCAAA-3=	5=-GGCCAGTCAACATTAAGTG-3=	8_9
11	5=-TGGAGCACATCCTTCCATTG-3=	5=-TTGCCCAAACTCCCATTAAG-3=	10
12	5=-TCACTGGGCACCTCAAGTTC-3=	5=-AGCAGGAATCCATCACCTCT-3=	11
13	5=-ATTTTGGGGACTATATCT-3=	5=-GATGTGATAGCCAGTCATTC-3=	12_13
14	5=-GTTCGGTGGAGATTAGGAG-3=	5=-TCTTATGCAAGCATAGGCTC-3=	14
15	5=-TTCACCTCCTGTTAGCGTA-3=	5=-ACCGAAGACATGCACATAGC-3=	15
16	5=-TTCACCTCCTGTTAGCGTA-3=	5=-CAAGACCTCACCTACTAGCC-3=	15_16
17	5=-ACAAGCACGTGAATACATATCAG-3=	5=-ACCCCTGTGTAGTTCTT-3=	17
18	5=-GTAAGATTTTTAACCCCTTG-3=	5=-GCCCAGGCATAGAGTTTC-3=	18_19
19	5=-GTATGGAGTATTTATGGAGT-3=	5=-TGTAAGTATGCGTTCAAT-3=	20_21
20	5=-GTGGTTGGCATTCTAGGT-3=	5=-CATAATAATTCCTCCCTATCA-3=	22_23
21	5=-CTGGGAACACACAGAATCCAAC-3=	5=-GGCTCCTGGGTATGTCAACA-3=	24
22	5=-GGCTTTTGTCTTGTTCAGACG-3=	5=-CTTGGTAAACGGCAGA-3=	25
23	5=-CCCGATCAAGTCAAAC-3=	5=-GGCATTCATGTCTACTTAGGA-3=	26
24	5=-GGAGGCAAGGATTGTC-3=	5=-TCTGCATACTGTGGACCTTAT-3=	27_28
25	5=-ACAGCTGCCAAGAGAGTCCAT-3=	5=-GCTCAAGTATCCCGAGTAGA-3=	29
26	5=-CCTTGCGGAAGCTCAACC-3=	5=-TGCCAGGCATCACCTAACACG-3=	30
27	5=-GTTTTGAAAGTCTGATCTG-3=	5=-AGGAAGTACGATCGAGGTA-3=	31_32

umns (Qiagene, Hilden, Germany), and sequenced on an ABI 3130 Genetic Analyzer (Applied Biosystems, Foster

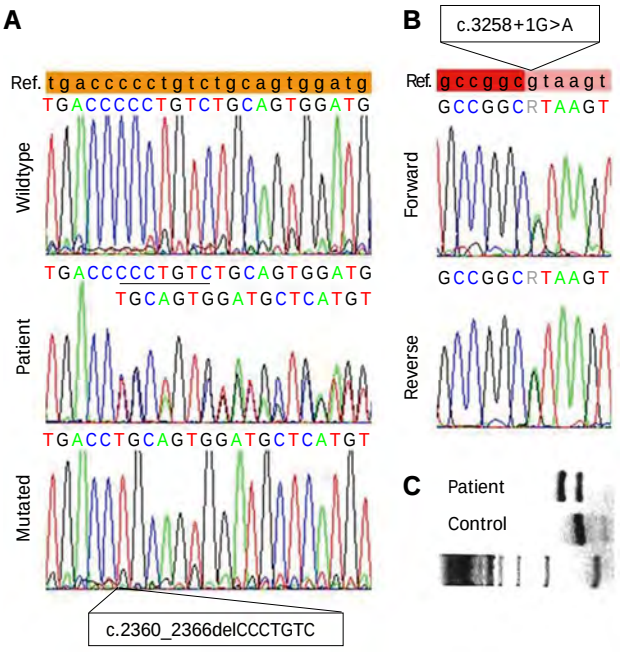


Figure 2 Mutation analysis results. A: Presence of a heterozygous deletion c.2360_2366delCCCTGTC in exon 18 of *ABCC2* (panel A, middle lane) was confirmed by sequence analysis of individual alleles separated by cloning (wildtype allele - panel A, upper lane; mutated allele - panel A, lower lane); B: In addition, a heterozygous splice site mutation c.3258+1G>A was detected in intron 23; C: Presence of the latter mutation was confirmed by polymerase chain reaction-Bsh1236I restriction fragment length polymorphism analysis.

City, CA). The obtained sequence was compared with the reference sequences GenBank NM_000392 (mRNA) and NT_030059 (genomic DNA). An exon with suspected deletion was cloned into a pCR4.1-TOPO plasmid vector (Invitrogen, Carlsbad, CA), and the wild-type and mutated alleles were sequenced separately. Presence of the second mutation was confirmed by PCR-restriction fragment length polymorphism analysis (PCR-Bsh1236I RFLP).

Analysis of the *ABCC2*/*MRP2* gene disclosed two novel mutations: a heterozygous deletion c.2360_2366delCCCTGTC in protein coding exon 18 (Figure 2A), and a heterozygous mutation c.3258+1G>A in intron 23 (Figure 2B and C). The former mutation in exon 18 was predicted to cause a reading frame shift and premature termination of DNA translation at position 803 with protein alteration p.Pro787LeufsX7. The latter mutation affecting the donor splice site of intron 23 was predicted to cause abnormal splicing of mRNA. Therefore, both mutations were considered as pathogenic. Histochemistry and mutation analysis accordingly established the diagnosis of DJS.

The patient suddenly died several months after being released from the hospital. The patient's autopsy revealed no liver metastases and confirmed the diagnosis of advanced atherosclerosis with serious involvement of coronary arteries with signs of chronic myocardial ischemia.

DISCUSSION

The diagnosis of DJS is indicated by the presence of fluctuating predominantly conjugated hyperbilirubinemia, an increased ratio of urinary coproporphyrin I to coproporphyrin Ⅲ, and a unique cholescintigram showing delayed visualization of the liver and biliary tract. Neither a percutaneous liver biopsy with evidence of melanin-like pigment and the absence of MRP2 protein nor mutation analysis are necessary for the diagnosis. Nonetheless, both methods may be helpful in complicated cases to establish the correct diagnosis and rule out more serious liver pathology.

In our patient, neither an examination of urinary coproporphyrin levels nor cholescintigraphy was performed. The diagnosis was based on biochemical evidence of fluctuating biphasic hyperbilirubinemia and characteristic findings in the liver biopsy, and further supported by disclosure of two pathogenic mutations by subsequent mutational analysis. Considering the clinical picture and the results of laboratory tests, the two mutations are supposed to be in transposition. Unfortunately, no living relatives of the patient were found to confirm our presumption.

As the clinical onset of DJS is most often seen during the teenage years or in early adulthood, the first recognition of the disease in old age is highly unusual. To the best of our knowledge, our patient is the oldest reported newly diagnosed DJS patient confirmed by mutation analysis.

The clinical presentation and laboratory findings of our case indicated the coincidence of DJS with mild cholestatic liver damage, the etiology of which remained obscure. The simultaneous occurrence of the syndrome with another hepatobiliary disease is well-known and the coincidence with another disease or pathologic stimulus can modify the clinical picture and results of laboratory tests, including histomorphology[21-24].

A significant fact in our case is the presence of colorectal adenocarcinoma with nodal metastases in a patient with chronic mixed hyperbilirubinemia. Numerous clinical trials have demonstrated the protective effects of hyperbilirubinemia against the development of sporadic colorectal cancer and an association of low bilirubin levels with an increased risk of colon cancer morbidity and mortality[10-12].

In addition, there are reports describing the protective effect of bilirubin on the development of atherosclerosis emphasizing the antioxidative and anti-inflammatory properties of bilirubin[2-5]. Interestingly, our patient, a lifelong normotensive non-smoker with normal lipidogram, suffered from ischemic heart disease and advanced atherosclerosis affecting both large arteries and peripheral circulation, especially coronary arteries.

In conclusion, our case demonstrates that DJS should be included in the differential diagnosis of liver diseases even in atypical age categories. The fact that our patient with DJS developed colorectal adenocarcinoma and clinically significant atherosclerosis indicates that Dubin-Johnson hyperbilirubinemia may not be sufficient to protect from atherogenesis and cancer development, even in the absence of established risk factors.

REFERENCES

1 **Stocker R**, Yamamoto Y, McDonagh AF, Glazer AN, Ames

BN. Bilirubin is an antioxidant of possible physiological importance. *Science* 1987; **235**: 1043-1046 [PMID: 3029864 DOI: 10.1126/science.3029864]
2 **Siow RC**, Sato H, Mann GE. Heme oxygenase-carbon monoxide signalling pathway in atherosclerosis: anti-atherogenic actions of bilirubin and carbon monoxide? *Cardiovasc Res* 1999; **41**: 385-394 [PMID: 10341838 DOI: 10.1016/S0008-6363(98)00278-8]
3 **Mayer M**. Association of serum bilirubin concentration with risk of coronary artery disease. *Clin Chem* 2000; **46**: 1723-1727 [PMID: 11067805]
4 **Vítek L**, Schwertner HA. Protective effects of serum bilirubin on peripheral vascular disease. *Ann Hepatol* 2008; **7**: 94-95 [PMID: 18376376]
5 **Lin JP**, Vitek L, Schwertner HA. Serum bilirubin and genes controlling bilirubin concentrations as biomarkers for cardiovascular disease. *Clin Chem* 2010; **56**: 1535-1543 [PMID: 20693308 DOI: 10.1373/clinchem.2010.151043]
6 **Ollinger R**, Kogler P, Troppmair J, Hermann M, Wurm M, Drasche A, Königsrainer I, Amberger A, Weiss H, Ofner D, Bach FH, Margreiter R. Bilirubin inhibits tumor cell growth via activation of ERK. *Cell Cycle* 2007; **6**: 3078-3085 [PMID: 18073533 DOI: 10.4161/cc.6.24.5022]
7 **Lacko M**, Roelofs HM, Te Morsche RH, Voogd AC, Ophuis MB, Peters WH, Manni JJ. Genetic polymorphism in the conjugating enzyme UGT1A1 and the risk of head and neck cancer. *Int J Cancer* 2010; **127**: 2815-2821 [PMID: 21351260 DOI: 10.1002/ijc.25296]
8 **Horsfall LJ**, Rait G, Walters K, Swallow DM, Pereira SP, Nazareth I, Petersen I. Serum bilirubin and risk of respiratory disease and death. *JAMA* 2011; **305**: 691-697 [PMID: 21325185 DOI: 10.1001/jama.2011.124]
9 **Keshavan P**, Schwemberger SJ, Smith DL, Babcock GF, Zucker SD. Unconjugated bilirubin induces apoptosis in colon cancer cells by triggering mitochondrial depolarization. *Int J Cancer* 2004; **112**: 433-445 [PMID: 15382069 DOI: 10.1002/ijc.20418]
10 **Temme EH**, Zhang J, Schouten EG, Kesteloot H. Serum bilirubin and 10-year mortality risk in a Belgian population. *Cancer Causes Control* 2001; **12**: 887-894 [PMID: 11808707]
11 **Zucker SD**, Horn PS, Sherman KE. Serum bilirubin levels in the U.S. population: gender effect and inverse correlation with colorectal cancer. *Hepatology* 2004; **40**: 827-835 [PMID: 15382174 DOI: 10.1002/hep.20407]
12 **Jirásková A**, Novotný J, Novotný L, Vodicka P, Pardini B, Naccarati A, Schwertner HA, Hubácek JA, Puncochárová L, Šmerhovský Z, Vítek L. Association of serum bilirubin and promoter variations in HMOX1 and UGT1A1 genes with sporadic colorectal cancer. *Int J Cancer* 2012; **131**: 1549-1555 [PMID: 22212955 DOI: 10.1002/ijc.27412]
13 **Ioannou GN**, Liou IW, Weiss NS. Serum bilirubin and colorectal cancer risk: a population-based cohort study. *Aliment Pharmacol Ther* 2006; **23**: 1637-1642 [PMID: 16696814 DOI: 10.1111/j.1365-2036.2006.02939.x]
14 **Dubin IN**, Johnson FB. Chronic idiopathic jaundice with unidentified pigment in liver cells; a new clinicopathologic entity with a report of 12 cases. *Medicine* (Baltimore) 1954; **33**: 155-197 [PMID: 13193360]
15 **Shani M**, Seligsohn U, Gilon E, Sheba C, Adam A. Dubin-Johnson syndrome in Israel. I. Clinical, laboratory, and genetic aspects of 101 cases. *Q J Med* 1970; **39**: 549-567 [PMID: 5532959]
16 **Paulusma CC**, Kool M, Bosma PJ, Scheffer GL, ter Borg F, Scheper RJ, Tytgat GN, Borst P, Baas F, Oude Elferink RP. A mutation in the human canalicular multispecific organic anion transporter gene causes the Dubin-Johnson syndrome. *Hepatology* 1997; **25**: 1539-1542 [PMID: 9185779 DOI: 10.1002/hep.510250635]
17 **Toh S**, Wada M, Uchiumi T, Inokuchi A, Makino Y, Horie Y, Adachi Y, Sakisaka S, Kuwano M. Genomic structure of the canalicular multispecific organic anion-transporter gene (MRP2/cMOAT) and mutations in the ATP-binding-cassette region in Dubin-Johnson syndrome. *Am J Hum Genet* 1999; **64**: 739-746 [PMID: 10053008 DOI: 10.1086/302292]
18 **Machida I**, Wakusawa S, Sanae F, Hayashi H, Kusakabe A, Ninomiya H, Yano M, Yoshioka K. Mutational analysis of the MRP2 gene and long-term follow-up of Dubin-Johnson syndrome in Japan. *J Gastroenterol* 2005; **40**: 366-370 [PMID: 15870973 DOI: 10.1007/s00535-004-1555-y]
19 **Haimi-Cohen Y**, Merlob P, Marcus-Eidlits T, Amir J. Dubin-Johnson syndrome as a cause of neonatal jaundice: the importance of coproporphyrins investigation. *Clin Pediatr* (Phila) 1998; **37**: 511-513 [PMID: 9729708 DOI: 10.1177/000992289803700809]
20 **Sprinz H**, Nelson RS. Persistent non-hemolytic hyperbilirubinemia associated with lipochrome-like pigment in liver cells: report of four cases. *Ann Intern Med* 1954; **41**: 952-962 [PMID: 13208040]
21 **Varma RR**, Grainger JM, Scheuer PJ. A case of the Dubin-Johnson syndrome complicated by acute hepatitis. *Gut* 1970; **11**: 817-821 [PMID: 5485831 DOI: 10.1136/gut.11.10.817]
22 **Watanabe S**, Nishioka M, Kodama T, Ando K, Numa Y, Fukumoto Y, Okita K, Takemoto T, Mizuta M. Clinicopathological studies of the Dubin-Johnson syndrome complicated with chronic hepatitis. *Gastroenterol Jpn* 1982; **17**: 576-584 [PMID: 7160601 DOI: 10.1007/BF02779135]
23 **Hunter FM**, Sparks RD, Flinner RL. Hepatitis with resulting mobilization of hepatic pigment in a patient with Dubin-Johnson syndrome. *Gastroenterology* 1964; **47**: 631-635 [PMID: 14234683]
24 **Cebecauerova D**, Jirasek T, Budisova L, Mandys V, Volf V, Novotna Z, Subhanova I, Hrebicek M, Elleder M, Jirsa M. Dual hereditary jaundice: simultaneous occurrence of mutations causing Gilbert's and Dubin-Johnson syndrome. *Gastroenterology* 2005; **129**: 315-320 [PMID: 16012956 DOI: 10.1053/j.gastro.2004.10.009]

P- Reviewer Reshetnyak VI **S- Editor** Gou SX
L- Editor Logan S **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i6.951

World J Gastroenterol 2013 February 14; 19(6): 951-954
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Successful balloon-occluded retrograde transvenous obliteration for bleeding duodenal varices using cyanoacrylate

Rintaro Hashimoto, Keitaro Sofue, Yoshito Takeuchi, Kentaro Shibamoto, Yasuaki Arai

Rintaro Hashimoto, Keitaro Sofue, Yoshito Takeuchi, Kentaro Shibamoto, Yasuaki Arai, Department of Diagnostic Radiology, National Cancer Center Central Hospital, Tokyo 104-0045, Japan
Author contributions: Hashimoto R wrote the manuscript and performed additional data analysis; Sofue K wrote the manuscript and revised it critically for important intellectual content; Takeuchi Y and Shibamoto K performed procedures; and Arai Y helped by final approval of the version to be published.
Correspondence to: Keitaro Sofue, MD, Department of Diagnostic Radiology, National Cancer Center Hospital, 5-1-1, Tsukiji, Chuo-ku, Tokyo 104-0045, Japan. keitarosofue@yahoo.co.jp
Telephone: +81-3-35422511 Fax: +81-3-35475989
Received: August 19, 2012 Revised: September 11, 2012
Accepted: October 30, 2012
Published online: February 14, 2013

Abstract

A 76-year-old woman with hepatitis C cirrhosis presented with tarry stools and hematemesis. An endoscopy demonstrated bleeding duodenal varices in the second portion of the duodenum. Contrast-enhanced computed tomography revealed markedly tortuous varices around the wall in the duodenum. Several afferent veins appeared to have developed, and the right ovarian vein draining into the inferior vena cava was detected as an efferent vein. Balloon-occluded retrograde transvenous obliteration (BRTO) of the varices using cyanoacrylate was successfully performed in combination with the temporary occlusion of the portal vein. Although no previous publications have used cyanoacrylate as an embolic agent for BRTO to control bleeding duodenal varices, this strategy can be considered as an alternative procedure to conventional BRTO using ethanolamine oleate when numerous afferent vessels that cannot be embolized are present.

© 2013 Baishideng. All rights reserved.

Key words: Bleeding duodenal varices; Balloon-occluded retrograde transvenous obliteration; Cyanoacrylate; Combination therapy; Temporary portal vein occlusion

Hashimoto R, Sofue K, Takeuchi Y, Shibamoto K, Arai Y. Successful balloon-occluded retrograde transvenous obliteration for bleeding duodenal varices using cyanoacrylate. *World J Gastroenterol* 2013; 19(6): 951-954 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i6/951.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i6.951

INTRODUCTION

Bleeding duodenal varices is a rare complication in patients with portal hypertension, occurring in only 0.4% of these patients, and is often life-threatening because of the difficulty in diagnosis and treatment[1]. Treatment options include a surgical procedure, endoscopic treatment[2], and endovascular treatment, including transjugular intrahepatic portosystemic shunts (TIPS)[3,4] and balloon-occluded retrograde transvenous obliteration (BRTO)[5-10]. Although several studies have reported successful results using BRTO alone[5-8], some difficult cases with large varices or numerous collaterals requiring a combined approach have been reported[7,9], and no previous publications have used cyanoacrylate as an embolic agent for BRTO to control bleeding duodenal varices. We herein report a case with bleeding duodenal varices that were successfully embolized using cyanoacrylate and BRTO in combination with temporary occlusion of the portal vein.

CASE REPORT

A 76-year-old woman with liver cirrhosis secondary to hepatitis C presented with tarry stools and hematemesis. An urgent endoscopy demonstrated bleeding varices in the second portion of the duodenum (Figure 1A). She had no esophageal or gastric varices. Although banding

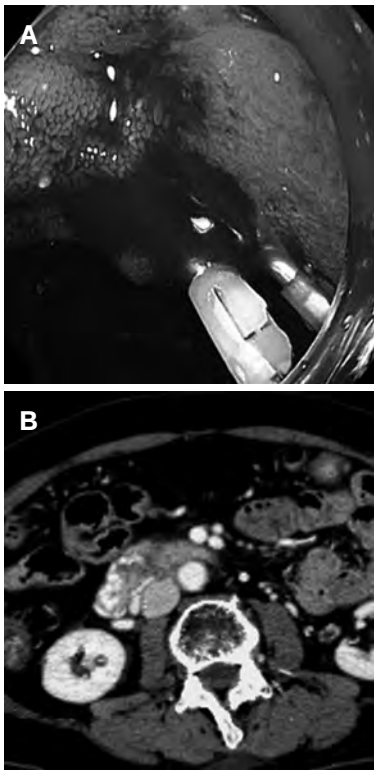


Figure 1 Endoscopy and computed tomography of the duodenum. A: Endoscopy demonstrates bleeding varices in the second portion of the duodenum; B: Contrast-enhanced computed tomography reveals markedly tortuous varices around the wall in the second and third portion of the duodenum.

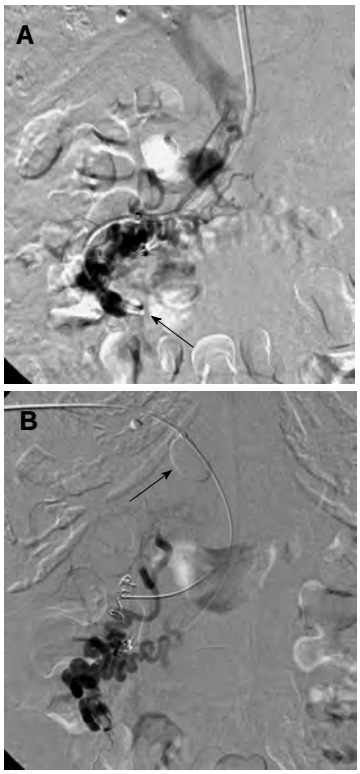


Figure 2 Balloon-occluded retrograde venography. A: Balloon-occluded retrograde venography (BRTV) shows the dilated efferent vein and the duodenal varices, but the contrast material quickly disappears through several afferent veins. Note that the balloon was inflated in the right ovarian vein (arrow); B: BRTV with occlusion of the main portal trunk (arrow) after embolization of one of the afferent veins reveals the complete opacification of the duodenal varices.

and clipping for the varices was attempted, the bleeding continued and frequent blood transfusions were required. Laboratory findings were as follows: red blood cell, 232 $\times 10^4/\mu L$; hemoglobin, 6.8 g/dL; hematocrit, 20.4%; platelets, 96 000/mL; total bilirubin, 1.36 mg/dL; serum albumin, 3.3 g/dL; and prothrombin time, 15.7 s (reference, 11.3 s). Neither ascites nor encephalopathy was observed. Child-Pugh's classification was graded as B. Contrast-enhanced computed tomography (CT) revealed markedly tortuous varices around the wall in the second and third portion of the duodenum (Figure 1B). Several afferent veins of the varices appeared to have developed, and the right ovarian vein draining into the inferior vena cava was detected as an efferent vein. We planned BRTO to embolize the duodenal varices after obtaining informed consent from the patient.

An 8-French guiding sheath introducer was inserted into the inferior vena cava *via* the right internal jugular vein. A 5.2-French, 9-mm cobra-shaped balloon catheter was inserted into the efferent vein through the right ovarian vein, and the balloon was inflated to occlude the efferent vein. Balloon-occluded retrograde venography (BRTV) showed that the dilated efferent vein and the duodenal varices were filled with contrast material, but the contrast material quickly disappeared through several afferent veins (Figure 2A). Because BRTO alone may have failed to achieve adequate sclerosant accumulation because of the leakage into the portal vein, antegrade transhepatic embolization of the afferent veins was attempted.

A 5-French sheath introducer was inserted through the left lateral portal branch, and one of the afferent veins was embolized using two microcoils (MicroNester coil; Cook, Inc, Bloomington, Indiana, United States). However, several remaining afferent veins could not be embolized, and the contrast material also disappeared through the afferent veins. We then placed a balloon catheter into the main portal trunk to control the hepatopetal flow of the afferent veins. BRTV with occlusion of the main portal trunk revealed the disappearance of the hepatopetal portal flow and complete opacification of the duodenal varices (Figure 2B). A microcatheter was coaxially advanced to the duodenal varices through the retrograde route, and a total of 4 mL of 20% cyanoacrylate with ethiodized oil was injected into the duodenal varices (Figure 3).

The following day, a contrast-enhanced CT examination confirmed the complete accumulation of the ethiodized oil replacement in the duodenal varices (Figure 4) and the patency of either portal vein or systemic circulation. Liver function was preserved after the procedure. Four days after the procedure, an endoscopy showed that hemostasis of the bleeding duodenal varices had been achieved. No evidence of bleeding of the duodenal varices was found on follow-up CT and endoscopy examinations performed four months after the procedure.



Figure 3 Radiograph after embolization of the duodenal varices demonstrates complete and adequate accumulation of the ethiodized oil in the varices.



Figure 4 Contrast-enhanced computed tomography after embolization of the duodenal varices shows the complete accumulation of ethiodized oil in the varices.

DISCUSSION

BRTO is an established endovascular treatment for gastric varices[11] but has only been described for the treatment of bleeding duodenal varices in a few reports with limited numbers of clinical patients[5-10]. The advantages of BRTO over TIPS for duodenal varices are that it can completely embolize targeted varices and that it does not reduce portal flow, avoiding further exacerbation of hepatic function and encephalopathy without a significant mortality rate[4,8]. However unlike gastric varices, successful treatment with BRTO alone for duodenal varices is not always feasible and often require combined therapies with an endoscopic or anterograde transhepatic approach, as significant communications or complex hemodynamics between the efferent and afferent veins often complicate treatment and necessitate combined therapy[7,9].

In the present case, some of the afferent veins may have enabled collateral hepatopetal flow during balloon occlusion of the afferent vein, and pressure among the duodenal varices varied, resulting in insufficient filling with the contrast material. At first, coil embolization was attempted *via* a transhepatic portal venous approach, as reported by previous investigators[5,7-9], but not all the afferent veins could be embolized because of the difficulty in catheterizing the tortuous vessels. Second, we performed temporary balloon occlusion of the portal vein. This method was effective because a change in the hemodynamics of the duodenal varices occurred. Temporary occlusion of the main portal trunk may increase the pressure of hepatopetal flow, and the direction of flow in the afferent veins changes from hepatopetal to hepatofugal. This mechanism is similar to that of temporary balloon occlusion of the splenic artery during BRTO for gastric varices to control the portal pressure gradient[12].

In our case, we used cyanoacrylate, not ethanolamine oleate, as a sclerosant. Every investigator has used ethanolamine oleate as the most suitable sclerosant during BRTO for duodenal varices[5-10]. However, ethanolamine oleate was not suitable in our case, because it requires several hours to achieve full effort and may increase the risk of portal venous thrombosis under temporary portal venous balloon occlusion. On the other hand, cyanoacrylate rapidly solidifies with fast polymerization upon exposure to an ionic solution[13], and we believe that this was the best way of minimizing the duration of portal venous occlusion. The potential shortcomings of cyanoacrylate are adhesion to the balloon catheter system or inadvertent embolization upon balloon removal. This should be kept in mind as a note of caution whenever attempting to use cyanoacrylate. To prevent this complication, it would be advantageous to ensure that a microcatheter is advanced to the targeted duodenal varices and only duodenal varices are embolized, with minimal volume of cyanoacrylate.

Endoscopic injection sclerotherapy using cyanoacrylate has been performed as an effective measure[2], but it has the drawback of perforation, tissue injury, and unclear visualization because of massive hemorrhage. Moreover, endoscopic injection of cyanoacrylate also carries a risk of embolism of either portal vein or systemic circulation[14]. Endovascular injection of cyanoacrylate can prevent untargeted embolization such as portal vein or pulmonary artery, confirming the hemodynamics of the duodenal varices using contrast material.

Bleeding duodenal varices is a rare condition that is difficult to diagnose and is potentially life-threatening. BRTO using cyanoacrylate was successfully performed for control of bleeding duodenal varices in the present case. This is an alternative procedure to conventional BRTO using ethanolamine oleate when insufficient filling of the varices with sclerosant occurs and several afferent vessels cannot be adequately embolized.

REFERENCES

1 **Hashizume M**, Tanoue K, Ohta M, Ueno K, Sugimachi K, Kashiwagi M, Sueishi K. Vascular anatomy of duodenal varices: angiographic and histopathological assessments. *Am J Gastroenterol* 1993; **88**: 1942-1945 [PMID: 8237946]

2 **Kang HY**, Lee WK, Kim YH, Kwon BW, Kang MS, Kim SB, Song IH. Ruptured duodenal varices arising from the main portal vein successfully treated with endoscopic injection sclerotherapy: a case report. *Korean J Hepatol* 2011; **17**: 152-156 [PMID: 21757987 DOI: 10.3350/kjhep.2011.17.2.152]

3 **Lopera JE**, Arthurs B, Scheuerman C, Sandoz C, Petersosn S, Castaneda-Zuniga W. Bleeding duodenal: varices treatment by TIPS and transcatheter embolization. *Cardiovasc Intervent Radiol* 2008; **31**: 431-434 [PMID: 17593432]

4 **Kim MJ**, Jang BK, Chung WJ, Hwang JS, Kim YH. Duodenal variceal bleeding after balloon-occluded retrograde transverse obliteration: treatment with transjugular intrahepatic portosystemic shunt. *World J Gastroenterol* 2012; **18**: 2877-2880 [PMID: 22719200 DOI: 10.3748/wjg.v18.i22.2877]

5 **Haruta I**, Isobe Y, Ueno E, Toda J, Mitsunaga A, Noguchi S, Kimura T, Shimizu K, Yamauchi K, Hayashi N. Balloon-occluded retrograde transvenous obliteration (BRTO), a promising nonsurgical therapy for ectopic varices: a case report of successful treatment of duodenal varices by BRTO. *Am J Gastroenterol* 1996; **91**: 2594-2597 [PMID: 8946993]

6 **Ohta M**, Yasumori K, Saku M, Saitsu H, Muranaka T, Yoshida K. Successful treatment of bleeding duodenal varices by balloon-occluded retrograde transvenous obliteration: a transjugular venous approach. *Surgery* 1999; **126**: 581-583 [PMID: 10486613 DOI: 10.1016/S0039-6060(99)70102-9]

7 **Ota K**, Okazaki M, Higashihara H, Kokawa H, Shirai Z, Anan A, Kitamura Y, Shijo H. Combination of transileocolic vein obliteration and balloon-occluded retrograde transvenous obliteration is effective for ruptured duodenal varices. *J Gastroenterol* 1999; **34**: 694-699 [PMID: 10588186 DOI: 10.1007/s005350050321]

8 **Sonomura T**, Horihata K, Yamahara K, Dozaiku T, Toyonaga T, Hiroka T, Sato M. Ruptured duodenal varices successfully treated with balloon-occluded retrograde transvenous obliteration: usefulness of microcatheters. *AJR Am J Roentgenol* 2003; **181**: 725-727 [PMID: 12933468]

9 **Zamora CA**, Sugimoto K, Tsurusaki M, Izaki K, Fukuda T, Matsumoto S, Kuwata Y, Kawasaki R, Taniguchi T, Hirota S, Sugimura K. Endovascular obliteration of bleeding duodenal varices in patients with liver cirrhosis. *Eur Radiol* 2006; **16**: 73-79 [PMID: 15856238 DOI: 10.1007/s00330-005-2781-2]

10 **Tanaka O**, Ohno K, Ohno T, Tomioka H, Shimizu S, Yamagami T, Nishimura T. Should balloon-occluded retrograde transvenous obliteration be the first-line interventional radiologic treatment for bleeding duodenal varices? A case report and review of the literature. *Acta Radiol* 2008; **49**: 32-36 [PMID: 17963080 DOI: 10.1080/02841850701633049]

11 **Fukuda T**, Hirota S, Sugimoto K, Matsumoto S, Zamora CA, Sugimura K. "Downgrading" of gastric varices with multiple collateral veins in balloon-occluded retrograde transvenous obliteration. *J Vasc Interv Radiol* 2005; **16**: 1379-1383 [PMID: 16221910 DOI: 10.1016/S1051-0443(07)61912-5]

12 **Kiyosue H**, Tanoue S, Kondo Y, Maruno M, Takaji R, Matsuoto S, Ueda S, Mori H. Balloon-occluded retrograde transvenous obliteration of complex gastric varices assisted by temporary balloon occlusion of the splenic artery. *J Vasc Interv Radiol* 2011; **22**: 1045-1048 [PMID: 21708324 DOI: 10.1016/j.jvir.2010.12.028]

13 **Pollak JS**, White RI. The use of cyanoacrylate adhesives in peripheral embolization. *J Vasc Interv Radiol* 2001; **12**: 907-913 [PMID: 11487670 DOI: 10.1016/S1051-0443(07)61568-1]

14 **Miyakoda K**, Takedatsu H, Emori K, Inoue H, Toyonaga A, Mitsuyama K, Tsuruta O, Sata M. N-butyl-2-cyanoacrylate (histoacryl) glue in the right atrium after endoscopic injection for a ruptured duodenal varix: complication of histoacryl injection. *Dig Endosc* 2012; **24**: 192 [PMID: 22507099 DOI: 10.1111/j.1443-1661.2011.01185.x]

P- Reviewer Rerknimitr R **S- Editor** Jiang L
L- Editor O'Neill M **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i6.955

World J Gastroenterol 2013 February 14; 19(6): 955-959
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Rescue endoscopic band ligation of iatrogenic gastric perforations following failed endoclip closure

Joung-Ho Han, Tae Hoon Lee, Yunho Jung, Suck-Ho Lee, Hyun Kim, Hye-Suk Han, Heebok Chae, Seon Mee Park, Seijin Youn

Joung-Ho Han, Hyun Kim, Hye-Suk Han, Heebok Chae, Seon Mee Park, Seijin Youn, Division of Gastroenterology, Department of Internal medicine, Chungbuk National University College of Medicine, Cheongju 361-711, South Korea
Tae Hoon Lee, Yunho Jung, Suck-Ho Lee, Division of Gastroenterology, Department of Internal Medicine, Soon Chun Hyang University College of Medicine, Cheonan Hospital, Cheonan 330-721, South Korea
Author contributions: Han JH and Lee TH contributed equally to this work; Jung Y, Park SM, Kim H, Han HS, Chae H, Lee SH and Youn S provided clinical advice and analyzed the data; Han JH and Lee SH performed the procedures; Han JH and Lee TH designed case report; and Lee TH and Han JH wrote the paper.
Supported by Grant of the Korea Healthcare Technology R and D Project, Ministry of Health and Welfare, South Korea, No. A100054
Correspondence to: Tae Hoon Lee, MD, PhD, Division of Gastroenterology, Department of Internal Medicine, Soon Chun Hyang University College of Medicine, Cheonan Hospital, 23-20 Bongmyung-dong, Cheonan-si, Cheonan 330-721, South Korea. thlee9@lycos.co.kr
Telephone: +82-41-5703662 Fax: +82-41-5745762
Received: October 3, 2012 Revised: November 6, 2012
Accepted: November 11, 2012
Published online: February 14, 2013

Abstract

Iatrogenic gastric perforation is one of the most serious complications during therapeutic endoscopy, despite significant advances in endoscopic techniques and devices. This case study evaluated the clinical efficacy and safety of the rescue endoscopic band ligation (EBL) technique in iatrogenic gastric wall perforation following the failure of primary endoclip closure. Five patients were enrolled in this study. These patients underwent emergency endoscopy following the onset of acute gastric wall perforation during endoscopic procedures. The outcome measurements were primary technical success and immediate or delayed procedure-related complications. Successful endoscopic closure using band ligation was reported in all patients, with no complication occurring. We conclude that EBL may be a feasible and safe alternate technique for the management of acute gastric perforation, especially in cases where closure is difficult with endoclips.

© 2013 Baishideng. All rights reserved.

Key words: Gastric perforation; Endoscopy; Band ligation

Han JH, Lee TH, Jung Y, Lee SH, Kim H, Han HS, Chae H, Park SM, Youn S. Rescue endoscopic band ligation of iatrogenic gastric perforations following failed endoclip closure. *World J Gastroenterol* 2013; 19(6): 955-959 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i6/955.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i6.955

INTRODUCTION

The management of gut perforation, including successful primary endoscopic repair, has been increasingly reported in endoscopic trials. Primary endoscopic therapy is now often recommended as an alternative to surgical intervention in select cases[1-3]. However, the commonly used endoclip technique for endoscopic closure has some limitations, depending on the perforation size and anatomic site. Endoscopic clip closure may be difficult for a large perforation, one with a tangential angle, and/or on a necrotic or ulcerated surface. Even when clipping is successful, dehiscence might occur due to strong wall tension. Newly developed devices have been introduced and show promise, but many of these devices are expensive, require additional equipment, and are not readily available in many countries[2,3].

Endoscopic band ligation (EBL) is commonly performed in the management of varix or Dieulafoy's bleed-

ing[4-6]. EBL is a safe and feasible method that can reduce procedure time and be useful in the primary repair of colonic or duodenal perforations[7-9]. Our group previously reported the use of EBL to successfully close two colonic perforations in which endoclip closure initially failed due to the presence of a large perforation and a severe tangential angle in endoscopic procedures assisted with a transparent cap[9].

In this case study, we evaluated the clinical efficacy and safety of the rescue EBL technique in iatrogenic gastric wall perforations in which primary endoclip closure failed.

CASE REPORT

Patients with acute gastric wall perforations were enrolled in this study between September 2011 and August 2012. Written informed consent was obtained from all patients and the study was approved by the institutional ethics committee.

All patients underwent emergency endoscopy following the onset of acute gastric wall perforation during endoscopic procedures. Primary endoscopic closure using an endoclip (Endoclip HX-600-090L; Olympus Optical Co., Ltd., Tokyo, Japan) was attempted initially, immediately upon recognition of the perforation. Patients in whom primary endoclip closure failed or was technically difficult subsequently underwent rescue EBL to achieve closure. After endoscopic confirmation of the perforation site, the endoscope was withdrawn and reinserted after attachment of a pneumoactive, single-band ligator (MD-48709; Akita Sumitomo Bakelite Co, Ltd, Tokyo, Japan). The hood of the ligation device was then placed over the target lesion site. Following successful ligation of the approximate targeted edge of the perforation, additional bands or clips were used to completely close the site (Figure 1). After endoscopic closure, patients' recovery was managed with vital sign monitoring, no oral intake, and intravenous fluid therapy with broad-spectrum intravenous antibiotics and acid suppression. Early oral intake was allowed when clinical symptoms such as abdominal pain or fever resolved, appetite and bowel function returned, and laboratory test values were normalized. Surgical intervention was planned if the patient's clinical condition deteriorated.

The outcome measurements were primary technical success and immediate or delayed procedure-related complications. All endoscopic procedures, including EBL, were performed by experienced faculty endoscopists in tertiary referral centers.

Table 1 presents the clinical characteristics of five patients who underwent rescue closure by EBL. Gut perforation rates following therapeutic endoscopic procedures were ranging from 0.9% to 1.8% in two participated units. Three patients underwent endoscopic mucosal resection with ligation (EMR-L) due to gastric adenoma (n = 2) or neuroendocrine tumor (n = 1); one patient received endoscopic submucosal dissection due to adenocarcinoma; and one patient underwent endoscopic biopsy due to a chronic gastric ulcer. The mean perforation size was 8.6 (range 5-11) mm. The primary causes of endoclip failure were difficulty in approximating the location of adjacent gastric mucosa due to wall tension and a tangential angle. In the case of ulcer base perforation occurred by biopsy (iatrogenic perforation following biopsy; Figure 2), the fibrotic ulcer base made endoscopic clipping difficult.

Table 1 Baseline patient characteristics

No.	Age (yr)/sex	Diagnosis	Cause	Perforation location	Size (mm)	Causes of clip failure
1	65/M	Neuroendocrine tumor	EMR-L	Fundus	9	Tangential angle and wall tension
2	52/M	Gastric adenoma	EMR-L	Angle	11	Severe belching
3	68/M	Gastric adenoma	EMR-L	Upper body, great curvature	8	Tangential angle
4	91/F	Gastric ulcer	Biopsy	Angle	5	Fibrotic tissue and tension
5	73/M	Adenocarcinoma	ESD	Antrum, great curvature	10	Friable mucosa

EMR-L: Endoscopic mucosal resection with ligation; ESD: Endoscopic submucosal dissection; M: Male; F: Female.

Table 2 Procedure outcomes of rescue endoscopic band ligation

No.	Procedure time for endoclips (s)	Procedure time for EBL (s)	No. of bands	No. of clips before/after EBL	Success	Days until diet resumption/discharge
1	198	66	1	3/3	Yes	3/7
2	102	42	2	2/1	Yes	4/5
3	138	78	1	0/4	Yes	2/4
4	894	334	2	6/0	Yes	7/14
5	353	39	1	1/2	Yes	4/7

EBL: Endoscopic band ligation.

The rescue EBL technique was performed successfully in all patients (Table 2). The mean procedure time for complete band ligation was 111.8 (range 39-334) s. Two band ligations were performed in the cases of large and ulcer base perforations. Additional endoclips were applied in four cases to achieve complete closure. No procedure-related complication or delayed dehiscence occurred following successful EBL with or without clipping. Patients resumed their normally scheduled diet an average of 4 (range 2-7) d after the procedure, and were discharged an average of 7.4 (range 4-14) d after the procedure.

DISCUSSION

Iatrogenic gut perforations occurring during endoscopic procedures are generally managed surgically. Although surgical operation remains the gold standard treatment for intestinal perforation, the clinician's familiarity with endoclips and their immediate availability and proper use

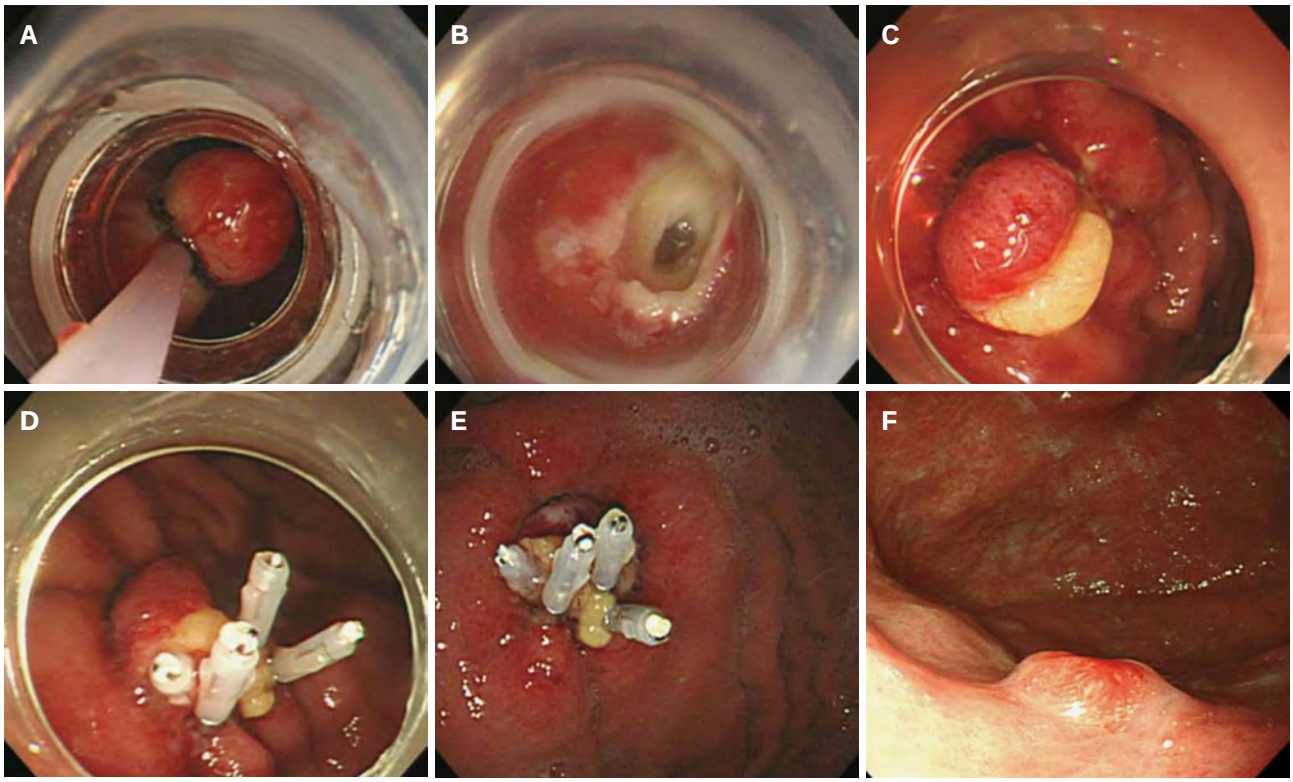


Figure 1 Endoscopic band ligation in iatrogenic gastric wall perforation. A: Endoscopic view of endoscopic mucosal resection with ligation (EMR-L) due to gastric adenoma on the greater curvature of the upper gastric body; B: Iatrogenic gastric wall perforation following EMR-L; C: Primary endoscopic band ligation (EBL) was successful following technical difficulty with endoclip closure; D: Additional clips were applied around the band and surrounding mucosa; E: Follow-up endoscopy 1 d later shows band and multiple clips, with no complication; F: Endoscopic view 1 mo after EBL, showing the absence of the band and clips.

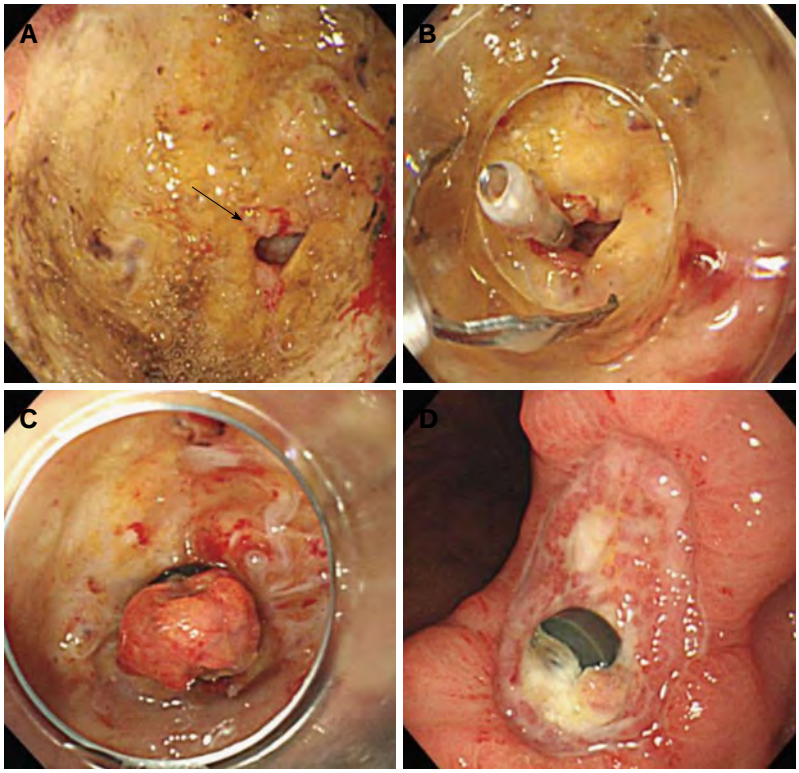


Figure 2 Endoscopic band ligation in iatrogenic ulcer base perforation following endoscopic biopsy. A: Iatrogenic gastric ulcer base perforation following biopsy (arrow); B: The fibrotic ulcer base made endoscopic clipping difficult; C: Successful endoscopic band ligation following technical difficulty with endoclip closure; D: Endoscopic view 2 wk later, showing the healed base of ulcer with remaining bands.

may replace surgery for a selected group of patients with a high surgical risk. The mainstays of treatment of early perforations without systemic upset are nasogastric suction, antibiotics, bowel rest, and parenteral nutrition. This type of conservative management may be undertaken in patients with asymptomatic perforations or localized

peritonitis that is expected to improve clinically without complication[1,10-14]. In presented cases, perforation was recognized immediately during procedure, and treated by endoscopic band and clipping successfully. So that, did not use nasogastic suction. However, in patients whose condition deteriorates despite conservative management or alternative endoscopic management, surgical treatment should be considered immediately.

Recent studies have reported high technical success rates for primary closure of an acute iatrogenic perforation with endoclips, newly developed devices, or a band[1,10-12]. Endoscopic repair using endoclips can be limited in large perforations or in those with tangential angles. A wide perforation is difficult to close because of slippage of the perforation edge from the clip while the clip is maneuvered across the defect to grasp the opposite edge of the perforation. Everted perforation edges also make it impossible to grasp the tissue with endoclips. Newly developed devices have recently been introduced and successfully used to close perforations[15]. These devices include through-the-scope (TTS) clips, such as the QuickClip 2 (Olympus Inc., Center Valley, PA, United States), the Resolution clip (Boston Scientific Inc., Natick, MA, United States), and the Tri-Clip and Instinct clip (Cook Medical, Winston-Salem, NC, United States); the over-the-scope clip (OTSC) system (Ovesco Endoscopy AG, Tubingen, Germany); and endoscopic suturing devices such as T-tags (Ethicon Endo-Surgery, Cincinnati, OH, United States) and the flexible Endo Stitch (Covidien, Mansfield, MA, United States). Closure of luminal perforation > 20 mm in size may be difficult. For larger gastric defects, TTS clips can be placed around the circumference of the perforation and lassoed together with a detachable plastic snare (Endo-loop; Olympus)[16]. Among the newly developed devices, OTSC was approved for the closure of perforation < 20 mm in size, and ex vivo studies shown that colon defects 10 to 30 mm in size can be closed with a single OTSC[2]. However, although some techniques have been developed to correct deficits in clip placement, they are not commonly practiced. Some of these devices may prove suitable for the closure of defects throughout the intestinal tract, but their use is limited by the endoscopist's experience, device availability, and cost[2]. Currently, no particular technique has demonstrated proven efficacy or greater reliability than other closure modalities.

EBL was first used in 1988 to treat bleeding from esophageal varices[15]. The simplicity of the technique and low complication rates compared with sclerotherapy have contributed to its growing popularity[4]. Technically, EBL is a simple procedure, even if the targeted site must be approached tangentially or is located in the posterior wall of the proximal body. EBL has been widely used in the management of non-variceal hemorrhage from Dieulafoy's ulcer, gastric angiodysplasia, and polypectomy-induced bleeding[4-6]. In addition, several reports have described the use of EBL in rectal and duodenal perforations during EMR[7-9]. Our group previously reported the use of EBL to successfully close two colonic perforations when endoscopic closure with endoclips initially failed[9]. Primary endoscopic repair can be difficult in some cases because the clips may not hold the tissue of large perforations together successfully, the tissue may slip in perforations with everted edges, or a fibrotic ulcer base may hinder successful manipulation. In contrast, acute perforations with no hardening can be readily closed with suction and band ligation.

Theoretically, EBL can readily approximate both edges of the perforation. Thus, complete suture to the remaining wall by additional bands or endoclips may be simple, even with a large perforation. EBL can also reduce procedure time in comparison with clipping. Immediate closure could prevent the need for surgery or the development of serious peritonitis caused by gastric content leakage. Additionally, in the case of ulcer base perforation, clipping of the fibrotic membrane is difficult due to strong wall tension, which may result in tearing or dehiscence after closure. EBL with suction could reduce such damage throughout the lesion. No dehiscence occurred after EBL in our cases. Finally, the use of additional clips to suture the perforation after EBL might not be necessary. Follow-up endoscopy has detected clip deterioration, with only the band holding the perforated mucosa tightly. Prudent banding may thus be important for successful closure, and one or more band ligations may be sufficient. However, due to the limited number of cases included in our study, we could not confirm that additional clipping is unnecessary, and we generally recommend additional clipping to maintain tight closure of the primary band.

Several factors can be identified as limitations in our study. Our case study was not comparative and included a limited number of patients, preventing us from drawing concrete conclusions. Also, this result cannot be generalized due to an experimental trial. Additionally, we did not evaluate closure dehiscence or ischemic necrosis following band ligation. Although ischemic necrosis induced by band ligation is limited to the mucosa or submucosa, further prospective studies should be conducted to examine whether the perforation risk may be lower with conventional sclerosant injection therapy, heater probe therapy, or electrocoagulation[4,17]. Secondary risks may also develop due to suction of the adjacent mesentery. None of these complications was observed in our study. In summary, EBL can be used as a rescue method to repair full-thickness perforations and may facilitate complete repair by enabling the approximation of the perforation when initial band ligation is not sufficient to achieve complete closure. EBL may be a feasible and safe alternate technique for the management of acute gastric perforation, especially in cases where closure with endoclips is difficult due to tangential angles, severe belching, or narrow space availability. To evaluate the suitability of EBL for wide clinical use, comparative controlled studies should be conducted.

REFERENCES

1 **Lee TH**, Bang BW, Jeong JI, Kim HG, Jeong S, Park SM, Lee DH, Park SH, Kim SJ. Primary endoscopic approximation suture under cap-assisted endoscopy of an ERCP-induced duodenal perforation. *World J Gastroenterol* 2010; **16**: 2305-2310 [PMID: 20458771]

2 **Baron TH**, Wong Kee Song LM, Zielinski MD, Emura F, Fotoohi M, Kozarek RA. A comprehensive approach to the management of acute endoscopic perforations (with videos). *Gastrointest Endosc* 2012; **76**: 838-859 [PMID: 22831858 DOI: 10.1016/j.gie.2012.04.476]

3 **von Renteln D**, Schmidt A, Vassiliou MC, Rudolph HU, Gieselmann M, Caca K. Endoscopic closure of large colonic perforations using an over-the-scope clip: a randomized controlled porcine study. *Endoscopy* 2009; **41**: 481-486 [PMID: 19533550]

4 **Van Stiegmann G**, Goff JS. Endoscopic esophageal varix ligation: preliminary clinical experience. *Gastrointest Endosc* 1988; **34**: 113-117 [PMID: 3259195]

5 **Brown GR**, Harford WV, Jones WF. Endoscopic band ligation of an actively bleeding Dieulafoy lesion. *Gastrointest Endosc* 1994; **40**: 501-503 [PMID: 7926548]

6 **Slivka A**, Parsons WG, Carr-Locke DL. Endoscopic band ligation for treatment of post-polypectomy hemorrhage. *Gastrointest Endosc* 1994; **40**: 230-232 [PMID: 8013830]

7 **Moon SY**, Park CH, Yoon SM, Chung Ys, Chung YW, Kim KO, Hahn TH, Yoo KS, Park SH, Kim JH, Park CK. Repair of an endoscopic submucosal dissection-induced rectal perforation with band ligation. *Gastrointest Endosc* 2009; **69**: 160-161; discussion 161 [PMID: 19111698]

8 **Fan CS**, Soon MS. Repair of a polypectomy-induced duodenal perforation with a combination of hemoclip and band ligation. *Gastrointest Endosc* 2007; **66**: 203-205 [PMID: 17521637]

9 **Han JH**, Park S, Youn S. Endoscopic closure of colon perforation with band ligation; salvage technique after endoclip failure. *Clin Gastroenterol Hepatol* 2011; **9**: e54-e55 [PMID: 21195795]

10 **Kaneko T**, Akamatsu T, Shimodaira K, Ueno T, Gotoh A, Mukawa K, Nakamura N, Kiyosawa K. Nonsurgical treatment of duodenal perforation by endoscopic repair using a clipping device. *Gastrointest Endosc* 1999; **50**: 410-413 [PMID: 10462667]

11 **Katsinelos P**, Paroutoglou G, Papaziogas B, Beltsis A, Dimiropoulos S, Atmatzidis K. Treatment of a duodenal perforation secondary to an endoscopic sphincterotomy with clips. *World J Gastroenterol* 2005; **11**: 6232-6234 [PMID: 16273659]

12 **Baron TH**, Gostout CJ, Herman L. Hemoclip repair of a sphincterotomy-induced duodenal perforation. *Gastrointest Endosc* 2000; **52**: 566-568 [PMID: 11023583]

13 **Iqbal CW**, Cullinane DC, Schiller HJ, Sawyer MD, Zietlow SP, Farley DR. Surgical management and outcomes of 165 colonoscopic perforations from a single institution. *Arch Surg* 2008; **143**: 701-706; discussion 706-707 [PMID: 18645114]

14 **Damore LJ**, Rantis PC, Vernava AM, Longo WE. Colonoscopic perforations. Etiology, diagnosis, and management. *Dis Colon Rectum* 1996; **39**: 1308-1314 [PMID: 8918445]

15 **Mangiavillano B**, Viaggi P, Masci E. Endoscopic closure of acute iatrogenic perforations during diagnostic and therapeutic endoscopy in the gastrointestinal tract using metallic clips: a literature review. *J Dig Dis* 2010; **11**: 12-18 [PMID: 20132426]

16 **Matsuda T**, Fujii T, Emura F, Kozu T, Saito Y, Ikematsu H, Saito D. Complete closure of a large defect after EMR of a lateral spreading colorectal tumor when using a two-channel colonoscope. *Gastrointest Endosc* 2004; **60**: 836-838 [PMID: 15557972]

17 **Akahoshi K**, Yoshinaga S, Fujimaru T, Harada N, Nawata H. Hypertonic saline-epinephrine injection plus endoscopic band ligation therapy for gastric Dieulafoy's lesion. *J Gastroenterol* 2003; **38**: 911-912 [PMID: 14564640]

P- Reviewers Cho YS, Drastich P, Farias AQ **S- Editor** Wen LL
L- Editor A **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i6.960

World J Gastroenterol 2013 February 14; 19(6): 960-963
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Misdiagnosis of left supraclavicular lymph node metastasis of hepatocellular carcinoma: A case report

Tao Liu, Jun-Fang Gao, Yong-Xiang Yi, Hai Ding, Wei Liu

Tao Liu, Yong-Xiang Yi, Hai Ding, Department of Hepatobiliary Surgery, the Second Affiliated Hospital of Southeast University, Nanjing 210003, Jiangsu Province, China
Jun-Fang Gao, Department of Hepatic Surgery and Liver Transplantation Centre, the First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China
Wei Liu, Department of Pathology, the Second Affiliated Hospital of Southeast University, Nanjing 210003, Jiangsu Province, China
Author contributions: Liu T conceptualized the case report and wrote the paper as a major contributor; Gao JF, Yi YX and Ding H were involved in drafting the article and revising it for intellectual content; Liu W carried out the pathological analysis; and all authors read and approved the final manuscript.
Correspondence to: Tao Liu, MD, Department of Hepatobiliary Surgery, the Second Affiliated Hospital of Southeast University, Nanjing 210003, Jiangsu Province, China. lt9821@163.com
Telephone: +86-25-83626393 Fax: +86-25-83626227
Received: November 1, 2012 Revised: December 18, 2012
Accepted: January 11, 2013
Published online: February 14, 2013

Abstract

Left supraclavicular lymph node metastasis is a rare presentation of hepatocellular carcinoma (HCC). This phenomenon is easily neglected in the clinic. A 56-year-old man presented with HCC. On examination, a 1cm long left supraclavicular lymph node was palpated. Auxiliary examination indicated a lesion located in the right lobe of the liver. Fine needle aspiration cytology (FNAC) of the enlarged lymph node was performed; however, only necrosis was found. Hepatectomy was performed and HCC was confirmed by Hematoxylin-Eosin staining. However, 14 d after surgery, significantly enlarged left supraclavicular lymph nodes, a new intrahepatic lesion, and pulmonary and mediastinal metastasis appeared. An excisional biopsy of the left supraclavicular lymph node was performed, and its findings confirmed metastatic HCC. The patient's HCC rapidly progressed and he died one month later. It is possible for HCC to metastasize to the left supraclavicular lymph node. Surgeons should always consider an overall physical examination. When left supraclavicular lymphadenopathy of unknown origin is encountered, FNAC should be performed initially. If the results are negative, an excisional biopsy and subsequent Positron emission tomography - computed tomography scanning should be performed. These are very important for making the correct diagnosis and for selecting reasonable therapies.

© 2013 Baishideng. All rights reserved.

Key words: Left supraclavicular lymph node; Metastasis; Hepatocellular carcinoma; Fine needle aspiration cytology; Misdiagnosis

Liu T, Gao JF, Yi YX, Ding H, Liu W. Misdiagnosis of left supraclavicular lymph node metastasis of hepatocellular carcinoma: A case report. *World J Gastroenterol* 2013; 19(6): 960-963 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i6/960.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i6.960

INTRODUCTION

Hepatocellular carcinoma (HCC) is a common malignancy of the digestive tract. The main route of spread is hematogenous, that is, by invasion of the portal venous system or hepatic venous system[1]. Lymphatic metastases are uncommon. For patients with HCC larger than 5 cm, tumor-related factors predict outcomes and survival[2]. Left supraclavicular lymphadenopathy may be the sign of a metastatic tumor, mostly from lung cancer, gastric cancer, nasopharyngeal cancer and breast cancer[3]. However, HCC rarely metastasizes to the left supraclavicular lymph node and few relevant reports are available in the literature. Here, we present a case of HCC that manifested as left supraclavicular lymphadenopathy and analyze the reasons for the initial misdiagnosis.

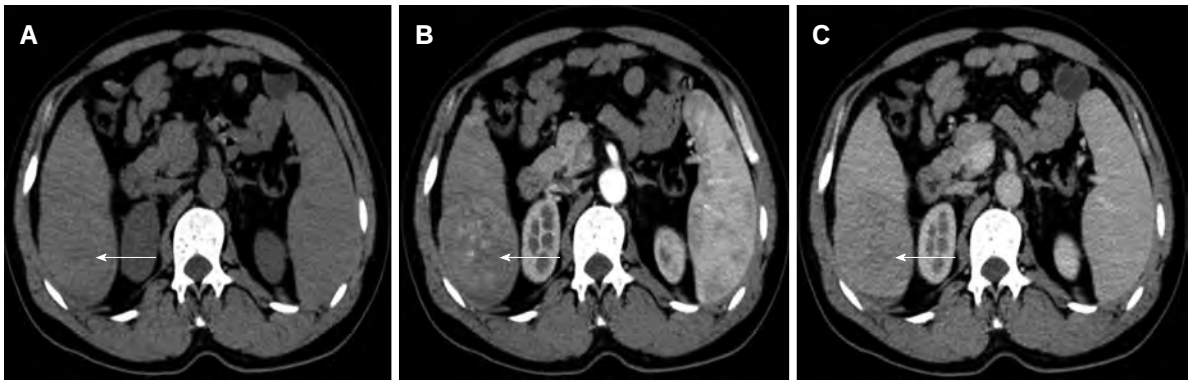


Figure 1 Abdominal computed tomographic images before surgery. A: Non-contrast-enhanced computed tomography scan showing the lesion (arrow) located in the right lobe of the liver; B: The lesion (arrow) showed enhancement during the hepatic arterial phase; C: The lesion (arrow) showed no enhancement during the portal phase.

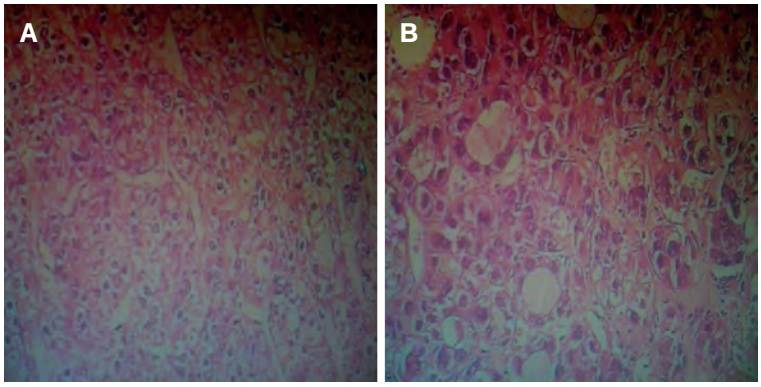


Figure 2 Histopathology of the primary tumor and enlarged left supraclavicular lymph node. A: Cancer cells having oval and single nuclei, with less mitosis and less heteromorphosis in the primary lesion; hematoxylin and eosin (HE) stain, ×400; B: The same changes in cell morphology in left supraclavicular lymph node; HE stain, ×400.

CASE REPORT

A 56-year-old man suffering from HCC was referred to our hospital. His chief complaint was abdominal pain. He had hypertension that was successfully treated with oral medication. On examination, a 1 cm long left supraclavicular lymph node was palpated and it was slightly tender. Examination of the chest did not reveal any signs of bronchospasm. Varicose veins on the thoracic and abdominal wall were not observed. Neurological examination did not demonstrate any deficit. Laboratory examination revealed a platelet count of 89×10^9/L. His alpha-fetoprotein (AFP) level was 695.4 ng/mL (normal < 10.9 ng/mL). Serum hepatitis B surface antigen was positive. HBV DNA quantification was within normal limits. Renal and liver function tests, coagulation function, electronic laryngoscopy and gastroscopy were also normal. Abdominal computed tomography indicated an 8 cm lesion located in the right lobe of the liver (VI segment) (Figure 1A), which was showed significant enhancement during the hepatic arterial phase (Figure 1B), but no enhancement during the portal phase (Figure 1C). The vascular system was normal, and no enlarged lymph nodes were found. No new lesions were found by contrast-enhanced ultrasound. A chest X-ray revealed no lung metastasis. Fine needle aspiration cytology (FNAC) of the enlarged left supraclavicular lymph node showed only necrosis. A hepatectomy was performed without hepatic hilar occlusion. Doppler ultrasound was used to define the tumor margin during the operation. The dissection line was 1 cm outside the tumor margin. Diagnosis was confirmed as HCC with moderate differentiation by Hematoxylin-Eosin staining (Figure 2A), and the resection margin was negative. Initially, the patient showed a good recovery. However, 14 d after surgery, enlarged left supraclavicular lymph nodes were observed to be firm and fixed. To exclude the possibility of malignant lymphoma, an excisional biopsy of the left supraclavicular lymph node was performed, and its findings confirmed metastatic HCC (Figure 2B). Thoracic computed tomography showed significantly enlarged left supraclavicular lymph nodes (Figure 3A), part of which were fused into masses (Figure 3B). Their diameters ranged from 2 cm to 6 cm. Enlarged lymph nodes appeared in mediastinum (Figure 3C) and round nodules were distributed in both lungs (Figure 3D and E). A new lesion was detected in the liver *via* Doppler ultrasound (Figure 3F). Laboratory examination revealed an AFP level > 1050 ng/mL. The patient rapidly declined and died one month later.

DISCUSSION

Distant metastasis of HCC occurs in three main ways: Hematogenous dissemination, lymphatic metastasis and implantation metastasis. Hematogenous dissemination is common, whereas lymphatic metastasis is uncommon. Lymphatic metastasis has been documented in 25.5% of patients at different stages of HCC. Hilar lymph nodes

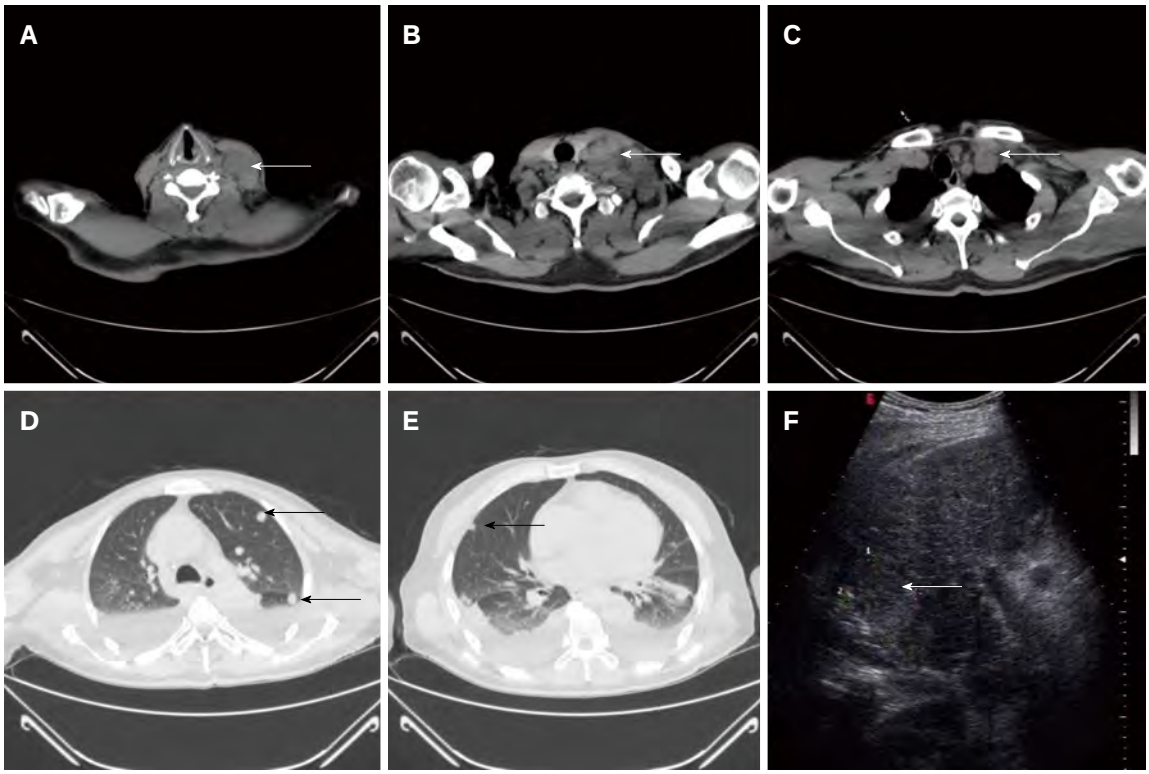


Figure 3 Thoracic computed tomographic and Doppler images after surgery. A: Non-contrast-enhanced computed tomography scan showing enlarged left supraclavicular lymph nodes (arrow), which were easily observed; B: Part of enlarged left supraclavicular lymph nodes (arrow) fused into the mass; C: Enlarged lymph nodes in the mediastinum (arrow) was observed; D and E: Round nodules (arrows) distributed in both lungs; F: A new intrahepatic lesion (arrow) was detected in the non-operating region *via* Doppler ultrasound.

and para-aortic nodes are the major metastatic sites[4]. Nowadays, a hepatectomy has become one of the most popular treatments for HCC, and understanding lymph node metastasis in HCC is indispensable at surgery for improving the patient's prognosis. A previous study revealed that the incidence of lymph node metastasis in operable HCC patients was low, but patients with lymph node metastasis had a poorer prognosis[5].

HCC metastasis to left supraclavicular lymph nodes, although uncommon, does occur, probably *via* the hepatic node and then through the thoracic duct[6]. Early discovery of distant metastasis is important for tumor staging, prognosis judgment and therapy determination in those patients with HCC. However, left supraclavicular lymphadenopathy is not the initial presentation in most conditions; therefore, overall physical examination, especially of the supraclavicular fossa, should not be overlooked. When the left supraclavicular lymph node is involved in the tumor, patients already have advanced disease. In this situation, it is more appropriate for patients to adopt non-operative treatment, including hepatic arterial chemoembolization, molecular targeted therapy, chemotherapy and radiotherapy, instead of surgery. For palpable left supraclavicular lymph nodes, FNAC is not only useful for diagnosing various lesions, but also can help in deciding on appropriate management[7]. However, the diagnosis may sometimes be difficult, with the potential for clinically important diagnostic errors. Therefore, FNAC can also show false negative results for positive lesions[8]. It is presumed that multiple factors can result in misdiagnosis[9], including inadequate specimens, poor smears, inaccurate puncture location, and lack of ancillary studies (cell blocks, immunocytochemistry and electron microscopy)[10].

For a patient with left supraclavicular lymphadenopathy who is highly suspected to have a metastatic tumor, FNAC can be used as a first line diagnostic modality in the evaluation of enlarged lymph nodes. If negative findings are obtained, an excisional biopsy is necessary, which is the gold standard for diagnosis, and Positron emission tomography - computed tomography (PET-CT) scanning should be performed, which allows identification of the primary site and metastatic lesions, including bone and soft tissue metastases, in a single examination.

In our case, an enlarged left supraclavicular lymph node was noticed preoperatively. The negative findings of FNAC and no further examination resulted in incorrect tumor staging. The disease progressed rapidly after surgery. Metastatic HCC was not confirmed until an excisional biopsy was performed.

In summary, we have presented an uncommon case of advanced HCC with left supraclavicular lymph node metastasis. When similar cases are encountered, FNAC should be performed initially. When the results of FNAC, are negative, further tests, such as an excisional biopsy and PET-CT scanning will help to achieve a definitive diagnosis. Such diagnoses will help to prioritize the patient's management protocol.

REFERENCES

1 **Kew MC**, Paterson AC. Unusual clinical presentations of hepatocellular carcinoma. *Trop Gastroenterol* 1985; **6**: 10-22 [PMID: 2992134]

2 **Schiffman SC**, Woodall CE, Kooby DA, Martin RC, Staley CA, Egnatashvili V, McMasters KM, Scoggins CR. Factors associated with recurrence and survival following hepatectomy for large hepatocellular carcinoma: a multicenter analysis. *J Surg Oncol* 2010; **101**: 105-110 [PMID: 20035538 DOI: 10.1002/jso.21461]

3 **Gupta N**, Rajwanshi A, Srinivasan R, Nijhawan R. Pathology of supraclavicular lymphadenopathy in Chandigarh, north India: an audit of 200 cases diagnosed by needle aspiration. *Cytopathology* 2006; **17**: 94-96 [PMID: 16548994 DOI: 10.1111/j.1365-2303.2006.00285.x]

4 **Watanabe J**, Nakashima O, Kojiro M. Clinicopathologic study on lymph node metastasis of hepatocellular carcinoma: a retrospective study of 660 consecutive autopsy cases. *Jpn J Clin Oncol* 1994; **24**: 37-41 [PMID: 8126919]

5 **Sun HC**, Zhuang PY, Qin LX, Ye QH, Wang L, Ren N, Zhang JB, Qian YB, Lu L, Fan J, Tang ZY. Incidence and prognostic values of lymph node metastasis in operable hepatocellular carcinoma and evaluation of routine complete lymphadenectomy. *J Surg Oncol* 2007; **96**: 37-45 [PMID: 17345597 DOI: 10.1002/jso.20772]

6 **Selvasekaran R**, Cherian JV, Venkataraman J. Metastasis of hepatocellular carcinoma to Virchow's node: have the tumor cells gone astray? *Hepatobiliary Pancreat Dis Int* 2007; **6**: 650-652 [PMID: 18086635]

7 **Gupta RK**, Naran S, Lallu S, Fauck R. The diagnostic value of fine needle aspiration cytology (FNAC) in the assessment of palpable supraclavicular lymph nodes: a study of 218 cases. *Cytopathology* 2003; **14**: 201-207 [PMID: 12873313 DOI: 10.1046/j.1365-2303.2003.00057.x]

8 **Ellison E**, LaPuerta P, Martin SE. Supraclavicular masses: results of a series of 309 cases biopsied by fine needle aspiration. *Head Neck* 1999; **21**: 239-246 [PMID: 10208667]

9 **Stanley MW**. Selected problems in fine needle aspiration of head and neck masses. *Mod Pathol* 2002; **15**: 342-350 [PMID: 11904347 DOI: 10.1038/modpathol.3880528]

10 **Mutreja D**, Nijhawan VS, Srinivasa V, Lakhtakia R, Subramanya H. Value of ancillary studies in the evaluation of fine-needle aspiration specimens: Our experience. *J Cytol* 2012; **29**: 103-110 [PMID: 22787289 DOI: 10.4103/0970-9371.97148]

P- Reviewers Julie NL, Hoogenraad TU **S- Editor** Huang XZ
L- Editor Stewart GJ **E- Editor** Li JY



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i6.964

World J Gastroenterol 2013 February 14; 19(6): 964-967
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Severe hepatic necrosis of unknown causes following ABO-incompatible liver transplantation

Hao Lu, Chuan-Yong Zhang, Wei Ding, Yun-Jie Lu, Guo-Qiang Li, Feng Zhang, Ling Lu

Hao Lu, Chuan-Yong Zhang, Wei Ding, Yun-Jie Lu, Guo-Qiang Li, Feng Zhang, Ling Lu, Liver Transplantation Center, First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China

Author contributions: Lu H, Zhang CY and Ding W contributed equally to this work; Zhang F and Lu L designed the research; Ding W and Lu YJ analyzed the data; Zhang CY, Li GQ and Zhang F performed the operation; and Lu H and Ding W wrote the paper.

Correspondence to: Ling Lu, MD, PhD, Liver Transplantation Center, First Affiliated Hospital of Nanjing Medical University, Nanjing 210029, Jiangsu Province, China. lvling@njmu.edu.cn
Telephone: +86-25-83672106 Fax: +86-25-83672106
Received: October 17, 2012 Revised: December 29, 2012
Accepted: January 11, 2013
Published online: February 14, 2013

Abstract

Emergency ABO-incompatible (ABO-I) liver transplantations (LTx) have been performed increasingly to treat severe liver failure. Herein, we report a case of severe hepatic necrosis after ABO-I LTx. A 53-year-old man with blood group O was diagnosed as having severe hepatitis B and acute-on-chronic liver failure, and underwent an emergency liver transplantation implanting a blood-group-B liver from a cardiac-death donor. A routine anti-rejection, anti-infection and anti-virus therapy was given after operation. On post-operative day (POD) 16, the recipient had fever and erythra. Laboratory and radiographic examinations suggested a severe hepatic necrosis of unknown causes. The patient was managed with a 10-d methylprednisolone pulse therapy. He was discharged on POD 35 with stable condition, and no recurrent disease was found during the follow-up.

© 2013 Baishideng. All rights reserved.

Key words: Liver transplantations; ABO-incompatible; Hepatic necrosis; Graft rejection; Pulse therapy

Lu H, Zhang CY, Ding W, Lu YJ, Li GQ, Zhang F, Lu L. Severe hepatic necrosis of unknown causes following ABO-incompatible liver transplantation. *World J Gastroenterol* 2013; 19(6): 964-967
Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i6/964.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i6.964

INTRODUCTION

Acute-on-chronic liver failure (ACLF) has a poor prognosis and a high mortality. It may result in multiple syndromes, including jaundice, coagulopathy, ascites, and even multiple organ dysfunction syndrome induced by precipitating factors (*e.g.*, hepatotropic virus reactivation, infection/sepsis, and hepatotoxic drugs), and secondary compensated liver diseases (*e.g.*, chronic hepatitis and stable liver cirrhosis). ACLF is characterized by acute deterioration of hepatic function, which develops aggressively[1,2]. Given the long-term survival without liver transplantation (LTx) or self-recovery in ACLF subjects is very low, emergency LTx has been introduced as a first-line therapy to significantly improve the short- and long-term survival of the patients[3], in spite of the recently emerging debate on the benefit of artificial liver support to improve the short-term prognosis[4-7].

ABO blood group is considered as a barrier to LTx. Immune rejection, especially antibody-mediated rejection (AMR), is the main cause of graft loss in ABO-incompatible (ABO-I) LTx. Due to the presence of A/B antigen on the surface of vascular endothelium, bile duct epithelium and liver sinusoidal endothelial cells, an ABO-I graft might be attacked by the existing hemagglutinin, causing damage to the blood vessel and bile duct of the graft, characterized by liver parenchymal necrosis and biliary strictures. Other causes of graft loss include vascular or biliary complications, and recurrence of viral hepatitis.

Therapeutic advancement has improved the outcomes of ABO-I LTx and made a long-term survival possible[8-10]. In this report, we present a case of severe hepatic necrosis of unknown reasons after ABO-I LTx. However,

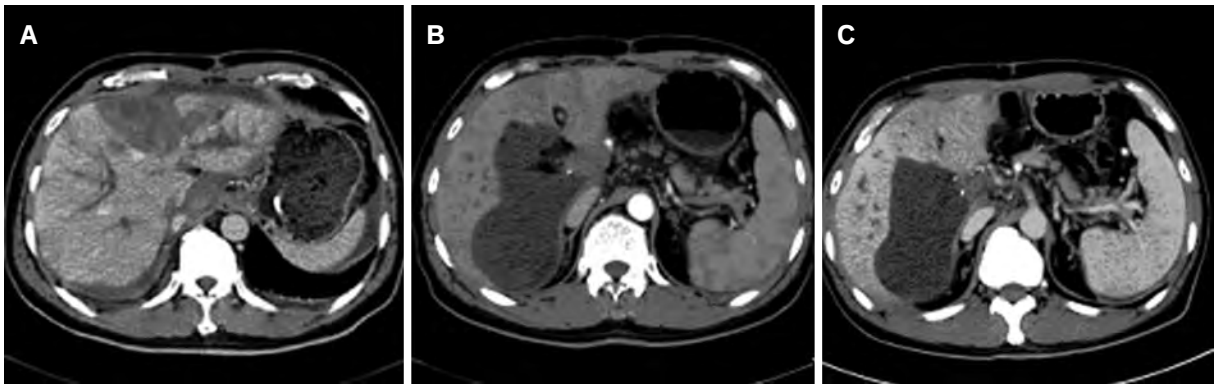


Figure 1 Computed tomography scan and computed tomography angiography images. A: Intrahepatic bile duct expansion and left lobe liver necrosis on post-operative day (POD) 19; B: A low-density cyst (6.1 cm × 13 cm) and a filling defect (2.1 cm × 1.9 cm) at portal area on POD 48; C: Computed tomography (CT) scan and CT angiography on POD 118 showed a smaller low-density cyst (5 cm × 10 cm) and a same-sized filling defect (2.1 cm × 1.9 cm) at portal area on POD 118 compared with earlier findings.

the patient was cured and discharged.

CASE REPORT

A 53-year-old male patient was transferred from the internal medical ward to the liver transplant unit after he was diagnosed as having severe hepatitis B and ACLF, and he then underwent an emergency ABO-I LTx. The liver graft was obtained from a cardiac-death donor with blood group B, which mismatched the recipient's blood group O. Cold ischemia time of the graft was 5 h. The modified piggy orthotopic liver transplantation was performed with a 60-min anhepatic phase and without splenectomy. Massive ascites (about 4000 mL), omentum and gallbladder edema, enlarged tough spleen and severe atrophic and cirrhotic liver were visualized after a right upper incision, confirming the diagnosis of ACLF. The operation was successful. Basiliximab 20 mg and methylprednisolone 1000 mg were administered for immune-induction and hepatitis B immunoglobulin (HB-Ig) 1000 U for prevention of hepatitis B virus (HBV) recurrence before anhepatic phase. A quadruple therapy was given to prevent rejection: (1) Rituximab 100 mg on post-operative day (POD) 1 and Basiliximab 20 mg on POD 4 for induction of immune tolerance; (2) Tacrolimus (Tac) at a dose adjusted according to a blood level of 10-15 ng/mL; (3) Methylprednisolone (MP) at a dose gradually reduced from 1000 mg *qd* to a maintenance of 10 mg *bid*; and (4) Mycophenolatemofetil, 0.5 g, *bid*. Other treatments included administration of antibiotics, antiviral therapy with lamivudine, proton pump inhibitor and liver-protective medication. Primary monitoring examinations included blood routine, liver function, coagulation function, HBV serum virology, serum drug level of Tac, B-ultrasound (B-US) and computed tomography (CT) scan. Pathological examination, lymphocyte subsets analysis and ImmuKnow Assay (T cell function test) results showed no rejection. On POD 16, the patient had fever and erythra. B-US displayed a sub-hepatic, pelvic and thoracic fluid sonolucent area, while CT scan showed abnormal left liver lobe infusion and slight intrahepatic bile duct dilatation (Figure 1). Both alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were elevated (ALT 54.3 U/L and AST 26.5 U/L *vs* ALT 1204.2 U/L and AST 1555.3 U/L at POD 12 and 16, respectively). All these results indicated a severe hepatic necrosis. After a 10-d MP pulse therapy, the patient's symptoms were relieved and aminotransferase was reduced. The patient was discharged on POD 35, and was followed up once a month. The patient is doing well without any recurrent disease.

DISCUSSION

The initial clinical impression of severe hepatic necrosis on POD 16 was rejection. AMR is triggered by alloantibody binding and compliment activation. In the case of ABO-I solid organ transplantation, A, B or AB blood group antigens expressed on graft could be detected by specific natural antibodies. Incompatible blood group antigens activated B cells directly with the help of T cells and resulted in transcription of cytokines and antibodies[11]. AMR can be caused by performed antibodies, anamnestic B-cell responses, early *de novo* and late *de novo* B-cell responses. The strength of isohemagglutinins and natural antibodies in ABO-I recipients can be detected and quantified by multiple methods. Antibody titer is reported to correlate with the survival of recipients. ABO-I living donor liver transplantation with high preoperative and postoperative immunoglobulin (Ig) G and IgM levels sustained bile duct complication and hepatic necrosis, respectively[12], while the safe value of isohemagglutinin titer is $\leqslant$ 1:16 for kidney transplantation[13,14] and $\leqslant$ 1:4 for pediatric heart transplantation[15,16]. In this case, on POD 16, the recipient run a fever, and had poor liver function, and CT scan showed intrahepatic bile duct dilatation and liver necrosis in the left lobe (Figure 1A). But lymphocyte subset analysis indicated a slight increase of T cells and a drop of B cells (CD3+ 77.9%, CD3+CD4+ 49.7%, CD3+CD8+ 23.9%, CD3-CD16+56 14.4% and CD19+ 1%), which was contradictory to AMR with an enhanced B cell function. Meanwhile, ImmuKnow Assay (T cell function tests) before LTx and on POD 34 and

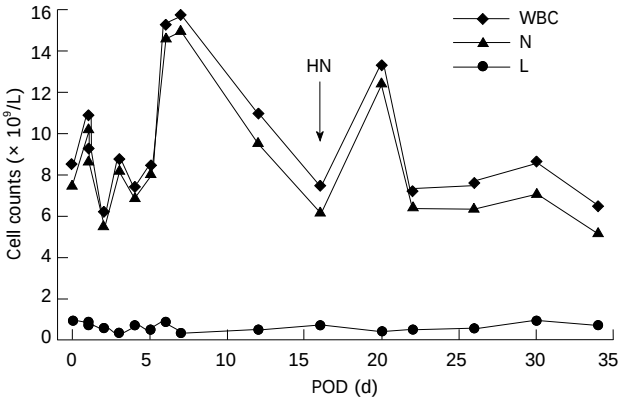


Figure 2 Blood routine test results after ABO-incompatibleliver transplantation. Due to treatment with tacrolimus and methylprednisolone, the number of lymphocyte stayed stable before and after the occurrence of severe hepatic necrosis, while the total number of white blood cell counts was mainly affected by neutrophil changes, which might be induced by non-specific reasons, *e.g.*, phagocytose and absorption of necrotic tissues. LTx: Liver transplantation; POD: Post-operative day; HN: Hepatic necrosis; WBC: White blood cell; L: Lymphocyte; N: Neutrophil.

90 were 156.7 ng/mL, 101.7 ng/mL and 70.3 ng/mL, respectively, suggesting a depression of T cell function after LTx resulting from immunosuppressive therapy[17,18]. The total number of white blood cell was mainly affected by neutrophils (Figure 2). Levels of IgG, IgA, IgM, C3 and C4 were normal or almost normal before LTx and on POD 118 (18.1 g/L, 3.45 g/L, 0.94 g/L, 0.20 g/L and 0.0159 g/L *vs* 7.1 g/L, 1.38 g/L, 0.51 g/L, 1.07 g/L and 0.305 g/L, respectively). Therefore, rejection was not considered as the cause of hepatic necrosis initially.

Another factor leading to severe hepatic necrosis after LTx was recurrent diseases. To prevent recurrent hepatitis B, HB-Ig and/or lamivudine was administered. A 10-year follow-up study in 24 individuals with chronic hepatitis B demonstrated the effectiveness of HB-Ig and/or lamivudine[19]. Nineteen patients were alive after the follow-up duration with well-functioning graft. None of them developed recurrent hepatitis B. Among them, 12 patients received HB-Ig and lamivudine, 3 received lamivudine only, 1 received adefovir, and 3 received no antivirals. HbsAg and HbcAb were both positive in the serum virology test in this patient before LTx. Antiviral treatments included HB-Ig 1000 U injection during LTx and lamivudine *p.o.* after operation. Post-LTx HBV examination results were all negative. Pathological examination of the explanted liver showed classic necrotic changes, without hepatocellular carcinoma. Taken together, there was no recurrent hepatitis or carcinoma till the last follow-up in this case, although CT scan showed dilation of bile duct and inflammation of left liver lobe (Figure 1A), which could result from recurrent viral hepatitis[20].

Blood supply disorders and biliary complications could also lead to hepatic necrosis after LTx. CT scan and CT angiography (CTA) also suggested normal hemodynamics on POD 3, but a low-density cystic echo (6.1 cm × 13 cm) and a filling defect (2.1 cm × 1.9 cm) at the portal area (Figure 1B). However, as shown in Figure 3A, total bilirubin and creatinine levels turned normal on

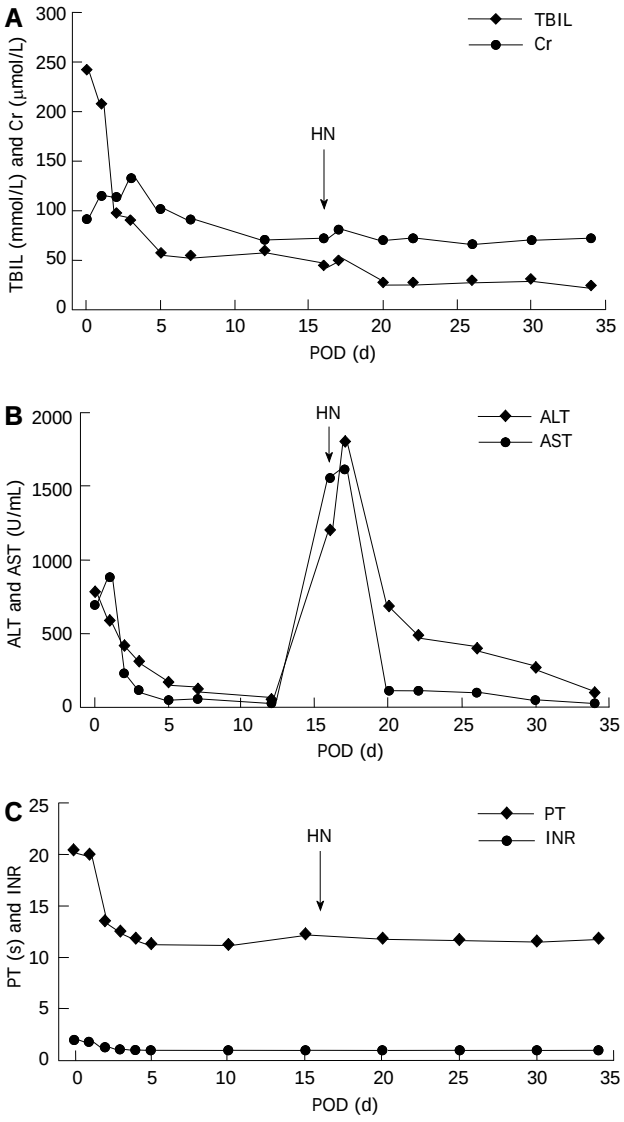


Figure 3 Total bilirubin and creatine, liver function, prothrombin time and international normalized ratio changes after liver transplantation. A: Total bilirubin (TBIL) and creatine (Cr) levels elevated after graft implantation and then decreased in 2 wk. Severe hepatic necrosis resulted in a slight rise, which was reversed by methylprednisolone(MP) pulse therapy; B:Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) dropped significantly within 5 d after liver transplantation (LTx), and then remained in a low level till post-operative day (POD) 16. Severe hepatic necrosis led to a sharp increase of both ALT and AST which were under control after 4-d intravenous drip of 500 mg MP. Dosage of MP was reduced gradually to 40 mg on POD 25; C: Prothrombin time (PT) and international normalized ratio (INR) changes after LTx. Coagulation function became normal within 5 d. HN: Hepatic necrosis.

POD 7 according to Li *et al*[21], and remained stable during hepatic necrosis after LTx. Besides, ALT and AST levels started to fall on POD 20, *i.e.*, 4 d after an MP pulse therapy for severe hepatic necrosis, indicating that liver function had been improved (Figure 3B). As demonstrated in Figure 3C, PT and INR became normal on POD 5[21], and B-US on POD 16 showed no sign of intrahepatic thrombosis and stricture. To prevent postoperative bleeding, hemocoagulase was injected which would not result in prothrombin increase and thrombosis. So we excluded vascular or biliary complications. Low molecular weight

heparin calcium injection was also given till POD 30 for 14 d. However, CT scan and CTA on POD 118 showed a smaller low-density cyst (5 cm × 10 cm) and a same-sized filling defect (2.1 cm × 1.9 cm) at portal area compared with the earlier findings (Figure 1C), which indicated no deterioration of blood supply.

In conclusion, coagulatory, immunologic and hepatic functions after LTx were complicated, and we have not encountered such case before at our center. This patient had severe hepatic necrosis on POD 16, which might be attributed to the rejection with atypical manifestation due to immune suppressive therapy. However, the patient recovered in liver function after treatment with MP, and discharged on POD 34. Till the last follow-up 10 mo after LTx, there were no symptoms or signs indicating bad prognosis. Although the mechanism for hepatic necrosis and recovery of similar cases needs further studies, our experience has provided evidence that emergency ABO-I LTx is an effective treatment option for ACLF, and secondary liver necrosis of unknown causes might be treated by MP.

REFERENCES

1 **Sarin SK**, Kumar A, Almeida JA, Chawla YK, Fan ST, Garg H, de Silva HJ, Hamid SS, Jalan R, Komolmit P, Lau GK, Liu Q, Madan K, Mohamed R, Ning Q, Rahman S, Rastogi A, Riordan SM, Sakhuja P, Samuel D, Shah S, Sharma BC, Sharma P, Takikawa Y, Thapa BR, Wai CT, Yuen MF. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the study of the liver (APASL). *Hepatol Int* 2009; **3**: 269-282 [PMID: 19669378 DOI: 10.1007/s12072-008-9106-x]

2 **Katoonizadeh A**, Laleman W, Verslype C, Wilmer A, Maleux G, Roskams T, Nevens F. Early features of acute-on-chronic alcoholic liver failure: a prospective cohort study. *Gut* 2010; **59**: 1561-1569 [PMID: 20675694 DOI: 10.1136/gut.2009.189639]

3 **Rajekar H**, Wai CT, Majeed TA, Lee KH, Wong SY, Leong SO, Singh R, Tay KH, Soosaynathan C, Tan KC. Prognostic factors in patients with acute liver failure undergoing live donor liver transplantation. *Transplant Proc* 2008; **40**: 2492-2493 [PMID: 18929776 DOI: 10.1016/j.transproceed.2008.07.009]

4 **Heemann U**, Treichel U, Loock J, Philipp T, Gerken G, Malago M, Klammt S, Loehr M, Liebe S, Mitzner S, Schmidt R, Stange J. Albumin dialysis in cirrhosis with superimposed acute liver injury: a prospective, controlled study. *Hepatology* 2002; **36**: 949-958 [PMID: 12297843 DOI: 10.1053/jhep.2002.36130]

5 **Hassanein TI**, Tofteng F, Brown RS, McGuire B, Lynch P, Mehta R, Larsen FS, Gornbein J, Stange J, Blei AT. Randomized controlled study of extracorporeal albumin dialysis for hepatic encephalopathy in advanced cirrhosis. *Hepatology* 2007; **46**: 1853-1862 [PMID: 17975845 DOI: 10.1002/hep.21930]

6 **Nevens F**, Laleman W. Artificial liver support devices as treatment option for liver failure. *Best Pract Res Clin Gastroenterol* 2012; **26**: 17-26 [PMID: 22482522 DOI: 10.1016/j.bpg.2012.01.002]

7 **Tritto G**, Davies NA, Jalan R. Liver replacement therapy. *Semin Respir Crit Care Med* 2012; **33**: 70-79 [PMID: 22447262 DOI: 10.1055/s-0032-1301736]

8 **Nakamura Y**, Hama K, Iwamoto H, Yokoyama T, Kihara Y, Konno O, Jojima Y, Shimazu M. Long-term recurrence-free survival after liver transplantation from an ABO-incompatible living donor for treatment of hepatocellular carcinoma exceeding Milano criteria in a patient with hepatitis B virus cirrhosis: a case report. *Transplant Proc* 2012; **44**: 565-569 [PMID: 22410070 DOI: 10.1016/j.transproceed.2012.01.029]

9 **Yamada N**, Urahashi T, Ihara Y, Sanada Y, Wakiya T, Okada N, Mizuta K. Veno-occlusive disease/sinusoidal obstruction syndrome associated with potential antibody-mediated rejection after pediatric living donor liver transplantation: a case report. *Transplant Proc* 2012; **44**: 810-813 [PMID: 22483502 DOI: 10.1016/j.transproceed.2012.01.008]

10 **Yamanouchi K**, Eguchi S, Takatsuki M, Kamohara Y, Hidaka M, Miyazaki K, Inokuma T, Tajima Y, Kanematsu T. Management of cytomegalovirus infection after living donor liver transplantation. *Hepatogastroenterology* 2012; **59**: 231-234 [PMID: 22251542 DOI: 10.5754/hge09582]

11 **Montgomery RA**, Cozzi E, West LJ, Warren DS. Humoral immunity and antibody-mediated rejection in solid organ transplantation. *Semin Immunol* 2011; **23**: 224-234 [PMID: 21958960 DOI: 10.1016/j.smim.2011.08.021]

12 **Kozaki K**, Egawa H, Ueda M, Oike F, Yoshizawa A, Fukatsu A, Takada Y. The role of apheresis therapy for ABO incompatible living donor liver transplantation: the Kyoto University experience. *Ther Apher Dial* 2006; **10**: 441-448 [PMID: 17096700 DOI: 10.1111/j.1744-9987.2006.00409.x]

13 **Montgomery RA**, Locke JE, King KE, Segev DL, Warren DS, Kraus ES, Cooper M, Simpkins CE, Singer AL, Stewart ZA, Melancon JK, Ratner L, Zachary AA, Haas M. ABO incompatible renal transplantation: a paradigm ready for broad implementation. *Transplantation* 2009; **87**: 1246-1255 [PMID: 19384174 DOI: 10.1097/TP.0b013e31819f2024]

14 **Warren DS**, Zachary AA, Sonnenday CJ, King KE, Cooper M, Ratner LE, Shirey RS, Haas M, Leffell MS, Montgomery RA. Successful renal transplantation across simultaneous ABO incompatible and positive crossmatch barriers. *Am J Transplant* 2004; **4**: 561-568 [PMID: 15023148 DOI: 10.1111/j.1600-6143.2004.00364.x]

15 **Irving C**, Parry G, Cassidy J, Hasan A, Griselli M, Kirk R. Outcomes following infant listing for cardiac transplantation: the impact of strategies introduced to counteract limited donor availability. *Arch Dis Child* 2010; **95**: 883-887 [PMID: 20656734 DOI: 10.1136/adc.2009.171348]

16 **Roche SL**, Burch M, O'Sullivan J, Wallis J, Parry G, Kirk R, Elliot M, Shaw N, Flett J, Hamilton JR, Hasan A. Multicenter experience of ABO-incompatible pediatric cardiac transplantation. *Am J Transplant* 2008; **8**: 208-215 [PMID: 18021280 DOI: 10.1111/j.1600-6143.2007.02040.x]

17 **Gralla J**, Huskey J, Wiseman AC. Trends in immune function assay (ImmuKnow; Cylex™) results in the first year post-transplant and relationship to BK virus infection. *Nephrol Dial Transplant* 2012; **27**: 2565-2570 [PMID: 22167591 DOI: 10.1093/ndt/gfr675]

18 **Berglund D**, Bengtsson M, Biglarnia A, Berglund E, Yamamoto S, von Zur-Mühlen B, Lorant T, Tufveson G. Screening of mortality in transplant patients using an assay for immune function. *Transpl Immunol* 2011; **24**: 246-250 [PMID: 21232600 DOI: 10.1016/j.trim.2010.12.005]

19 **Yataco M**, Bonatti H, Machicao V, Mendez J, Stauffer J, Nguyen J, Ghabril M, Aranda-Michel J, Dickson R. Long term survival and complications after liver transplantation in patients with chronic hepatitis B. *Ann Transplant* 2010; **15**: 27-34 [PMID: 20657516]

20 **Petrovic LM**. Recurrent diseases following liver transplantation: current concepts. *Curr Opin Organ Transplant* 2012; **17**: 293-302 [PMID: 22498649 DOI: 10.1097/MOT.0b013e32835365f6]

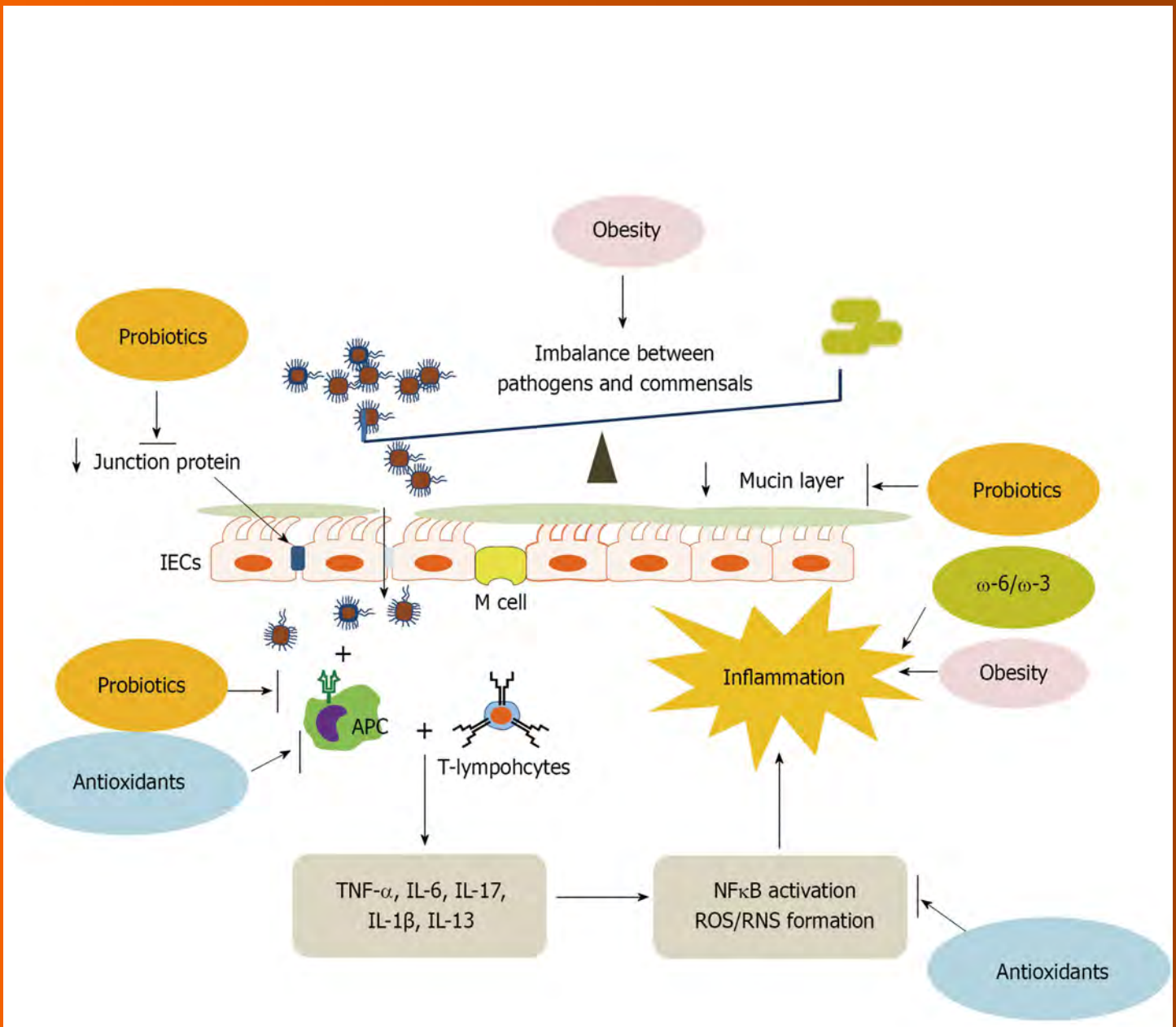
21 **Li C**, Mi K, Wen Tf, Yan Ln, Li B, Yang Jy, Xu Mq, Wang Wt, Wei Yg. Outcomes of patients with benign liver diseases undergoing living donor versus deceased donor liver transplantation. *PLoS One* 2011; **6**: e27366 [PMID: 22087299 DOI: 10.1371/journal.pone.0027366]

P- Reviewer Ramsay M **S- Editor** Gou SX **L- Editor** A **E- Editor** Li JY

ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

World J Gastroenterol 2013 February 21; 19(7): 968-1151



Baishideng Publishing Group
www.wjgnet.com

WJG World Journal of Gastroenterology

Contents

Weekly Volume 19 Number 7 February 21, 2013

EDITORIAL

968 Histological healing in inflammatory bowel disease: A still unfulfilled promise
Villanacci V, Antonelli E, Geboes K, Casella G, Bassotti G

TOPIC HIGHLIGHT

979 Colorectal cancer mortality in Hong Kong of China, Japan, South Korea, and Singapore
Shin A, Jung KW, Won YJ

984 Molecular mechanisms of chemopreventive phytochemicals against gastroenterological cancer development
Chung MY, Lim TG, Lee KW

994 Nutritional modulators of ulcerative colitis: Clinical efficacies and mechanistic view
Sung MK, Park MY

1005 Vitamin B6 and colorectal cancer: Current evidence and future directions
Zhang XH, Ma J, Smith-Warner SA, Lee JE, Giovannucci E

1011 Dietary flavonoid intake and risk of stomach and colorectal cancer
Woo HD, Kim J

1020 Consumption of red and processed meat and esophageal cancer risk: Meta-analysis
Choi Y, Song S, Song Y, Lee JE

1030 Current trends in the development and application of molecular technologies for cancer epigenetics
Jang H, Shin H

REVIEW

1040 Use of thiopurines in inflammatory bowel disease
Frei P, Biedermann L, Nielsen OH, Rogler G

MINIREVIEW

1049 Tailoring the area of hepatic resection using inflow and outflow modulation
Donadon M, Procopio F, Torzilli G

ORIGINAL ARTICLE

1056 *Pseudomonas fluorescens*-like bacteria from the stomach: A microbiological and molecular study
Patel SK, Pratap CB, Verma AK, Jain AK, Dixit VK, Nath G

1068 Long interspersed nuclear element ORF-1 protein promotes proliferation and resistance to chemotherapy in hepatocellular carcinoma
Feng F, Lu YY, Zhang F, Gao XD, Zhang CF, Meredith A, Xu ZX, Yang YT, Chang XJ, Wang H, Qu JH, Zeng Z, Yang JL, Wang CP, Zhu YF, Cui JJ, Yang YP

BRIEF ARTICLE

1079 Papillary thyroid cancer and inflammatory bowel disease: Is there a relationship?
Sonu IS, Blonski W, Lin MV, Lewis J, Aberra F, Lichtenstein GR

1085 Efficacy and safety of Chlorella supplementation in adults with chronic hepatitis C virus infection
Azocar J, Diaz A

1091 Efficacy of infliximab in acute severe ulcerative colitis: A single-centre experience
Halpin SJ, Hamlin PJ, Greer DP, Warren L, Ford AC

1098 Pegylated interferon alfa and ribavirin for children with chronic hepatitis C
Rosen I, Kori M, Eshach Adiv O, Yerushalmi B, Zion N, Shaoul R

1104 Granulocyte-colony stimulating factor therapy improves survival in patients with hepatitis B virus-associated acute-on-chronic liver failure
Duan XZ, Liu FF, Tong JJ, Yang HZ, Chen J, Liu XY, Mao YL, Xin SJ, Hu JH

1111 Risk factors for hepatic decompensation in patients with primary biliary cirrhosis
Shi TY, Zhang LN, Chen H, Wang L, Shen M, Zhang X, Zhang FC

1119 A temporary self-expanding metallic stent for malignant colorectal obstruction
Ding XL, Li YD, Yang RM, Li FB, Zhang MQ

META-ANALYSIS

1124 Roux-en-Y versus Billroth Ⅰ reconstruction after distal gastrectomy for gastric cancer: A meta-analysis
Xiong JJ, Altaf K, Javed MA, Nunes QM, Huang W, Mai G, Tan CL, Mukherjee R, Sutton R, Hu WM, Liu XB

CASE REPORT

1135 Gastrointestinal sarcoidosis associated with pneumatosis cystoides intestinalis
Rahim H, Khan M, Hudgins J, Lee K, Du L, Amorosa L

1140 Transjugular intrahepatic portosystemic shunt in refractory chylothorax due to liver cirrhosis
Lutz P, Strunk H, Schild HH, Sauerbruch T

1143 Cytomegalovirus-associated gastric ulcer: A side effect of steroid injections for pyloric stenosis
Mori H, Fujihara S, Nishiyama N, Kobara H, Oryu M, Kato K, Rafiq K, Masaki T

1147 Isolated fever induced by mesalamine treatment
Slim R, Amara J, Nasnas R, Honein K, Jaoude JB, Yaghi C, Daniel F, Sayegh R

LETTERS TO THE EDITOR

1150 Management of duodenal ulcer bleeding resistant to endoscopy: Surgery is dead!
Loffroy R

World Journal of Gastroenterology
Volume 19 Number 7 February 21, 2013

Contents

APPENDIX	I-VI	Instructions to authors
ABOUT COVER		Sung MK, Park MY. Nutritional modulators of ulcerative colitis: Clinical efficacies and mechanistic view. *World J Gastroenterol* 2013; 19(7): 994-1004 http://www.wjgnet.com/1007-9327/full/v19/i7/994.htm

AIMS AND SCOPE

World Journal of Gastroenterology (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. *WJG* was established on October 1, 1995. It is published weekly on the 7th, 14th, 21st, and 28th each month. The *WJG* Editorial Board consists of 1352 experts in gastroenterology and hepatology from 64 countries.

The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.

INDEXING/ABSTRACTING

World Journal of Gastroenterology is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, Digital Object Identifier, and Directory of Open Access Journals. ISI, Journal Citation Reports®, Gastroenterology and Hepatology, 2011 Impact Factor: 2.471 (32/74); Total Cites: 16951 (7/74); Current Articles: 677 (1/74); and Eigenfactor® Score: 0.06035 (5/74).

FLYLEAF	I-IX	Editorial Board

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Shuai Ma*
Responsible Electronic Editor: *Li Xiong*
Proofing Editor-in-Chief: *Lian-Sheng Ma*
Responsible Science Editor: *Su-Xin Gou*
Proofing Editorial Office Director: *Jin-Lei Wang*

NAME OF JOURNAL
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

EDITOR-IN-CHIEF
Ferruccio Bonino, MD, PhD, Professor of Gastroenterology, Director of Liver and Digestive Disease Division, Department of Internal Medicine, University of Pisa, Director of General Medicine 2 Unit University Hospital of Pisa, Via Roma 67, 56124 Pisa, Italy

Myung-Hwan Kim, MD, PhD, Professor, Head, Department of Gastroenterology, Director, Center for Biliary Diseases, University of Ulsan College of Medicine, Asan Medical Center, 388-1 Pungnap-2dong, Songpa-gu, Seoul 138-736, South Korea

Kjell Öberg, MD, PhD, Professor, Department of Endocrine Oncology, Uppsala University Hospital, SE-751 85 Uppsala, Sweden

Matt D Rutter, MBBS, MD, FRCP, Consultant Gastroenterologist, Senior Lecturer, Director, Tees Bowel Cancer Screening Centre, University Hospital of North Tees, Durham University, Stockton-on-Tees, Cleveland TS19 8PE, United Kingdom

Andrzej S Tarnawski, MD, PhD, DSc (Med), Professor of Medicine, Chief Gastroenterology, VA Long Beach Health Care System, University of California, Irvine, CA, 5901 E. Seventh Str., Long Beach, CA 90822, United States

EDITORIAL OFFICE
Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
World Journal of Gastroenterology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Co., Limited
Room 1701, 17/F, Henan Building,
No.90 Jaffe Road, Wanchai, Hong Kong, China
Fax: +852-31158812
Telephone: +852-58042046
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

PUBLICATION DATE
February 21, 2013

COPYRIGHT
© 2013 Baishideng. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT
All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i7.968

World J Gastroenterol 2013 February 21; 19(7): 968-978
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

EDITORIAL

Histological healing in inflammatory bowel disease: A still unfulfilled promise

Vincenzo Villanacci, Elisabetta Antonelli, Karel Geboes, Giovanni Casella, Gabrio Bassotti

Vincenzo Villanacci, Department of Pathology, Spedali Civili of Brescia, 25100 Brescia, Italy
Elisabetta Antonelli, Gabrio Bassotti, Gastroenterology and Hepatology Section, Department of Clinical and Experimental Medicine, University of Perugia, 06156 Perugia, Italy
Karel Geboes, Department of Pathology, University Hospital KU Leuven and UZ Gent, 3000 Leuven, Belgium
Giovanni Casella, Division of Internal Medicine, Desio General Hospital, 20033 Desio, Italy
Author contributions: Bassotti G and Villanacci V designed the research project and wrote the paper; Antonelli E, Geboes K and Casella G critically reviewed the manuscript for important intellectual content; all authors approved the final draft of the manuscript.
Correspondence to: Gabrio Bassotti, Professor, Gastroenterology and Hepatology Section, Department of Clinical and Experimental Medicine, University of Perugia, Ospedale Santa Maria della Misericordia, Piazzale Menghini 1, 06156 Perugia, Italy. gabassot@tin.it
Telephone: +39-75-5784423 Fax: +39-75-5847570
Received: June 27, 2012 Revised: September 11, 2012
Accepted: September 19, 2012
Published online: February 21, 2013

Abstract

Treatment of inflammatory bowel disease (IBD) is traditionally based on several drugs, including salicylates, corticosteroids, and antibiotics; in addition, the therapeutic armamentarium has considerably evolved with the advent of newer, effective therapeutic measures (such as the biological agents) that are able to improve in a considerable manner both the clinical and endoscopic variables. Thus, mucosal healing, at least considered from an endoscopic point of view, is today regarded as the ultimate endpoint for treatment of these conditions. However, it is also increasingly clear that endoscopic healing is not necessarily paralleled by histological healing; There are few doubts that the latter should be considered as a true, objective healing and the ultimate goal to reach when treating patients with IBD. Unfortunately, and surprisingly, only a few, incomplete, and somewhat conflicting data exist on this topic, especially because there is still the need to standardize both histological assessment and the severity grading of these disorders; Issues that have not been yet been resolved for clinical practice and therapeutic trials. Hopefully, with the help of an increased awareness on the clinical researchers' side, and the availability of dedicated pathologists on the other side, this matter will be effectively faced and resolved in the near future.

© 2013 Baishideng. All rights reserved.

Key words: Crohn's disease; Healing; Histology; Inflammatory bowel diseases; Ulcerative colitis

Villanacci V, Antonelli E, Geboes K, Casella G, Bassotti G. Histological healing in inflammatory bowel disease: A still unfulfilled promise. *World J Gastroenterol* 2013; 19(7): 968-978 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i7/968.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i7.968

INTRODUCTION

The medical treatment of inflammatory bowel disease (IBD), including Crohn's disease (CD) and ulcerative colitis (UC), is based on several different approaches relying on aminosalicylates, antibiotics, corticosteroids, and immunomodulatory agents, in addition to the newest biological agents[1]. At present, there is also a strong impulse toward treatment paradigms directed to achieve specific targets, such as a decrease in hospital admissions, the need for surgery, and mucosal healing[2]. The latter point seems to be particularly important, because reaching this goal would also produce a domino effect, resulting in less complications, hospitalization, and surgical procedures related to IBD[3].

However, mucosal healing could be considered as an ambiguous term, because it may be related to both the endoscopic and histological aspects, and at present

a standardized definition for IBD patients does not exist[4]. Actually, by considering mucosal healing in IBD, histological remission is not recommended as primary endpoint for therapeutic trials[5], whereas the suggested endpoint is to obtain a genuine endoscopic healing (defined as absence of friability, blood, erosions, and ulcers in all visualized segments)[5].

Strictly speaking, the optimal treatment goal should be the complete resolution of the inflammatory process, and this is reached only when confirmed by the corresponding histological assessment. In fact, the presence of endoscopic healing does not necessarily imply that this also happens at a microscopic level[6], as also shown (for instance) in celiac disease, in which a gluten-free diet restores the normal endoscopic aspect, but rarely does the microscopic assessment show completely normal features[7].

Moreover, there are objective reasons that probably hamper the use of histological healing as an end point for therapeutic trials. For instance, it has been recently stated that, concerning histopathological assessment of IBD, "…bewildering variations can be observed in the terminology employed to report either individual lesions or diagnostic categories"[8]. In addition, significant differences may exist between UC and CD concerning this aspect. In fact, in UC (in which lesions are usually limited to the mucosa) mucosal/histological healing may represent the ultimate therapeutic goal, whereas in CD (a transmural process) mucosal/histological healing should be achieved as a minimum therapeutic target[9]. Thus, even considering the potentiality of the new therapeutic approaches, the importance of achieving also histological healing might add further value to future trials[10].

A generally accepted definition of histological mucosal healing does however not exist. Histological healing could be defined as "a normal mucosa" or as a disappearance of inflammation, and hence, "a mucosa with limited architectural abnormalities but normally differentiated epithelial cells and no signs of active inflammation (presence of neutrophils) or an increased density of lymphocytes and plasma cells". Usually, histological assessment, when performed during drug trials, has focused mainly on improvement and regression of inflammatory features. The assessment is usually based on the analysis of microscopic sections stained routinely with haematoxylin and eosin. This is a cheap and simple technique which is widely available.

ONLINE SEARCH

We made a comprehensive online search of Medline and the Science Citation Index using the keywords "inflammatory bowel disease", "ulcerative colitis", "Crohn' s disease", "mesalazine", "immunosuppressant", "biologics", "infliximab", "azathioprine", "corticosteroids", "prednisolone", "beclomethasone", "endoscopy", "histologic", "mucosal", and "healing" in various combinations with the Boolean operators "and", "or", and "not". We included only articles that related to human studies, and we performed manual cross referencing, selecting articles published in English between January 1965 and April 2012. A search of non-English language articles and journals older than 1965 was also performed in our library. We excluded letters, and we reviewed abstracts only when the full papers were unavailable.

HISTOPATHOLOGICAL CONSIDERATIONS

An important point is that most literature on this topic originates from only rectal sampling[6]. Thus, it is important that the pathologist receives an adequate number of biopsies correctly oriented from several sites, including the rectum and the terminal ileum. The biopsies should always be accompanied by a report including the age of the patient, clinical information, duration of disease, and type of treatment[11].

The pathological assessment of IBD basically relies on two types of lesions, combined with each other in various ways, represented by architectural abnormalities (that include crypt branching/shortening, decreased crypt density, and irregular mucosal surface) and inflammatory features (transmucosal increase of lamina propria mononuclear cells and the presence of epithelioid granulomas)[6,12].

Moreover, it should be always kept in mind that the histological features of IBD are variable in time, due to both the natural evolution of the disease and the different therapeutic measures. For instance, crypt distortion in UC is usually absent in early stages[13], and the development of architectural abnormalities may take up to 2 mo to appear[14]. Also, decreased crypt density is usually not observed in the first week of the disease, and it is present in about 75% of UC patients with longstanding disease in remission[15]. Again, irregular mucosal surface (present in about 40% of IBD patients) is generally observed in subjects presenting with symptoms for > 2 wk, and it is more common in UC than in CD[6,16,17]. Thus, the absence of mucosal architectural abnormalities in the early phases may present difficulties in the differential diagnosis with acute colitis and during follow-up[18].

Concerning inflammatory features, an increase in cellularity of the lamina propria (especially when localized in its basal third) allows us to distinguish longstanding IBD patients from normal subjects in almost 90% of cases[19]. Another useful clue is an increase of plasma cells, a population increased in IBD rectal samples compared to controls[20]; indeed, blind evaluation of IBD and control specimens has revealed that > 50% of patients displayed basal plasmacytosis[17]. However, it must always kept in mind that inflammatory features also are not constant over time; for instance, a prospective study has demonstrated that focal basal plasmacytosis was found in 40% of IBD patients with symptoms for < 2 wk, but disappeared after 1 year follow-up in half of those without relapse[13]. Granulomas, a distinctive feature of CD, are not constantly present, and are more frequent in

Table 1 Score for histological assessment of ulcerative colitis

No significant inflammation	Mucosa free from active inflammation; no erosions or crypt abscesses; surface and glandular epithelial cells intact; general architecture of the mucosa often disturbed; edema and fibrosis of the lamina propria with occasional foci of lymphocytes
Mild to moderate inflammation	Epithelium usually intact; Glandular tubules irregularly arranged and often showing increased proliferative activity; Edema, vascular congestion, and interstitial hemorrhage presented in the lamina propria; Lymphocytes, plasma cells, and eosinophils increased in number, neutrophils often present, but less numerous than in the more severely affected specimens; Variation in intensity of inflammatory change in individual specimens giving a range of appearances from relatively quiescent to active inflammation
Severe inflammation	Mucosal surface often irregular due to edema, interstitial, hemorrhage, or inflammatory exudate in the lamina, propria; Small epithelial breaches common, sometimes with frank erosions and purulent exudate; Neutrophils and eosinophils passing through the damaged epithelium; Areas of flattened and cuboidal cells especially found near erosions; Mucosa showing heavy interstitial infiltration by lymphocytes, plasma cells, eosinophils and neutrophils; Glandular abnormalities (neutrophilic invasion of the tubules, epithelial focal degeneration and shedding of necrotic/viable cells into the glandular lumina; crypt abscesses (neutrophils, eosinophils, and epithelial debris); Sometimes, breaking down of the wall of the tubule with inflammatory exudate passing from the tubule into the lamina propria

Adapted from Truelove *et al*[23], 1956.

children and in the early phases of the disease[6,21].

It must also be stressed that in CD, being a transmural disease, mucosal healing may not be sufficient even though the mucosa may return to almost normal after treatment. Deeply situated lesions may persist, and endoscopy with biopsy can however not assess the deeper layers of the digestive tract[22].

An important point to be addressed is that, to date, there is no standardized histological scoring system for the assessment of disease activity in IBD, although many scores have been proposed. A scoring system was firstly introduced many years ago for UC[23], and in subsequent years many other histological scores have been proposed, all based on routine haematoxylin-eosin (HE) staining. As a result of the more homogeneous distribution of the lesions, most of these scores have been described in UC, many during the 1980s[24-28], a few in the 1990s[29-32], and only two more recently[33,34], although some proposal for CD also exist[35-37]. However, even recent consensus conferences on CD and UC have not included a formal histological score for the pathological evaluation of these patients[38-41].

This is partly due to the possible impact of sampling error, to the limits of routine HE staining, and to the existence of some poorly known variables. Histologically, disease activity is usually based upon the combination of the presence of neutrophils and epithelial damage, because neutrophils can be recognized reliably and it is known that they release molecules that are capable of damaging the tissue. Other cells, however, might also have deleterious effects. The activity status of cells such as macrophages and lymphocytes can however not be assessed on HE-stained sections. The influence of eosinophils is still unclear. Elevated levels of eosinophils have been observed in colonic biopsy samples from UC patients, and increased numbers of these cells and eosinophil-derived granular proteins (major basic protein, eosinophil cationic protein, eosinophil peroxidase, and eosinophil derived neurotoxin) have been shown to correlate with morphological changes in the gastrointestinal tract, disease severity, and gastrointestinal dysfunction[42,43].

Finally, the natural evolution of the disease and its histological features are not well known. Inflammatory features may however decrease over time and the distribution pattern may change[44].

HISTOLOGICAL HEALING IN UC

Corticosteroids

These agents were introduced early in the therapeutic armamentarium of IBD, and are still commonly used in the acute phases; moreover, their ability to induce at least macroscopic mucosal healing at endoscopy is well recognized[45]. Concerning histological healing, only a few studies are available in literature, and none recent.

The first study to tackle this topic in 40 patients with active UC investigated the effect of 1 wk treatment with rectally administered hydrocortisone hemisuccinate sodium[46]. Histological variables were analyzed by a previously described scoring system[23] (Table 1), and showed significant improvement compared to baseline and to placebo in patients treated with steroids, in whom there was a 50% decrease in severe grading and 64% in moderate grading. Moreover, 55% shifted toward mild grading after treatment. However, no normalization of histological variables was reported in this study. Interestingly, the authors of the histological scoring system stated that "…this system of grading depends to a large extent on subjective judgments"[23].

In a study of 215 patients with UC, cell counts revealed that acute inflammation as indicated by neutrophils was decreased most notably following treatment with prednisone (and/or 6-mercaptopurine). Chronic inflammation as indicated by the presence of plasma cells was also reduced after prednisone and equally after 6-mercaptopurine and salicylazosulfapyridine. The authors further demonstrated an increase of epithelial goblet cells and lamina propria macrophages during healing[47]. Although these results are particularly interesting, the method used is time consuming and probably difficult to apply routinely.

Another 2-wk study compared the effects of aqueous hydrocortisone enema and a suspension of hydrocortisone in an inert foam base in 30 UC patients with proctosigmoiditis[48]. Histological assessment was carried

Table 2 Modified scoring system for histological assessment of ulcerative colitis

Inflammation grade	Score	Intensity	Histological criteria
Active inflammation	0	Normal	Neutrophils not present in crypt or surface epithelium and no exudate, erosion or ulceration
	1	Low grade	Neutrophils present transmigrating through the crypt epithelium or within crypt lumina in < 20% of crypts; no erosions or ulcers
	2	Moderate	Neutrophilic infiltration in > 20% of crypts or presence of erosions
	3	High grade	Presence of ulcers
Chronic inflammation	0	No increase	Normal number of chronic inflammatory cells present primarily in the superficial lamina propria
	1	Moderate	Moderate number of mononuclear cells Aggregated between crypts at the base of the lamina propria
	2	Severe	Marked increase in chronic inflammation shown by sheets of chronic cells
Crypt distortion	0	None	Crypts had normal outlines with only artifactual irregularities
	1	Mild	Scattered or rare crypts showing irregular (bent, forked) outline
	2	Moderate	Approximately 25%-50% of crypts with an irregular outline
	3	Severe	> 50% of crypts with an irregular outline

Adapted from Hanauer *et al*[50], 1998.

out by means of an active inflammation score (0-3 for each of the following three characteristics: polymorph infiltration, mucus depletion, and superficial epithelial degeneration, allocating a score of 0-9 to each biopsy). In the enema group there was a significant ($P < 0.05$) improvement in active inflammation score (from 4.4 to 2.5) compared to baseline, but this was not observed in the foam group (inflammation score 5.2 *vs* 4.9). However, this apparent superiority for enema treatment was not confirmed when the two treatments were directly compared.

Lee *et al*[49] subsequently compared the effect of mesalazine foam enema (2 g) and prednisolone foam enema (20 mg) in 295 patients with relapsing distal UC. After 4 wk treatment, histological remission (score 0), using the above scoring system[48] was obtained in 27% of patients in the mesalazine group and 21% of patients in the prednisolone group.

In another study, 233 patients with active distal UC/proctitis were randomized to either a placebo enema or budesonide enema at a dose of 0.5 mg/100 mL, 2.0 mg/100 mL, or 8.0 mg/100 mL[50]. Biopsy specimens were histologically graded for active inflammation, chronic inflammation, and crypt distortion, and graded according to a modification of the Truelove and Richards scoring system[23] (Table 2). After treatment, total histopathological score (defined as the sum of the three above components) significantly improved in the 2.0 and 8.0 mg groups, compared to placebo, whereas no differences were found for the 0.5 mg dose. However, remission rate in this study did not include histological remission.

A study conducted on 17 patients with severe UC compared the effect on unfractionated heparin and corticosteroids, and demonstrated an improvement of histopathological grading (that, however, was not formally described), not significantly different between the two treatments (63% in the heparin group and 50% in those treated with steroids) at the end of the study[51].

Rizzello *et al*[52] have evaluated the effect of oral 5-aminosalicylic acid (5-ASA) (3.2 g/d) compared to oral beclometasone dipropionate (5 mg/d) and to placebo in 118 patients with extensive or left-sided mild to moderate UC. After 4 wk of treatment, the histological score[23] was significantly improved compared to baseline, but no differences between treatment groups were observed and no specific histological description was given.

In a more recent study, two budesonide formulations (foam and enema) were compared in 449 patients with distal UC/proctitis[53]. Histological assessment was conducted according to the score proposed by Riley *et al*[29], which takes into account six features [acute inflammatory cell infiltrate (neutrophils in the lamina propria), crypt abscesses, mucin depletion, surface epithelial integrity, chronic inflammatory cell infiltrate (round cells in the lamina propria), and crypt architectural irregularities], each graded on a 4-point scale (0 = none; 1 = mild; 2 = moderate; 3 = severe). At the end of treatment, a similar histological improvement was obtained in both groups (51% foam, 57% enema). Again, no specific histological description was given.

Overall, by considering the above studies, although steroid treatment seems to improve histological abnormalities in UC patients, true remission rates are probably quite low, and no more than 30%.

Salicylates

Overall, not many studies are available in the literature that have assessed the effect of these drugs on histological healing in UC, and it should be stressed that these studies included different subtypes of patients and used different pharmacological formulations and modes of administration. The study by Sommers *et al*[47] mentioned earlier did show some beneficial effects on inflammatory cell cell counts in the lamina propria.

The first study to assess this topic was that of Rao *et al*[54], who compared the effects of olsalazine (2 g/d) and sulfasalazine (3 g/d) in 37 patients with mild to moderate active distal UC. The degree of inflammation in rectal biopsies was graded as absent (without inflammation), mild (chronic glandular damage with definite increase in inflammatory cells), moderate (mild inflam-

mation plus small foci of ulceration), or severe (moderate inflammation in the presence of crypt abscesses and widespread ulceration). In both groups, a similar histological improvement (44% for olsalazine and 46% for sulfasalazine) was seen after 1 mo, even though this was not as impressive as the improvement of endoscopic appearance or clinical response. However, no histological details were given in this study, apart from generic statements (improved, unchanged, worsened), nor it was stated whether histological remission was achieved.

In a 12-wk multicenter double-blind parallel group study, the effects of balsalazide (6.75 g/d) or sulfasalazine (3 g/d) were compared in 57 UC patients, stratified for disease severity; topical and/or oral steroids were administered if clinically needed[55]. Histological assessment (only graded as 0, normal; 1, mild UC; 2, moderate UC; 3, severe UC) showed a similar improvement in both groups at the end of treatment. No histological details were given.

The same authors carried out another study with the same drug regimens (but without concomitant use of steroids) in 50 patients with mild to moderate UC[56]. Pathological assessment was used to identify patients in whom clinical remission was also associated with histological remission. Rectal biopsies, obtained at baseline and after 8 wk) were graded on a 4-point scale (0, normal; 1, minimal inflammation but not active disease; 2, moderate inflammation; 3, severe inflammation); all patients included in the study had at least mild inflammation at baseline, with 58% displaying severe inflammation. After treatment, histological grades improvement was observed in both groups, but only 34% of patients were free from inflammation. No other histological details were given.

In another study, 264 patients with distal active UC were treated with 5-ASA foam or liquid enema[57]. Histological assessment (no details were given), performed after 4 wk, showed similar improvement in both groups (46% generically defined remission in the foam group and 50% in the enema group).

An 8-wk double-blind multicenter trial compared three doses of 5-ASA (1.5, 3 and 4.5 g/d) in 321 patients with active UC[58]. Colonic biopsies were obtained at baseline and at the end of the study, and were assessed according to a previously described scoring system[29], considering histological improvement as reduction of at least one point of the histological activity index. At the end of study period, histological improvement was documented in 42% patients in the 1.5 g group, 65% of the 3 g group, and 63% of the 4.5 g group. Once again, no details on histological assessment were given.

Other authors compared the effects of slow-release (MMx) mesalazine with topical 5-ASA in 79 patients with active left-side UC[59]. The tissue inflammatory response was evaluated according a previously described score[26], and graded as follows: grade 1, normal mucosa; grade 2, enhanced glands with intraepithelial granulocytes. In the stroma, enhancement beyond normal of lymphocytes, plasma cells, or eosinophils (slight inflammation); grade 3, goblet cells depletion, loss of tubular parallelism, reduced mucin production in some glands, intraepithelial granulocytes, marked increase of inflammatory cells in the stroma (intermediate inflammation); grade 4, marked gland and mucosal atrophy, evident crypt abscesses and pus on the surface, massive increase of acute inflammatory cells and follicle formation in deeper cell layers (severe inflammation); and grade 5, ulceration with pus, gland and mucosal atrophy, crypt abscesses, extensive stromal inflammation, and deep follicles (fulminant inflammation). Histological remission (defined generically according to the above score) was obtained in only 15% patients of the MMx group and in 8.0% of those receiving enemas. No other histological details were given.

A more recent study compared two different scheduling dosages (3 g *oid* or 1 g *tid*) of mesalazine granules in 380 UC patients[60]. Biopsy samples were obtained at the start and the end of the trial, and were scored according to previously published criteria[29], with the total histological index based on the more severely affected colonic segment. Histological remission (grade 0), observed in 35% patients in the oid group and in 41% of the tid group, did not show significant differences between the two regimens.

Overall, the available evidence for salicylates in histological healing of UC patients suggests that an improvement may be obtained in 30%-60% of patients (obviously depending on the formulation and the dose scheduling), but the actual healing rate is lower (10%-30%).

Immunomodulators

There are still only a few data on histological healing with immunomodulatory agents. A study conducted in 32 patients with refractory active UC[61] showed that after 6 mo azathioprine treatment, 78% of patients in remission were free of histological inflammation graded according to previously described criteria[23]. However, after a median of 4 years follow-up, histological relapse was found in almost 90% of these patients. No histological details were given.

Another study compared the effects of intravenous cyclosporine A to those of methylprednisolone in 30 patients with severe UC[62]. Histological assessment, carried out according to a previously described scoring system[33] (Table 3), was done at baseline, and after 7 and 30 d therapy; after 1 wk no effects were seen, and only after 1 mo therapy was there a significant decrease in inflammatory cells, and severity of epithelial damage was similarly observed in both groups, although only small changes in the architectural mucosal disturbances were seen. Overall, it seems however that these agents have a beneficial effect on mucosal histology.

Biological agents

Concerning biological therapy, although anti-tumor necrosis factor (TNF)-α therapy can lead to endoscopically assessed mucosal healing in patients with UC[63,64],

Table 3 Histological scoring system for ulcerative colitis and Crohn's disease

Ulcerative colitis	
Grade 0	Structural (architectural change)
0	No abnormality
0.1	Mild abnormality
0.2	Mild or moderate diffuse or multifocal abnormalities
0.3	Severe diffuse or multifocal abnormalities
Grade 1	Chronic inflammatory infiltrate
1	No increase
1.1	Mild but unequivocal increase
1.2	Moderate increase
1.3	Marked increase
Grade 2	Lamina propria neutrophils and eosinophils
2A. Eosinophils	
2A.0	No increase
2A.1	Mild but unequivocal increase
2A.2	Moderate increase
2A.3	Marked increase
2B. Neutrophils	
2B.0	None
2B.1	Mild but unequivocal increase
2B.2	Moderate increase
2B.3	Marked increase
Grade 3	Neutrophils in epithelium
3	None
3.1	< 5% crypt involved
3.2	< 50% crypt involved
3.3	> 50% crypt involved
Grade 4	Crypt destruction
4	None
4.1	Probable-local excess of neutrophils in part of crypt
4.2	Probable-marked attenuation
4.3	Unequivocal crypt destruction
Grade 5	Erosion or ulceration
5	No erosion, ulceration, or granulation tissue
5.1	Recovering epithelium plus adjacent inflammation
5.2	Probable erosion-focally stripped
5.3	Unequivocal erosion
5.4	Ulcer or granulation tissue
Crohn's disease	
Epithelial damage	
0	Normal
1	Focal pathology
2	Extensive pathology
Architectural changes	
0	Normal
1	Moderately disturbed (< 50%)
2	Severely disturbed (> 50%)
Infiltration of mononuclear cells in the lamina propria	
0	Normal
1	Moderate increase
2	Severe increase
Infiltration of polymorphonuclear cells in the lamina propria	
0	Normal
1	Moderate increase
2	Severe increase
Polymorphonuclear cells in the epithelium	
1	In surface epithelium
2	Cryptitis
3	Crypt abscess
Presence of erosions and/or ulcers	
0	No
1	Yes
Presence of granuloma	
0	No
1	Yes
No. of biopsy specimens affected	
0	None (0 of 6)
1	< 33% (1 or 2 of 6)
2	33%-66% (3 or 4 of 6)
3	> 66% (5 or 6 of 6)

Each topic scored independently. Moderate increase, up to twice the number of cells that can normally be expected; severe increase, more than twice the normal number of cells.

histological data are still scarce. A 10-wk study conducted on nine moderate to severe UC patients[65], using a score that included polymorphonuclear infiltration of the epithelium and lamina propria, crypt abscesses, loss of glandular parallelism, crypt shortening and/or ramification, mucus epithelial depletion, involvement of muscularis mucosae and/or submucosa. Each histological variable was scored from 0 (normal) to 1 (mild), 2 (moderate) and 3 (severe). In addition, the total number of neutrophils, lymphocytes, and plasma cells in the lamina propria was counted in five high-power fields. At week 10, histological score significantly decreased only in responders (67% of patients), but normal architecture was observed only in 33% of these patients. The histological improvement was mainly due to a decrease in neutrophils associated with restoration of normal crypt architecture and mucus content in epithelial cells. No significant reduction of mononuclear cell infiltration was observed in responders and non-responders.

Another study took into account the ultrastructural features of the colon in seven patients with UC refractory to standard treatment, before and after 2 wk treatment with infliximab[66]. Before treatment, severe alterations of the epithelium were present, such as microvilli depletion, shattering of epithelial junctions, cytoplasmic vacuolization, dilatation of the endoplasmic reticulum, pycnotic nuclei, altered structure of mitochondria and Golgi complexes, in addition to rarefaction of the goblet cells with abnormal mucus formation and secretion. The chorion showed structural alteration of component cells, obstructed capillaries, erythrocyte extravasation, and many plasmocytes and neutrophils. After infliximab, there was improvement in morphology and function of the epithelial organelles, rich mucus secretion, and recovery of the chorionic components.

Miscellaneous

D'Ovidio *et al*[67] have evaluated the effects of granulocyte-monocyte apheresis in 12 patients with mild to moderate UC refractory to therapy/steroid dependent. After 6 wk, complete histological remission, defined as grade 1 according to the score of Florén *et al*[26], was obtained in six (50%) patients, and histological improvement in five (42%) patients. Once again, no further details on histology were given. In general, it seems that various drugs can lead to microscopic healing and a normal mucosa in a minority of patients (with a maximum of approximately 30%).

HISTOLOGICAL HEALING IN CD

Corticosteroids

These agents are frequently employed for treatment of disease flares; however, clinical and endoscopic data demonstrate that mucosal healing in CD is unlikely (less than one third of treated patients) after steroid treatment[68]. Moreover, there have been few studies evaluating histological healing in CD after steroid treatment.

In a study comparing 1 year treatment with budeso-

nide or azathioprine in 77 CD patients, histological assessment was carried out with a previously developed score[37] (Table 3). At the end of the study, the histological score fell significantly versus baseline only in the azathioprine group and was significantly lower than in the budesonide group at the end of the study[69]. This improvement was observed for all acute parameters (epithelial damage, acute lamina propria inflammatory cell infiltration, erosions and/or ulcers) irrespective of disease location, whereas glandular architecture and the presence (but not the degree) of chronic inflammation were not influenced by azathioprine treatment.

A 2-wk regimen of 20 mg oral prednisone showed, in individual biopsies of 30 CD patients undergoing surveillance colonoscopy, a decrease in the overall histological activity (assessed by a previously reported scoring system[33]) compared to placebo, whereas no significant differences were found with respect to the overall severity of inflammation[70]. No other details on histological assessment were given.

Immunomodulators

These agent represent to date the cornerstone of treatment in CD, thus researchers' interest has given rise to a few studies aimed at evaluating their effects on histological healing as well.

Azathioprine: A first study assessed the effect of 6 mo of therapy in 19 patients with severe postoperative recurrent CD[71]. Histological evaluation was done in seven patients (four with complete mucosal healing and three with near-complete healing at endoscopy). Only in patients with complete healing did comparison of the biopsy specimens taken before and after azathioprine show persistent mucosal architectural changes with complete disappearance of the inflammatory infiltrate. The same authors then reported pre/post-therapy data on 20 CD patients with colitis-ileocolitis taking azathioprine for at least 9 mo[72], using a histological disease severity score[37]. A decrease in histological score paralleled endoscopic healing, even though it was not as complete as shown by endoscopy. In patients with endoscopic complete healing, the colon showed a global decrease in histological score from 10 to 3 and inflammatory score from 5 to 2; in the ileum, global histological score decreased from 7 to 2 and inflammatory score decreased from 2 to 0. No significant changes were documented in patients without endoscopic healing while taking azathioprine. The study of Mantzaris *et al*[69], comparing azathioprine with budesonide, has been discussed above.

Methotrexate: Methotrexate has been even less frequently investigated in this setting. A pilot study evaluated the effects of adding this agent to standard treatment (steroids, mesalazine) in 14 CD patients with refractory disease[73]; four patients (28.5%) were reported to have normal histology at 12 wk. Another study compared the effects of methotrexate with those of azathioprine and infliximab in 40 patients with CD[74]. After 3 mo treatment, the scoring of microscopic activity[37] was similar in the three groups. No further histological data or data on histological healing were given.

Biological agents

A few studies have been carried out on biological agents. D'Haens *et al*[75] investigated histological healing in 30 CD patients treated with infliximab in a placebo-controlled trial. After 4 wk intravenous administration of 5, 10 or 20 mg/kg infliximab or placebo as a single infusion, inflammatory infiltrate disappeared only in infliximab-treated patients but architectural changes persisted in most of them. Another study from the same group evaluated the effects of a single infusion of infliximab in 15 patients with steroid-refractory CD[76]. Histological activity scores, assessed 4 wk after infusion showed, compared to placebo, a significant decrease mainly due to the reduction of inflammatory activity. Immunohistochemical assessment also revealed a decrease in inflammatory features due to global reduction of $CD4^+$ and $CD8^+$ T lymphocytes and $CD68^+$ mononuclear cells.

A follow-up study conducted in CD patients evaluated histological improvement by a previously described scoring system[37] after 54 wk of treatment with infliximab, and showed that histological mucosal healing was associated with a consistent decrease in the expression of inflammatory markers[77]. The use of etanercept (another anti-TNF-α agent) in 10 patients with refractory active CD did not result in any significant histological improvement after 2 wk of therapy[78].

Miscellaneous

Yamamoto *et al*[79] evaluated 28 patients with active CD, treated with an elemental diet for 4 wk. Histology was evaluated by a previously described scoring system[80], taking into consideration enterocyte appearance, extent of crypt inflammation, and the intensity of mononuclear and neutrophilic lamina propria inflammation. Each feature was given a score of 0-3, depending on the severity of the abnormality. The scores were added, and a total score was calculated. Grade 0 (no inflammation) was assigned a total score of 0 or 1, grade 1 (mild) was scored 2-4, grade 2 (moderate) was scored 5-8, and grade 3 (severe) was scored 9-12. Histological healing was defined as grade 0, and histological improvement (including healing) was defined as a decrease of at least 1 grade. At the end of treatment, histological healing and improvement rates were 19% and 54% in the terminal ileum and 20% and 55% in the large bowel, respectively.

A retrospective study carried out in 39 patients with refractory severe colonic CD evaluated the effect of anti-*Mycobacterium avium* ss *paratuberculosis* therapy[81]. Histological acute and chronic ileitis or colitis were recorded as the most severe inflammation found in available biopsies, and were generically reported by a pathologist as ranging from inactive chronic inflammation to mild, moderate, and severe active inflammation. After an aver-

age 3 years of therapy, 15/39 (38.5%) patients showed a marked reduction of acute and chronic inflammatory infiltrates; however, only in six (15.4%) patients was normalization of histological variables observed. No other histological variables or details were given.

HISTOLOGICAL HEALING IN IBD: WHERE ARE WE, AND WHERE ARE WE GOING?

Although mucosal healing has been associated with positive outcomes in IBD, most of the supporting data are retrospective and largely based on endoscopic assessment; in addition, it is not clear whether complete mucosal healing produces better outcomes than partial healing[82]. It has been stated that, in clinical practice, evaluation of mucosal healing (again, by an endoscopic point of view) should be considered in patients with persistent symptoms despite adequate therapy and when treatment discontinuation is being considered[83].

However, it must be stressed that histological assessment of mucosal healing, the only way to establish in an objective manner that the mucosa has reverted to a normal state, is at present not recommended as a primary end point for therapeutic trials because of the lack of a standardized approach[5]. Thus, it is unknown whether patients who achieve and maintain deep remission may stop treatment without having further/future problems[84].

As reported above, true histological healing may be grossly obtained in only about 30% of treated patients; this may justify the high (20%-40%) loss of response to even newer therapeutic approaches[85]. A recent study has shown that adding histological and mucosal gene panel assessment may predict the lack of response to infliximab (directed against TNF-α) treatment in patients with UC or Crohn's colitis, whereas no such predictive gene set could be identified for ileal disease[86,87].

Therefore, adding histological healing as an endpoint, at least in clinical trials, would substantially improve the evaluation of the actual effectiveness of a given treatment. Histological assessment is cheap and widely available. The features of disease activity are known. They include the presence of neutrophils in the lamina propria and the epithelium, epithelial cell damage (loss of cells, mucin depletion, cryptitis, crypt abscesses, erosion), and an increase in lymphocytes and plasma cells. Some of these features such as the presence of neutrophils can reliably be evaluated with good interobserver agreement. In addition, histology could help the management of the patient. Some histological variables are indeed associated with relapse. They include persistence of neutrophils, basal plasmacytosis and persistence of eosinophils in the lamina propria[29,88,89]. Furthermore, microscopic activity may be related to the development of dysplasia[90].

We realize that adding data on histological healing to investigations and trials on IBD patients might be a relatively difficult goal to achieve, and that such an approach would increase the investigators' burden. Furthermore, there are several unresolved issues. It has been shown that UC may become a discontinuous inflammatory process during its natural course or following medical treatment. This means that several biopsies would be needed for an adequate microscopic evaluation; the optimal number of samples has however not yet been determined. Although routine HE staining is a good tool for identification of neutrophils, epithelial damage and even eosinophils as markers of active disease, it is less appropriate for the evaluation of lymphocytes. The presence of lymphocytes can be determined adequately but the activation status is beyond the scope of routine histology and requires immunohistochemical staining. Yet, the application of this technique and the markers needed are not yet well determined.

CONCLUSION

In conclusion, it appears to us that histological healing should be considered as an end point in the treatment of IBD patients, especially for UC. Therefore, additional studies looking for optimal sampling, standardizing scores and features should be performed.

REFERENCES

1 **Pithadia AB**, Jain S. Treatment of inflammatory bowel disease (IBD). *Pharmacol Rep* 2011; **63**: 629-642 [PMID: 21857074]
2 **Taylor KM**, Irving PM. Optimization of conventional therapy in patients with IBD. *Nat Rev Gastroenterol Hepatol* 2011; **8**: 646-656 [PMID: 21970871 DOI: 10.1038/nrgastro.2011.172]
3 **Sandborn WJ**, Rutgeerts P, Feagan BG, Reinisch W, Olson A, Johanns J, Lu J, Horgan K, Rachmilewitz D, Hanauer SB, Lichtenstein GR, de Villiers WJ, Present D, Sands BE, Colombel JF. Colectomy rate comparison after treatment of ulcerative colitis with placebo or infliximab. *Gastroenterology* 2009; **137**: 1250-1260; quiz 1520 [PMID: 19596014 DOI: 10.1053/j.gastro.2009.06.061]
4 **D'Haens G**, Sandborn WJ, Feagan BG, Geboes K, Hanauer SB, Irvine EJ, Lémann M, Marteau P, Rutgeerts P, Schölmerich J, Sutherland LR. A review of activity indices and efficacy end points for clinical trials of medical therapy in adults with ulcerative colitis. *Gastroenterology* 2007; **132**: 763-786 [PMID: 17258735 DOI: 10.1053/j.gastro.2006.12.038]
5 **Kornbluth A**, Sachar DB. Ulcerative colitis practice guidelines in adults (update): American College of Gastroenterology, Practice Parameters Committee. *Am J Gastroenterol* 2004; **99**: 1371-1385 [PMID: 15233681 DOI: 10.1111/j.1572-0241.2004.40036.x]
6 **Geboes K**, Dalle I. Influence of treatment on morphological features of mucosal inflammation. *Gut* 2002; **50** Suppl 3: III37-III42 [PMID: 11953331 DOI: 10.1136/gut.50.suppl_3.iii37]
7 **Bardella MT**, Velio P, Cesana BM, Prampolini L, Casella G, Di Bella C, Lanzini A, Gambarotti M, Bassotti G, Villanacci V. Coeliac disease: a histological follow-up study. *Histopathology* 2007; **50**: 465-471 [PMID: 17448022 DOI: 10.1111/j.1365-2559.2007.02621.x]
8 **Cornaggia M**, Leutner M, Mescoli C, Sturniolo GC, Gullotta R. Chronic idiopathic inflammatory bowel diseases: the histology report. *Dig Liver Dis* 2011; **43** Suppl 4: S293-S303 [PMID: 21459335 DOI: 10.1016/S1590-8658(11)60585-9]
9 **Pineton de Chambrun G**, Peyrin-Biroulet L, Lémann M, Colombel JF. Clinical implications of mucosal healing for the management of IBD. *Nat Rev Gastroenterol Hepatol* 2010; **7**: 15-29 [PMID: 19949430 DOI: 10.1038/nrgastro.2009.203]
10 **Vatn MH**. Mucosal healing: impact on the natural course or therapeutic strategies. *Dig Dis* 2009; **27**: 470-475 [PMID:

19897962 DOI: 10.1159/000233285]
11 **Villanacci V**, Manenti S, Antonelli E, Chiudinelli M, Giuliano V, Bassotti G. Non-IBD colitides: clinically useful histopathological clues. *Rev Esp Enferm Dig* 2011; **103**: 366-372 [PMID: 21770683 DOI: 10.4321/S1130-01082011000700006]
12 **Jenkins D**, Balsitis M, Gallivan S, Dixon MF, Gilmour HM, Shepherd NA, Theodossi A, Williams GT. Guidelines for the initial biopsy diagnosis of suspected chronic idiopathic inflammatory bowel disease. The British Society of Gastroenterology Initiative. *J Clin Pathol* 1997; **50**: 93-105 [PMID: 9155688 DOI: 10.1136/jcp.50.2.93]
13 **Schumacher G**, Kollberg B, Sandstedt B. A prospective study of first attacks of inflammatory bowel disease and infectious colitis. Histologic course during the 1st year after presentation. *Scand J Gastroenterol* 1994; **29**: 318-332 [PMID: 8047806 DOI: 10.3109/00365529409094843]
14 **Theodossi A**, Spiegelhalter DJ, Jass J, Firth J, Dixon M, Leader M, Levison DA, Lindley R, Filipe I, Price A. Observer variation and discriminatory value of biopsy features in inflammatory bowel disease. *Gut* 1994; **35**: 961-968 [PMID: 8063225 DOI: 10.1136/gut.35.7.961]
15 **Rubio CA**, Johansson C, Kock Y. A quantitative method of estimating inflammation in the rectal mucosa. III. Chronic ulcerative colitis. *Scand J Gastroenterol* 1982; **17**: 1083-1087 [PMID: 7167739]
16 **Surawicz CM**, Belic L. Rectal biopsy helps to distinguish acute self-limited colitis from idiopathic inflammatory bowel disease. *Gastroenterology* 1984; **86**: 104-113 [PMID: 6689653]
17 **Seldenrijk CA**, Morson BC, Meuwissen SG, Schipper NW, Lindeman J, Meijer CJ. Histopathological evaluation of colonic mucosal biopsy specimens in chronic inflammatory bowel disease: diagnostic implications. *Gut* 1991; **32**: 1514-1520 [PMID: 1773958 DOI: 10.1136/gut.32.12.1514]
18 **Rubio CA**, Johansson C, Uribe A, Kock Y. A quantitative method of estimating inflammation in the rectal mucosa. IV. Ulcerative colitis in remission. *Scand J Gastroenterol* 1984; **19**: 525-530 [PMID: 6463576]
19 **Jenkins D**, Goodall A, Drew K, Scott BB. What is colitis? Statistical approach to distinguishing clinically important inflammatory change in rectal biopsy specimens. *J Clin Pathol* 1988; **41**: 72-79 [PMID: 3343381 DOI: 10.1136/jcp.41.1.72]
20 **Scott BB**, Goodall A, Stephenson P, Jenkins D. Rectal mucosal plasma cells in inflammatory bowel disease. *Gut* 1983; **24**: 519-524 [PMID: 6852632 DOI: 10.1136/gut.24.6.519]
21 **Rubio CA**, Orrego A, Nesi G, Finkel Y. Frequency of epithelioid granulomas in colonoscopic biopsy specimens from paediatric and adult patients with Crohn's colitis. *J Clin Pathol* 2007; **60**: 1268-1272 [PMID: 17293387 DOI: 10.1136/jcp.2006.045336]
22 **Frizelle FA**, Ing A, Gearry RB, Whitehead M, Faragher IG, Dobbs B. Immunomodulation does not alter histology in resected Crohn's disease. *Tech Coloproctol* 2009; **13**: 295-300 [PMID: 19774438 DOI: 10.1007/s10151-009-0538-7]
23 **Truelove SC**, Richards WC. Biopsy studies in ulcerative colitis. *Br Med J* 1956; **1**: 1315-1318 [PMID: 13316140 DOI: 10.1136/bmj.1.4979.1315]
24 **Powell-Tuck J**, Day DW, Buckell NA, Wadsworth J, Lennard-Jones JE. Correlations between defined sigmoidoscopic appearances and other measures of disease activity in ulcerative colitis. *Dig Dis Sci* 1982; **27**: 533-537 [PMID: 6979471 DOI: 10.1007/BF01296733]
25 **Gomes P**, du Boulay C, Smith CL, Holdstock G. Relationship between disease activity indices and colonoscopic findings in patients with colonic inflammatory bowel disease. *Gut* 1986; **27**: 92-95 [PMID: 3949241 DOI: 10.1136/gut.27.1.92]
26 **Florén CH**, Benoni C, Willén R. Histologic and colonoscopic assessment of disease extension in ulcerative colitis. *Scand J Gastroenterol* 1987; **22**: 459-462 [PMID: 3602926 DOI: 10.3109/00365528708991491]
27 **Danielsson A**, Hellers G, Lyrenäs E, Löfberg R, Nilsson A, Olsson O, Olsson SA, Persson T, Salde L, Naesdal J. A controlled randomized trial of budesonide versus prednisolone retention enemas in active distal ulcerative colitis. *Scand J Gastroenterol* 1987; **22**: 987-992 [PMID: 3317784 DOI: 10.3109/00365528708991947]
28 **Riley SA**, Mani V, Goodman MJ, Herd ME, Dutt S, Turnberg LA. Comparison of delayed-release 5-aminosalicylic acid (mesalazine) and sulfasalazine as maintenance treatment for patients with ulcerative colitis. *Gastroenterology* 1988; **94**: 1383-1389 [PMID: 2896139]
29 **Riley SA**, Mani V, Goodman MJ, Dutt S, Herd ME. Microscopic activity in ulcerative colitis: what does it mean? *Gut* 1991; **32**: 174-178 [PMID: 1864537 DOI: 10.1136/gut.32.2.174]
30 **Odze R**, Antonioli D, Peppercorn M, Goldman H. Effect of topical 5-aminosalicylic acid (5-ASA) therapy on rectal mucosal biopsy morphology in chronic ulcerative colitis. *Am J Surg Pathol* 1993; **17**: 869-875 [PMID: 8352372 DOI: 10.1097/00000478-199309000-00002]
31 **Beattie RM**, Schiffrin EJ, Donnet-Hughes A, Huggett AC, Domizio P, MacDonald TT, Walker-Smith JA. Polymeric nutrition as the primary therapy in children with small bowel Crohn's disease. *Aliment Pharmacol Ther* 1994; **8**: 609-615 [PMID: 7696450 DOI: 10.1111/j.1365-2036.1994.tb00338.x]
32 **Mulder CJ**, Fockens P, Meijer JW, van der Heide H, Wiltink EH, Tytgat GN. Beclomethasone dipropionate (3 mg) versus 5-aminosalicylic acid (2 g) versus the combination of both (3 mg/2 g) as retention enemas in active ulcerative proctitis. *Eur J Gastroenterol Hepatol* 1996; **8**: 549-553 [PMID: 8823568 DOI: 10.1097/00042737-199606000-00010]
33 **Geboes K**, Riddell R, Ost A, Jensfelt B, Persson T, Löfberg R. A reproducible grading scale for histological assessment of inflammation in ulcerative colitis. *Gut* 2000; **47**: 404-409 [PMID: 10940279 DOI: 10.1136/gut.47.3.404]
34 **D'Argenio G**, Cosenza V, Riegler G, Della Valle N, Deritis F, Mazzacca G. Serum transglutaminase correlates with endoscopic and histopathologic grading in patients with ulcerative colitis. *Dig Dis Sci* 2001; **46**: 649-657 [PMID: 11318547]
35 **Nicholls S**, Domizio P, Williams CB, Dawnay A, Braegger CP, MacDonald TT, Walker-Smith JA. Cyclosporin as initial treatment for Crohn's disease. *Arch Dis Child* 1994; **71**: 243-247 [PMID: 7979499 DOI: 10.1136/adc.71.3.243]
36 **Breese EJ**, Michie CA, Nicholls SW, Williams CB, Domizio P, Walker-Smith JA, MacDonald TT. The effect of treatment on lymphokine-secreting cells in the intestinal mucosa of children with Crohn's disease. *Aliment Pharmacol Ther* 1995; **9**: 547-552 [PMID: 8580276 DOI: 10.1111/j.1365-2036.1995.tb00419.x]
37 **D'Haens GR**, Geboes K, Peeters M, Baert F, Penninckx F, Rutgeerts P. Early lesions of recurrent Crohn's disease caused by infusion of intestinal contents in excluded ileum. *Gastroenterology* 1998; **114**: 262-267 [PMID: 9453485 DOI: 10.1016/S0016-5085(98)70476-7]
38 **Stange EF**, Travis SP, Vermeire S, Beglinger C, Kupcinkas L, Geboes K, Barakauskiene A, Villanacci V, Von Herbay A, Warren BF, Gasche C, Tilg H, Schreiber SW, Schölmerich J, Reinisch W. European evidence based consensus on the diagnosis and management of Crohn's disease: definitions and diagnosis. *Gut* 2006; **55** Suppl 1: i1-15 [PMID: 16481628 DOI: 10.1136/gut.2005.081950a]
39 **Stange EF**, Travis SP, Vermeire S, Reinisch W, Geboes K, Barakauskiene A, Feakins R, Fléjou JF, Herfarth H, Hommes DW, Kupcinskas L, Lakatos PL, Mantzaris GJ, Schreiber S, Villanacci V, Warren BF. European evidence-based Consensus on the diagnosis and management of ulcerative colitis: Definitions and diagnosis. *J Crohns Colitis* 2008; **2**: 1-23 [PMID: 21172194 DOI: 10.1016/j.crohns.2007.11.001]
40 **Peyrin-Biroulet L**, Ferrante M, Magro F, Campbell S, Franchimont D, Fidder H, Strid H, Ardizzone S, Veereman-Wauters

G, Chevaux JB, Allez M, Danese S, Sturm A. Results from the 2nd Scientific Workshop of the ECCO. I: Impact of mucosal healing on the course of inflammatory bowel disease. *J Crohns Colitis* 2011; **5**: 477-483 [PMID: 21939925 DOI: 10.1016/j.crohns.2011.06.009]

41 **Daperno M**, Castiglione F, de Ridder L, Dotan I, Färkkilä M, Florholmen J, Fraser G, Fries W, Hebuterne X, Lakatos PL, Panés J, Rimola J, Louis E. Results of the 2nd part Scientific Workshop of the ECCO. II: Measures and markers of prediction to achieve, detect, and monitor intestinal healing in inflammatory bowel disease. *J Crohns Colitis* 2011; **5**: 484-498 [PMID: 21939926 DOI: 10.1016/j.crohns.2011.07.003]

42 **Saitoh O**, Kojima K, Sugi K, Matsuse R, Uchida K, Tabata K, Nakagawa K, Kayazawa M, Hirata I, Katsu K. Fecal eosinophil granule-derived proteins reflect disease activity in inflammatory bowel disease. *Am J Gastroenterol* 1999; **94**: 3513-3520 [PMID: 10606313 DOI: 10.1111/j.1572-0241.1999.01640.x]

43 **Carvalho AT**, Elia CC, de Souza HS, Elias PR, Pontes EL, Lukashok HP, de Freitas FC, Lapa e Silva JR. Immunohistochemical study of intestinal eosinophils in inflammatory bowel disease. *J Clin Gastroenterol* 2003; **36**: 120-125 [PMID: 12544193 DOI: 10.1097/00004836-200302000-00006]

44 **Kleer CG**, Appelman HD. Ulcerative colitis: patterns of involvement in colorectal biopsies and changes with time. *Am J Surg Pathol* 1998; **22**: 983-989 [PMID: 9706978 DOI: 10.1097/00000478-199808000-00008]

45 **Regueiro M**, Loftus EV, Steinhart AH, Cohen RD. Medical management of left-sided ulcerative colitis and ulcerative proctitis: critical evaluation of therapeutic trials. *Inflamm Bowel Dis* 2006; **12**: 979-994 [PMID: 17012969 DOI: 10.1097/01.mib.0000231495.92013.5e]

46 **TRUELOVE SC**, HAMBLING MH. Treatment of ulcerative colitis with local hydrocortisone hemisuccinate sodium; a report on a controlled therapeutic trial. *Br Med J* 1958; **2**: 1072-1077 [PMID: 13584853 DOI: 10.1136/bmj.2.5104.1072]

47 **Sommers SC**, Korelitz BI. Mucosal-cell counts in ulcerative and granulomatous colitis. *Am J Clin Pathol* 1975; **63**: 359-365 [PMID: 234674]

48 **Ruddell WS**, Dickinson RJ, Dixon MF, Axon AT. Treatment of distal ulcerative colitis (proctosigmoiditis) in relapse: comparison of hydrocortisone enemas and rectal hydrocortisone foam. *Gut* 1980; **21**: 885-889 [PMID: 7002739 DOI: 10.1136/gut.21.10.885]

49 **Lee FI**, Jewell DP, Mani V, Keighley MR, Kingston RD, Record CO, Grace RH, Daniels S, Patterson J, Smith K. A randomised trial comparing mesalazine and prednisolone foam enemas in patients with acute distal ulcerative colitis. *Gut* 1996; **38**: 229-233 [PMID: 8801202 DOI: 10.1136/gut.38.2.229]

50 **Hanauer SB**, Robinson M, Pruitt R, Lazenby AJ, Persson T, Nilsson LG, Walton-Bowen K, Haskell LP, Levine JG. Budesonide enema for the treatment of active, distal ulcerative colitis and proctitis: a dose-ranging study. U.S. Budesonide enema study group. *Gastroenterology* 1998; **115**: 525-532 [PMID: 9721148 DOI: 10.1016/S0016-5085(98)70131-3]

51 **Ang YS**, Mahmud N, White B, Byrne M, Kelly A, Lawler M, McDonald GS, Smith OP, Keeling PW. Randomized comparison of unfractionated heparin with corticosteroids in severe active inflammatory bowel disease. *Aliment Pharmacol Ther* 2000; **14**: 1015-1022 [PMID: 10930895 DOI: 10.1046/j.1365-2036.2000.00802.x]

52 **Rizzello F**, Gionchetti P, D'Arienzo A, Manguso F, Di Matteo G, Annese V, Valpiani D, Casetti T, Adamo S, Prada A, Castiglione GN, Varoli G, Campieri M. Oral beclometasone dipropionate in the treatment of active ulcerative colitis: a double-blind placebo-controlled study. *Aliment Pharmacol Ther* 2002; **16**: 1109-1116 [PMID: 12030952 DOI: 10.1046/j.1365-2036.2002.01298.x]

53 **Gross V**, Bar-Meir S, Lavy A, Mickisch O, Tulassay Z, Pronai L, Kupcinskas L, Kiudelis G, Pokrotnieks J, Kovács A, Faszczyk M, Razbadauskas A, Margus B, Stolte M, Müller R, Greinwald R. Budesonide foam versus budesonide enema in active ulcerative proctitis and proctosigmoiditis. *Aliment Pharmacol Ther* 2006; **23**: 303-312 [PMID: 16393311 DOI: 10.1111/j.1365-2036.2006.02743.x]

54 **Rao SS**, Dundas SA, Holdsworth CD, Cann PA, Palmer KR, Corbett CL. Olsalazine or sulphasalazine in first attacks of ulcerative colitis? A double blind study. *Gut* 1989; **30**: 675-679 [PMID: 2567266 DOI: 10.1136/gut.30.5.675]

55 **Green JR**, Mansfield JC, Gibson JA, Kerr GD, Thornton PC. A double-blind comparison of balsalazide, 6.75 g daily, and sulfasalazine, 3 g daily, in patients with newly diagnosed or relapsed active ulcerative colitis. *Aliment Pharmacol Ther* 2002; **16**: 61-68 [PMID: 11856079]

56 **Mansfield JC**, Giaffer MH, Cann PA, McKenna D, Thornton PC, Holdsworth CD. A double-blind comparison of balsalazide, 6.75 g, and sulfasalazine, 3 g, as sole therapy in the management of ulcerative colitis. *Aliment Pharmacol Ther* 2002; **16**: 69-77 [PMID: 11856080 DOI: 10.1046/j.1365-2036.2002.01151.x]

57 **Malchow H**, Gertz B. A new mesalazine foam enema (Claversal Foam) compared with a standard liquid enema in patients with active distal ulcerative colitis. *Aliment Pharmacol Ther* 2002; **16**: 415-423 [PMID: 11876694 DOI: 10.1046/j.1365-2036.2002.01199.x]

58 **Kruis W**, Bar-Meir S, Feher J, Mickisch O, Mlitz H, Faszczyk M, Chowers Y, Lengyele G, Kovacs A, Lakatos L, Stolte M, Vieth M, Greinwald R. The optimal dose of 5-aminosalicylic acid in active ulcerative colitis: a dose-finding study with newly developed mesalamine. *Clin Gastroenterol Hepatol* 2003; **1**: 36-43 [PMID: 15017515 DOI: 10.1053/jcgh.2003.50006]

59 **Prantera C**, Viscido A, Biancone L, Francavilla A, Giglio L, Campieri M. A new oral delivery system for 5-ASA: preliminary clinical findings for MMx. *Inflamm Bowel Dis* 2005; **11**: 421-427 [PMID: 15867580 DOI: 10.1097/01.MIB.0000158386.25660.1e]

60 **Kruis W**, Kiudelis G, Rácz I, Gorelov IA, Pokrotnieks J, Horynski M, Batovsky M, Kykal J, Boehm S, Greinwald R, Mueller R. Once daily versus three times daily mesalazine granules in active ulcerative colitis: a double-blind, double-dummy, randomised, non-inferiority trial. *Gut* 2009; **58**: 233-240 [PMID: 18832520 DOI: 10.1136/gut.2008.154302]

61 **Paoluzi OA**, Pica R, Marcheggiano A, Crispino P, Iacopini F, Iannoni C, Rivera M, Paoluzi P. Azathioprine or methotrexate in the treatment of patients with steroid-dependent or steroid-resistant ulcerative colitis: results of an open-label study on efficacy and tolerability in inducing and maintaining remission. *Aliment Pharmacol Ther* 2002; **16**: 1751-1759 [PMID: 12269968 DOI: 10.1046/j.1365-2036.2002.01340.x]

62 **D'Haens G**, Lemmens L, Geboes K, Vandeputte L, Van Acker F, Mortelmans L, Peeters M, Vermeire S, Penninckx F, Nevens F, Hiele M, Rutgeerts P. Intravenous cyclosporine versus intravenous corticosteroids as single therapy for severe attacks of ulcerative colitis. *Gastroenterology* 2001; **120**: 1323-1329 [PMID: 11313301 DOI: 10.1053/gast.2001.23983]

63 **Hanauer SB**. The role of biologics in ulcerative colitis. *Dig Dis* 2010; **28**: 497-500 [PMID: 20926878 DOI: 10.1159/000320408]

64 **Colombel JF**, Rutgeerts P, Reinisch W, Esser D, Wang Y, Lang Y, Marano CW, Strauss R, Oddens BJ, Feagan BG, Hanauer SB, Lichtenstein GR, Present D, Sands BE, Sandborn WJ. Early mucosal healing with infliximab is associated with improved long-term clinical outcomes in ulcerative colitis. *Gastroenterology* 2011; **141**: 1194-1201 [PMID: 21723220 DOI: 10.1053/j.gastro.2011.06.054]

65 **Hassan C**, Ierardi E, Burattini O, De Francesco V, Zullo A, Stoppino G, Panella C, Morini S. Tumour necrosis factor alpha down-regulation parallels inflammatory regression in ulcerative colitis patients treated with infliximab. *Dig Liver Dis* 2007; **39**: 811-817 [PMID: 17652038 DOI: 10.1016/j.dld.2007.06.003]

66 **Fratila OC**, Craciun C. Ultrastructural evidence of mucosal healing after infliximab in patients with ulcerative colitis. *J Gastrointestin Liver Dis* 2010; **19**: 147-153 [PMID: 20593047]

67 **D'Ovidio V**, Aratari A, Viscido A, Marcheggiano A, Papi C, Capurso L, Caprilli R. Mucosal features and granulocyte-monocyte-apheresis in steroid-dependent/refractory ulcerative colitis. *Dig Liver Dis* 2006; **38**: 389-394 [PMID: 16569521 DOI: 10.1016/j.dld.2005.12.005]

68 **Modigliani R**, Mary JY, Simon JF, Cortot A, Soule JC, Gendre JP, Rene E. Clinical, biological, and endoscopic picture of attacks of Crohn's disease. Evolution on prednisolone. Groupe d'Etude Thérapeutique des Affections Inflammatoires Digestives. *Gastroenterology* 1990; **98**: 811-818 [PMID: 2179031 DOI: 10.1016/0016-5085(90)90002-I]

69 **Mantzaris GJ**, Christidou A, Sfakianakis M, Roussos A, Koilakou S, Petraki K, Polyzou P. Azathioprine is superior to budesonide in achieving and maintaining mucosal healing and histologic remission in steroid-dependent Crohn's disease. *Inflamm Bowel Dis* 2009; **15**: 375-382 [PMID: 19009634 DOI: 10.1002/ibd.20777]

70 **Baars JE**, Vogelaar L, Wolfhagen FH, Biermann K, Kuipers EJ, van der Woude CJ. A short course of corticosteroids prior to surveillance colonoscopy to decrease mucosal inflammation in inflammatory bowel disease patients: results from a randomized controlled trial. *J Crohns Colitis* 2010; **4**: 661-668 [PMID: 21122577 DOI: 10.1016/j.crohns.2010.07.011]

71 **D'Haens G**, Geboes K, Ponette E, Penninckx F, Rutgeerts P. Healing of severe recurrent ileitis with azathioprine therapy in patients with Crohn's disease. *Gastroenterology* 1997; **112**: 1475-1481 [PMID: 9136824]

72 **D'Haens G**, Geboes K, Rutgeerts P. Endoscopic and histologic healing of Crohn's (ileo-) colitis with azathioprine. *Gastrointest Endosc* 1999; **50**: 667-671 [PMID: 10536324 DOI: 10.1016/S0016-5107(99)80017-0]

73 **Kozarek RA**, Patterson DJ, Gelfand MD, Botoman VA, Ball TJ, Wilske KR. Methotrexate induces clinical and histologic remission in patients with refractory inflammatory bowel disease. *Ann Intern Med* 1989; **110**: 353-356 [PMID: 2492786]

74 **Laharie D**, Reffet A, Belleannée G, Chabrun E, Subtil C, Razaire S, Capdepont M, de Lédinghen V. Mucosal healing with methotrexate in Crohn's disease: a prospective comparative study with azathioprine and infliximab. *Aliment Pharmacol Ther* 2011; **33**: 714-721 [PMID: 21235604 DOI: 10.1111/j.1365-2036.2010.04569.x]

75 **D'Haens G**, Van Deventer S, Van Hogezand R, Chalmers D, Kothe C, Baert F, Braakman T, Schaible T, Geboes K, Rutgeerts P. Endoscopic and histological healing with infliximab anti-tumor necrosis factor antibodies in Crohn's disease: A European multicenter trial. *Gastroenterology* 1999; **116**: 1029-1034 [PMID: 10220494]

76 **Baert FJ**, D'Haens GR, Peeters M, Hiele MI, Schaible TF, Shealy D, Geboes K, Rutgeerts PJ. Tumor necrosis factor alpha antibody (infliximab) therapy profoundly down-regulates the inflammation in Crohn's ileocolitis. *Gastroenterology* 1999; **116**: 22-28 [PMID: 9869598 DOI: 10.1016/S0016-5085(99)70224-6]

77 **Geboes K**, Rutgeerts P, Opdenakker G, Olson A, Patel K, Wagner CL, Marano CW. Endoscopic and histologic evidence of persistent mucosal healing and correlation with clinical improvement following sustained infliximab treatment for Crohn's disease. *Curr Med Res Opin* 2005; **21**: 1741-1754 [PMID: 16307694 DOI: 10.1185/030079905X65457]

78 **D'Haens G**, Swijsen C, Noman M, Lemmens L, Ceuppens J, Agbahiwe H, Geboes K, Rutgeerts P. Etanercept in the treatment of active refractory Crohn's disease: a single-center pilot trial. *Am J Gastroenterol* 2001; **96**: 2564-2568 [PMID: 11569676 DOI: 10.1111/j.1572-0241.2001.04705.x]

79 **Yamamoto T**, Nakahigashi M, Umegae S, Kitagawa T, Matsumoto K. Impact of elemental diet on mucosal inflammation in patients with active Crohn's disease: cytokine production and endoscopic and histological findings. *Inflamm Bowel Dis* 2005; **11**: 580-588 [PMID: 15905706 DOI: 10.1097/01.MIB.0000161307.58327.96]

80 **Saverymuttu SH**, Camilleri M, Rees H, Lavender JP, Hodgson HJ, Chadwick VS. Indium 111-granulocyte scanning in the assessment of disease extent and disease activity in inflammatory bowel disease. A comparison with colonoscopy, histology, and fecal indium 111-granulocyte excretion. *Gastroenterology* 1986; **90**: 1121-1128 [PMID: 3956932]

81 **Borody TJ**, Bilkey S, Wettstein AR, Leis S, Pang G, Tye S. Anti-mycobacterial therapy in Crohn's disease heals mucosa with longitudinal scars. *Dig Liver Dis* 2007; **39**: 438-444 [PMID: 17369114 DOI: 10.1016/j.dld.2007.01.008]

82 **Stidham RW**, Higgins PD. Value of mucosal assessment and biomarkers in inflammatory bowel disease. *Expert Rev Gastroenterol Hepatol* 2010; **4**: 285-291 [PMID: 20528116 DOI: 10.1586/egh.10.22]

83 **van Assche G**, Vermeire S, Rutgeerts P. Mucosal healing and anti TNFs in IBD. *Curr Drug Targets* 2010; **11**: 227-233 [PMID: 20210770 DOI: 10.2174/138945010790309902]

84 **Clarke K**, Regueiro M. Stopping immunomodulators and biologics in inflammatory bowel disease patients in remission. *Inflamm Bowel Dis* 2012; **18**: 174-179 [PMID: 21674731 DOI: 10.1002/ibd.21792]

85 **Danese S**, Fiorino G, Reinisch W. Review article: Causative factors and the clinical management of patients with Crohn's disease who lose response to anti-TNF-α therapy. *Aliment Pharmacol Ther* 2011; **34**: 1-10 [PMID: 21539588 DOI: 10.1111/j.1365-2036.2011.04679.x]

86 **Arijs I**, Li K, Toedter G, Quintens R, Van Lommel L, Van Steen K, Leemans P, De Hertogh G, Lemaire K, Ferrante M, Schnitzler F, Thorrez L, Ma K, Song XY, Marano C, Van Assche G, Vermeire S, Geboes K, Schuit F, Baribaud F, Rutgeerts P. Mucosal gene signatures to predict response to infliximab in patients with ulcerative colitis. *Gut* 2009; **58**: 1612-1619 [PMID: 19700435 DOI: 10.1136/gut.2009.178665]

87 **Arijs I**, Quintens R, Van Lommel L, Van Steen K, De Hertogh G, Lemaire K, Schraenen A, Perrier C, Van Assche G, Vermeire S, Geboes K, Schuit F, Rutgeerts P. Predictive value of epithelial gene expression profiles for response to infliximab in Crohn's disease. *Inflamm Bowel Dis* 2010; **16**: 2090-2098 [PMID: 20848504 DOI: 10.1002/ibd.21301]

88 **Bitton A**, Peppercorn MA, Antonioli DA, Niles JL, Shah S, Bousvaros A, Ransil B, Wild G, Cohen A, Edwardes MD, Stevens AC. Clinical, biological, and histologic parameters as predictors of relapse in ulcerative colitis. *Gastroenterology* 2001; **120**: 13-20 [PMID: 11208709 DOI: 10.1053/gast.2001.20912]

89 **Azad S**, Sood N, Sood A. Biological and histological parameters as predictors of relapse in ulcerative colitis: a prospective study. *Saudi J Gastroenterol* 2011; **17**: 194-198 [PMID: 21546723 DOI: 10.4103/1319-3767.80383]

90 **Gupta RB**, Harpaz N, Itzkowitz S, Hossain S, Matula S, Kornbluth A, Bodian C, Ullman T. Histologic inflammation is a risk factor for progression to colorectal neoplasia in ulcerative colitis: a cohort study. *Gastroenterology* 2007; **133**: 1099-1105; quiz 1340-1341 [PMID: 17919486 DOI: 10.1053/j.gastro.2007.08.001]

P- Reviewers Shehata MM, Costa F
S- Editor Gou SX **L- Editor** Kerr C **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i7.979

World J Gastroenterol 2013 February 21; 19(7): 979-983
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

TOPIC HIGHLIGHT

Jung Eun Lee, ScD, Assistant Professor, *Series Editor*

Colorectal cancer mortality in Hong Kong of China, Japan, South Korea, and Singapore

Aesun Shin, Kyu-Won Jung, Young-Joo Won

Aesun Shin, Kyu-Won Jung, Young-Joo Won, Cancer Registration and Statistics Branch, National Cancer Control Institute, National Cancer Center, Goyang-si 410-769, South Korea
Aesun Shin, Molecular Epidemiology Branch, Division of Cancer Epidemiology and Prevention, National Cancer Center Research Institute, Goyang-si 410-769, South Korea
Author contributions: Shin A contributed to the study design, literature review, manuscript writing, and final revision of the article; Jung KW contributed to the study design, data analysis and critical revision of the manuscript; Won YJ contributed to the study design, and critical revision of the manuscript.
Supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology, No. 2010-0010276; A Research Grant from the National Cancer Center of Korea, No. 1010160
Correspondence to: Kyu-Won Jung, Branch Chief, Cancer Registration and Statistics Branch, National Cancer Control Institute, National Cancer Center, 323 Ilsanro, Ilsandong-gu, Goyang-si 410-769, South Korea. ara@ncc.re.kr
Telephone: +82-31-9202170 Fax: +82-31-9202179
Received: September 19, 2012 Revised: January 8, 2013
Accepted: January 23, 2013
Published online: February 21, 2013

Abstract

To clarify the trend in colorectal cancer mortality in Asian countries. We analyzed the colorectal cancer mortality in four Asian countries using the World Health Organization mortality database and the Korea National Statistics Office database. The annual age-standardized rates and truncated rated for the three age groups (30-49, 50-69 and ⩾ 70 years) for Hong Kong of China (1969-2009), Japan (1955-2009), South Korea (1985-2006), and Singapore (1966-2009) were estimated. A joinpoint regression model was used to detect significant trends in mortality rates. Colorectal cancer mortality in men started to decrease in 1992 in Japan followed by Singapore and Hong Kong of China in 1995. The mortality rates in women stared to decrease in 1980 in Singapore, followed by Hong Kong of China and Japan in 1996. In all countries and both genders, except for women in Singapore, the decrease in mortality began in the younger age groups. The colorectal cancer mortality in the four studied Asian countries has started to decrease, and the decrease occurred first in the younger age groups.

© 2013 Baishideng. All rights reserved.

Key words: Colorectal cancer; Mortality; Joinpoint regression; Trends; Early detection of cancer; Mass screening

Shin A, Jung KW, Won YJ. Colorectal cancer mortality in Hong Kong of China, Japan, South Korea, and Singapore. *World J Gastroenterol* 2013; 19(7): 979-983 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i7/979.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i7.979

INTRODUCTION

Colorectal cancer is responsible for 8% of all cancer deaths worldwide, with an estimated 608 000 deaths annually, and it is ranked as the fourth most common cause of death from cancer[1]. It has been estimated that the numbers of deaths due to colorectal cancer will reach approximately 376 700 by 2020 in Asia[1].

A decline in colorectal cancer mortality has been observed in most western and northern European countries[2] and the United States[3]. Among Asian countries, the mortality started to decrease in the early 1990s in Japan[4,5]. The trends in colorectal mortality differ by geographic region, sex, and age group[6]. In this review, we analyzed the trends in colorectal cancer mortality in 4 Asian countries stratified by sex and age groups.

DATA ANALYSIS

Colorectal cancer mortality data were extracted from the World Health Organization (WHO) mortality data-

base[7]. The 3 countries or regions with data available for the longest period were Japan (since 1955), Singapore (since 1966) and Hong Kong of China (since 1969) were included in the analysis. In addition, Korean data were available from the WHO mortality database for 1985 to 2006 and from the Korea National Statistics Office for 2007 to 2010 and were included in the study[8]. The annual age-standardized rates (ASR) and the truncated rates for the 3 age groups (30-49, 50-69 and ⩾ 70 years) were estimated using the world standard population.

The trends in colorectal cancer mortality were tested using joinpoint regression models, using Joinpoint software version 3.5.3. The software was developed by the Surveillance Research Program of the United States National Cancer Institute and is based on the Poisson assumption[9]. A maximum of 3 joinpoints was allowed, and the default settings were used.

Table 1 shows the age-adjusted mortality rates for colorectal cancer in the 4 countries. Singapore showed the highest female mortality rates of the 4 countries from 1966 to 2009. Singapore also showed the highest male mortality during the same period, except for 2005, when males in Hong Kong of China showed a slightly higher rate. Korean men and women showed the lowest rates among the 4 countries.

Table 2 shows the results for the joinpoint regression analyses. Colorectal cancer mortality in men started to decrease in 1992 in Japan, followed by Singapore and Hong Kong of China in 1995. The mortality rates in women stared to decrease in 1980 in Singapore, followed by Hong Kong of China and Japan in 1996. In South Korea, the mortality rates plateaued in 2002 in men and started to decrease in 2004 in women.

In men, the decline in mortality started for younger age groups first. In Japan, mortality started to decrease in 1977 for the 30-49 years age group, in 1995 for the 50-69 years age group, and in 1998 for the 70 and older age group (Table 2). A similar trend was observed for Hong Kong of China, Singapore and South Korea. Similarly, a significant decrease in APC was first observed for younger age groups in Hong Kong of China, Japan and South Korea in women. For example, in Hong Kong of China, female mortality began to decrease in 1992 for the 30-49 years age group, in 1994 for the 50-69 years age group, and in 2002 for the 70 years and older age group. Whereas, for women in Singapore, the decrease began in 1980 in the 50-69 years age group, which was 4 years earlier than among the 30-49 years age group (Table 2). Figure 1 shows the trends in colorectal cancer mortality rates for the 3 age groups in Hong Kong of China, Japan, South Korea, and Singapore between 1955 and 2010.

DISCUSSION

Colorectal cancer mortality in the European Union has declined since the early 1980s. These decreases were observed in most western and northern European countries, whereas a persistent excess in mortality was observed in Hungary and the Czech Republic[6]. In the United States, colorectal cancer mortality rates in white men began to decline in 1978[10]. The APC for white men was -0.6% between 1973 and 1978 and -2.0% in 1986[10]. The decline in the mortality of white women was more rapid, with an APC of -2.1% between 1973 and 1997[10].

In Asia, a reduction in colorectal cancer mortality has been observed for economically advanced regions, such as Japan, Hong Kong of China, Singapore, and more recently, in South Korea. Notably, the decline started in younger age groups. A more favorable mortality trend and a consequent widening of the survival gap between the elderly and middle age groups were observed in Europe[2,6,11].

In contrast, the incidence rates for colorectal cancer in these countries have increased, except for men and women in Japan and women in Hong Kong of China[4,12-14]. Singapore experienced a sharp increase in the colorectal cancer incidence between 1968 and 2002, particularly among older men[12]. In Hong Kong of China, the ASR peaked in 1994 and has since declined in women, whereas the ASR progressively increased in men[14]. The increase was notable among men above 60 years old and women above 70 years old[14]. Similarly, the APCs for the incidence rate of colorectal cancer was prominent among older Korean men and women between 1999 and 2009[13]. Japan is the only Asian country in which the incidence rates for colorectal cancer have decreased in both men and women[4]. The Osaka Cancer Registry data showed that the overall colorectal cancer incidence in women has decreased since 1995. In men, the rate has been stabilized since 1996[9].

Changes in risk factors, particularly those related to lifestyle, have been suspected as main contributors to the colorectal cancer increase. Among modifiable lifestyle factors, alcohol consumption, obesity, cigarette smoking, and dietary habits (*e.g.*, red meat and processed meat consumption) have been associated with colorectal cancer risk[15,16]. In a study for population-attributable fractions of cancer in Japan, 31%-33% of the colorectal cancer incidence or mortality was explained by known preventable risk factors, such as alcohol consumption, cigarette smoking, obesity, and physical inactivity[17] when men and women combined. Among them, alcohol consumption was attributed for the greatest portion, followed by cigarette smoking and obesity. In a Chinese study, the population-attributable fraction of known preventable risk factors for cancer death was 14.6% for colon cancer and 2.2% for rectal cancer[18]. Alcohol consumption was accountable for 32.9% of male colorectal cancer and 2.1% of female colorectal cancer in Japan[17]. In China, alcohol consumption was accountable for 2.1% and 0.2% of colorectal cancer cases in men and women, respectively[19]. The prevalence and amount of alcohol consumption in these countries and South Korea have not decreased during the last few decades[17,18,20]. However, the prevalence of obesity, particularly in men, and a sedentary lifestyle has increased in Hong Kong of China, South Korea, and Japan[14,17,21]. Fortunately, the prevalence of cigarette smoking has declined in South Korea, Japan, and China[17,18,20]. These changes may explain the transition in the colorectal cancer epidemiology in these Asian countries.

Table 1 Colorectal cancer mortality rates in Hong Kong of China, Japan, South Korea and Singapore, 1955-2010 (age-standardized mortality rates per 100 000)

Country or area	Period	Men				Women			
		1955	1975	1995	2005	1955	1975	1995	2005
Hong Kong (China)	1969-2009	-	29.7	40.7	37.8	-	18.8	26.1	23.4
Japan	1955-2009	14.5	23.8	38.2	34.8	12.5	18.3	22.3	20.8
South Korea	1985-2010	-	-	8.6	30.5	-	-	11.8	17.2
Singapore	1966-2009	-	37.2	52.1	36.8		26.1	31.7	27.5

Table 2 Joinpoint analysis for colorectal cancer mortality at all ages and at age 30-49, 50-69 and ≥ 70 year in Hong Kong of China, Japan, South Korea and Singapore, 1955-2010

	Trend 1		Trend 2		Trend 3		Trend 4	
	Year	APC	Year	APC	Year	APC	Year	APC
Men								
All ages								
Hong Kong (China)	1969-1995	1.3[1]	1995-2009	-0.1				
Japan	1955-1975	2.0[1]	1975-1992	1.2[1]	1992-2004	-0.4[1]	2004-2009	-2.21
South Korea	1985-2002	7.9[1]	2002-2010	0.3				
Singapore	1966-1995	1.4[1]	1995-2009	-1.5[1]				
30-49 yr								
Hong Kong (China)	1969-2009	-1.1[1]						
Japan	1955-1960	-1.0	1960-1977	3.8[1]	1977-1998	-0.5[1]	1998-2009	-2.51
South Korea	1985-2002	2.5[1]	2002-2010	-2.1				
Singapore	1966-1989	0.5	1989-2009	-2.6[1]				
50-69 yr								
Hong Kong (China)	1969-1994	1.2[1]	1994-2009	-0.4				
Japan	1955-1963	0.4	1963-1995	2.9[1]	1995-2009	-1.6[1]		
South Korea	1985-2003	6.5[1]	2003-2010	-1.4[1]				
Singapore	1966-1989	2.1[1]	1989-2009	-1.2[1]				
≥ 70 yr								
Hong Kong (China)	1969-2001	1.9[1]	2001-2009	-0.8				
Japan	1955-1980	3.4[1]	1980-1998	2.0[1]	1998-2009	-0.9[1]		
South Korea	1985-1994	14.0[1]	1994-1998	5.9	1998-2002	12.2[1]	2002-2010	1.11
Singapore	1966-1995	1.7[1]	1995-2009	-0.9				
Women								
All ages								
Hong Kong (China)	1969-1996	1.5[1]	1996-2009	-1.7[1]				
Japan	1955-1960	14.3[1]	1960-1983	2.9[1]	1983-1996	0.9[1]	1996-2009	-0.71
South Korea	1985-1994	9.9[1]	1994-2004	4.3[1]	2004-2010	-1.2[1]		
Singapore	1966-1980	4.7[1]	1980-1998	-0.2	1998-2009	-2.6[1]		
30-49 yr								
Hong Kong (China)	1969-1992	0.4	1992-2009	-2.4[1]				
Japan	1955-1963	0.2	1963-1974	3.1[1]	1974-1991	0.3	1991-2009	-2.11
South Korea	1985-1991	8.8[1]	1991-2010	-0.6				
Singapore	1966-1984	2.8	1984-2009	-3.2[1]				
50-69 yr								
Hong Kong (China)	1969-1994	1.0[1]	1994-2009	-2.3[1]				
Japan	1955-1963	0.4	1963-1992	1.4[1]	1992-2004	-0.8[1]	2004-2009	-2.51
South Korea	1985-1994	8.3[1]	1994-2003	3.3[1]	2003-2010	-1.1		
Singapore	1966-1980	4.4[1]	1980-2009	-1.8[1]				
≥ 70 yr								
Hong Kong (China)	1969-2002	2.5[1]	2002-2009	-1.9[1]				
Japan	1955-1975	3.1[1]	1975-1999	1.1[1]	1999-2009	-0.9[1]		
South Korea	1985-1993	16.9[1]	1993-2003	7.7[1]	2003-2010	0.0		
Singapore	1966-1981	5.8[1]	1981-2000	0.9[1]	2000-2009	-2.6[1]		

[1]$P < 0.05$ *vs* the younger age groups. APC: Annual percent change.

A rapid increase in the incidence among older age groups may reflect the accumulated exposure to risk factors[22]. In contrast, a major contributor to the mortality reduction for colorectal cancer in the younger generation is the adaptation of screening programs. In Japan, a colorectal cancer screening program using a fecal occult blood test (FOBT) has been in place since 1992 under the Health Services Law for the Aged[23]. Colorectal cancer screening programs were introduced as a part of the National Cancer Screening Program for Medical Aid

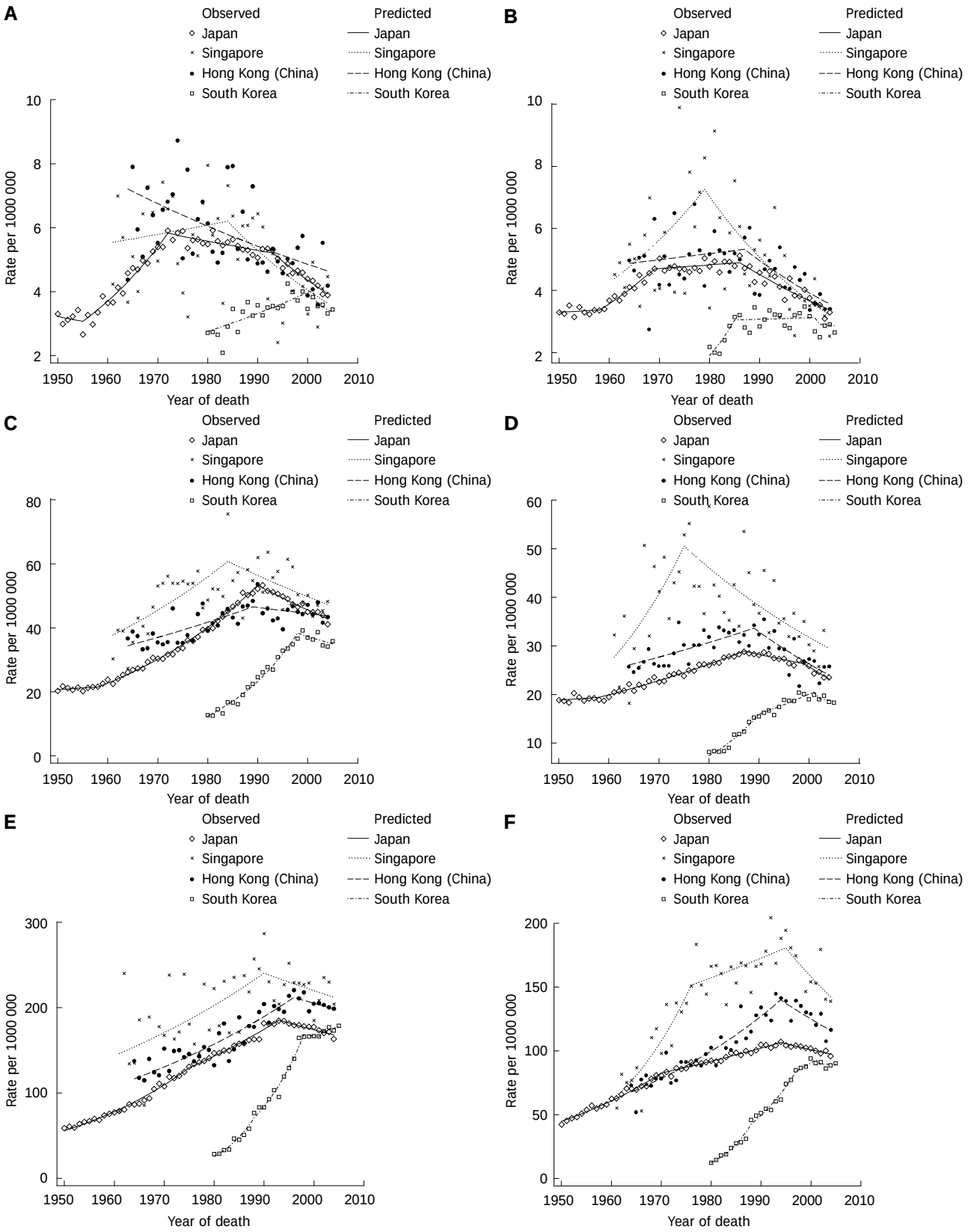


Figure 1 Trends in age-standardized colorectal cancer mortality rates per 100 000 in Hong Kong of China, Japan, South Korea and Singapore, 1955-2010. A: Men 30-49 yr; B: Men 50-69 yr; C: Men ≥ 70 yr; D: Women 30-49 yr; E: Women 50-69 yr; F: Women ≥ 70 yr.

recipients and National Health Insurance beneficiaries in the lower income bracket in 2004 in South Korea[24]. The FOBT is provided free of charge as a primary modality for men and women aged 50 years or older. FOBT-positive individuals were provided follow up by either colonoscopy or a double-contrast barium enema[24]. According to the Korean National Cancer Screening Survey, which covers both organized and opportunistic cancer screen-

ing programs, the lifetime screening rates for colorectal cancer were 25.3% in 2004 and 54.2% in 2010[25]. Although colorectal cancer screening for the average-risk population is recommended to start at 50 years of age, a national screening program is not available in Singapore[26]. However, compliance with opportunistic screening in Singapore was reasonably high[26].

CONCLUSION

In Hong Kong of China, Japan, South Korea and Singapore in which economic development and the westernized lifestyle were adopted early, colorectal cancer mortality has started to decrease. The decline or stabilization of mortality occurred the earliest in younger age groups and in women. The most important contributor to the decline in mortality is the introduction of colorectal cancer screening programs, although the role of the transition of lifestyle risk factors needs to be addressed.

REFERENCES

1 **Ferlay J,** Shin HR, Bray F, Forman D, Mathers C, Parkin DM. GLOBOCAN 2008 v1.2, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 10. Lyon, France: IARC Press, 2010

2 **Bosetti C**, Levi F, Rosato V, Bertuccio P, Lucchini F, Negri E, La Vecchia C. Recent trends in colorectal cancer mortality in Europe. *Int J Cancer* 2011; **129**: 180-191 [PMID: 20824701 DOI: 10.1002/ijc.25653]

3 **Jemal A**, Siegel R, Xu J, Ward E. Cancer statistics, 2010. *CA Cancer J Clin* ; **60**: 277-300 [PMID: 20610543]

4 **Ito Y**, Ioka A, Nakayama T, Tsukuma H, Nakamura T. Comparison of trends in cancer incidence and mortality in Osaka, Japan, using an age-period-cohort model. *Asian Pac J Cancer Prev* 2011; **12**: 879-888 [PMID: 21790220]

5 **Qiu D**, Katanoda K, Marugame T, Sobue T. A Joinpoint regression analysis of long-term trends in cancer mortality in Japan (1958-2004). *Int J Cancer* 2009; **124**: 443-448 [PMID: 18844218 DOI: 10.1002/ijc.23911.]

6 **La Vecchia C**, Bosetti C, Lucchini F, Bertuccio P, Negri E, Boyle P, Levi F. Cancer mortality in Europe, 2000-2004, and an overview of trends since 1975. *Ann Oncol* 2010; **21**: 1323-1360 [PMID: 19948741 DOI: 10.1093/annonc/mdp530]

7 World Health Organization Statistical Information System - WHO mortality database: Tables. [updated 2012 Jul 9; cited 2012 Jul 31]. Available from: http: //www.who.int/healthinfo/morttables/en

8 Korea National Statistical Office [cited 2011 Jul 21]. Available from: http: //www.kosis.kr

9 **National Cancer Institute.** Joinpoint Regression Program version 3.5.3 2012 (Aug 6, 2012). Available from: http: //surveillance.cancer.gov/joinpoint

10 **Ries LA**, Wingo PA, Miller DS, Howe HL, Weir HK, Rosenberg HM, Vernon SW, Cronin K, Edwards BK. The annual report to the nation on the status of cancer, 1973-1997, with a special section on colorectal cancer. *Cancer* 2000; **88**: 2398-2424 [PMID: 10820364]

11 **Quaglia A**, Tavilla A, Shack L, Brenner H, Janssen-Heijnen M, Allemani C, Colonna M, Grande E, Grosclaude P, Vercelli M. The cancer survival gap between elderly and middle-aged patients in Europe is widening. *Eur J Cancer* 2009; **45**: 1006-1016 [PMID: 19121578 DOI: 10.1016/j.ejca.2008.11.028]

12 **de Kok IM**, Wong CS, Chia KS, Sim X, Tan CS, Kiemeney LA, Verkooijen HM. Gender differences in the trend of colorectal cancer incidence in Singapore, 1968-2002. *Int J Colorectal Dis* 2008; **23**: 461-467 [PMID: 18185939 DOI: 10.1007/s00384-007-0421-9]

13 **Jung KW**, Park S, Kong HJ, Won YJ, Lee JY, Seo HG, Lee JS. Cancer statistics in Korea: incidence, mortality, survival, and prevalence in 2009. *Cancer Res Treat* 2012; **44**: 11-24 [PMID: 22500156 DOI: 10.4143/crt.2012.44.1.11]

14 **Yee YK**, Gu Q, Hung I, Tan VP, Chan P, Hsu A, Pang R, Lam CS, Wong BC. Trend of colorectal cancer in Hong Kong: 1983-2006. *J Gastroenterol Hepatol* 2010; **25**: 923-927 [PMID: 20074150 DOI: 10.1111/j.1440-1746.2009.06130.x]

15 **Chan AT**, Giovannucci EL. Primary prevention of colorectal cancer. *Gastroenterology* 2010; **138**: 2029-2043.e10 [PMID: 20420944 DOI: 10.1053/j.gastro.2010.01.057]

16 **Shin A**, Joo J, Bak J, Yang HR, Kim J, Park S, Nam BH. Site-specific risk factors for colorectal cancer in a Korean population. *PLoS One* 2011; **6**: e23196 [PMID: 21853085 DOI: 10.1371/journal.pone.0023196PONE-D-11-07835]

17 **Inoue M**, Sawada N, Matsuda T, Iwasaki M, Sasazuki S, Shimazu T, Shibuya K, Tsugane S. Attributable causes of cancer in Japan in 2005--systematic assessment to estimate current burden of cancer attributable to known preventable risk factors in Japan. *Ann Oncol* 2012; **23**: 1362-1369 [PMID: 22048150 DOI: 10.1093/annonc/mdr437]

18 **Wang JB**, Jiang Y, Liang H, Li P, Xiao HJ, Ji J, Xiang W, Shi JF, Fan YG, Li L, Wang D, Deng SS, Chen WQ, Wei WQ, Qiao YL, Boffetta P. Attributable causes of cancer in China. *Ann Oncol* 2012; **23**: 2983-2989 [PMID: 22689178 DOI: 10.1093/annonc/mds139]

19 **Liang H**, Wang J, Xiao H, Wang D, Wei W, Qiao Y, Boffetta P. Estimation of cancer incidence and mortality attributable to alcohol drinking in China. *BMC Public Health* 2010; **10**: 730 [PMID: 21108783 DOI: 10.1186/1471-2458-10-730]

20 **Shin A**, Cho ER, Kim J, Sung J, Park KW, Lim MK, Shin HR. Factors associated with awareness of infection status among chronic hepatitis B and C carriers in Korea. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 1894-1898 [PMID: 19454614 DOI: 10.1158/1055-9965.EPI-08-1228]

21 **Ministry of Health and Welfare.** Report on National Health and Nutritional Examination, 2008. Available from: URL: http://knhanes.cdc.go.kr/knhanes/index.do

22 **Wei EK**, Wolin KY, Colditz GA. Time course of risk factors in cancer etiology and progression. *J Clin Oncol* 2010; **28**: 4052-4057 [PMID: 20644083 DOI: 10.1200/JCO.2009.26.9324]

23 **Saito H**. Colorectal cancer screening using immunochemical faecal occult blood testing in Japan. *J Med Screen* 2006; **13** Suppl 1: S6-S7 [PMID: 17227634]

24 **Choi KS**, Lee HY, Jun JK, Shin A, Park EC. Adherence to follow-up after a positive fecal occult blood test in an organized colorectal cancer screening program in Korea, 2004-2008. *J Gastroenterol Hepatol* 2012; **27**: 1070-1077 [PMID: 22004224 DOI: 10.1111/j.1440-1746.2011.06944.x]

25 **Lee EH**, Lee HY, Choi KS, Jun JK, Park EC, Lee JS. Trends in Cancer Screening Rates among Korean Men and Women: Results from the Korean National Cancer Screening Survey (KNCSS), 2004-2010. *Cancer Res Treat* 2011; **43**: 141-147 [PMID: 22022290 DOI: 10.4143/crt.2011.43.3.141]

26 **Yeoh KG**, Chew L, Wang SC. Cancer screening in Singapore, with particular reference to breast, cervical and colorectal cancer screening. *J Med Screen* 2006; **13** Suppl 1: S14-S19 [PMID: 17227636]

P- Reviewers Mantoo SK, Trinder D
S- Editor Gou SX **L- Editor** A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i7.984

World J Gastroenterol 2013 February 21; 19(7): 984-993
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

TOPIC HIGHLIGHT

Jung Eun Lee, ScD, Assistant Professor, *Series Editor*

Molecular mechanisms of chemopreventive phytochemicals against gastroenterological cancer development

Min-Yu Chung, Tae Gyu Lim, Ki Won Lee

Min-Yu Chung, Ki Won Lee, WCU Biomodulation Major, Center for Food and Bioconvergence, Department of Agricultural Biotechnology, Seoul National University, Seoul 151-742, South Korea
Tae Gyu Lim, Ki Won Lee, Advanced Institutes of Convergence Technology, Seoul National University, Suwon 443-270, South Korea
Author contributions: Chung MY, Lim TG and Lee KW contributed equally to this work.
Supported by Grants from the Next-Generation BioGreen 21 Program (Plant Molecular Breeding Center, No. PJ008187), Rural Development Administration; the Leap Research Program (2010-0029233) and World Class University Program (Grant R31-2008-00-10056-0) through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology, South Korea
Correspondence to: Ki Won Lee, Associate Professor, WCU Biomodulation Major, Center for Food and Bioconvergence, Department of Agricultural Biotechnology, Seoul National University, San 56-1 Shinlim-dong, Gwanak-gu, Seoul 151-742, South Korea. kiwon@snu.ac.kr
Telephone: +82-2-8804661 Fax: +82-2-8786178
Received: September 19, 2012 Revised: January 14, 2013
Accepted: January 23, 2013
Published online: February 21, 2013

Abstract

Cancer is one of the leading causes of death worldwide. Commonly used cancer treatments, including chemotherapy and radiation therapy, often have side effects and a complete cure is sometimes impossible. Therefore, prevention, suppression, and/or delaying the onset of the disease are important. The onset of gastroenterological cancers is closely associated with an individual's lifestyle. Thus, changing lifestyle, specifically the consumption of fruits and vegetables, can help to protect against the development of gastroenterological cancers. In particular, naturally occurring bioactive compounds, including curcumin, resveratrol, isothiocyanates, (-)-epigallocatechin gallate and sulforaphane, are regarded as promising chemopreventive agents. Hence, regular consumption of these natural bioactive compounds found in foods can contribute to prevention, suppression, and/or delay of gastroenterological cancer development. In this review, we will summarize natural phytochemicals possessing potential antioxidant and/or anti-inflammatory and anti-carcinogenic activities, which are exerted by regulating or targeting specific molecules against gastroenterological cancers, including esophageal, gastric and colon cancers.

© 2013 Baishideng. All rights reserved.

Key words: Curcumin; Resveratrol; (-)-Epigallocatechin gallate; Isothiocyanates; Sulforaphane; Gastroenterological cancers; Molecular target

Chung MY, Lim TG, Lee KW. Molecular mechanisms of chemopreventive phytochemicals against gastroenterological cancer development. *World J Gastroenterol* 2013; 19(7): 984-993 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i7/984.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i7.984

INTRODUCTION

Cancer is a leading cause of death worldwide. Surgery, chemotherapy and radiation therapy are commonly used cancer treatments. However, these can cause a number of side effects, and complete cure is often infeasible for most patients suffering from specific cancers. Epidemiological studies have suggested that regular consumption of vegetables, fruits, red wine and tea is associated with lower incidences of many chronic diseases, including cancers[1-4]. A recent review reported that natural bioactive compounds found in various foods can activate or deactivate molecular signaling cascades by targeting small molecules in cancer cells[5]. A number of natural phytochemicals, including isoflavones, gingerol, (-)-epi-

gallocatechin gallate (EGCG), quercetin, resveratrol, and curcumin have been identified to be chemopreventive and their significant health benefits are an active field of research[5]. In particular, gastroenterological cancers are closely associated with lower consumption of fruits or vegetables. Therefore, in this review, we will summarize the natural phytochemicals possessing potential antioxidant and/or anti-inflammatory and anti-carcinogenic activities, which act by regulating or targeting specific molecules against gastroenterological cancers, including esophageal, gastric and colon.

PHYTOCHEMICALS IN ESOPHAGEAL CANCER

Esophageal cancer is the eighth most common cancer and is the sixth most common cause of cancer-related deaths[6]. Due to the lack of symptoms, individuals are rarely aware of their condition until the metastatic stages of the disease[7]. Despite developments in current cancer treatments, including chemotherapy, radiation therapy, and surgery (esophagogastric resection), patients with esophageal adenocarcinoma are not often cured of the disease[8]. Statistical analysis for the past 5 years shows that Americans and Europeans with esophageal cancer have relatively low survival rates, 10%-15% and 10% respectively[9]. Moreover, this suggests that the esophageal tumors are resistant to regular therapies and, thus, alternative strategies for the treatment and/or prevention of esophageal cancer are required.

Major risk factors for esophageal cancer are chewing and smoking tobacco, drinking alcoholic beverages[10], low consumption of fruits and vegetables[1] and consumption of salt-cured, salt-pickled, and moldy foods[11]. Therefore, quitting cigarette smoking, reducing alcohol consumption, increasing fruit and vegetable consumption, and avoiding foods containing nitrosamines and nitrosamine precursors are critical for the prevention of this disease. In addition to such lifestyle changes, identifying foods or food constituents that can help to prevent, suppress, and/or delay the onset of esophageal cancer is essential[12,13]. Recent attention has focused on the beneficial actions of natural phytochemicals, such as isothiocyanates, curcumin, and resveratrol, against esophageal cancer (Table 1). Although the underlying molecular mechanisms have not been fully understood, such natural chemicals are known to protect against disease progression by targeting specific proteins.

Isothiocyanates

Isothiocyanates are naturally occurring phytochemicals in cruciferous vegetables, including Chinese watercress, cabbage, Brussels sprouts, turnips and cauliflower[14]. In the gastrointestinal tract, isothiocyanates are released from their precursor *via* hydrolysis catalyzed by myrosinase. Of its metabolites, phenethyl isothiocyanate (PEITC) has been reported to be rapidly absorbed and distributed in mice following oral administration[15]. Studies have demonstrated that PEITC (> 1.0 μmol/g diet) protects against esophageal cancer by inhibiting tumor incidence and multiplicity in rats treated with N-nitrosobenzylmethylanime (NMBA), the most potent inducer of esophageal tumors and is commonly used to study the pathogenesis of esophageal cancer[16].

Several studies of the molecular mechanism whereby PEITC inhibits NMBA-induced esophageal tumorigenesis have revealed that PEITC suppresses the activity of cytochrome P450 enzymes in rats with NMBA-induced esophageal cancer[11,17,18], and also inhibits DNA methylation by inhibiting the formation of the pro-mutagenic adduct O^6-methylguanine in rat esophageal DNA[16]. Significant correlations between DNA adduct formation and tumor multiplicity have been observed in rat lungs as well as esophagi[18], indicating that DNA adduct formation probably contributes to tumor incidence and multiplicity. Collectively, PEITC is likely to have anti-carcinogenic activity *via* regulation of P450 enzyme activity and inhibition of DNA damage, contributing to the prevention of esophageal cancer. However, no direct target has been identified. Hence, future investigation is needed to elucidate the molecular target(s) of isothiocyanates or their metabolites in the prevention and/or treatment of esophageal cancer.

EGCG

Polyphenols are major components of tea. One-third of the dry weight of green or black tea is composed of polyphenols, which have powerful antioxidant and anti-inflammatory potential[19]. Wang *et al*[20] reported that both decaffeinated green and black tea consumption reduced esophageal tumorigenesis and molecular events in rats treated with N-nitrosomethylbenzylamine, which is probably due to the suppression of tumor incidence and multiplicity[21]. EGCG is the most abundant and active constituent among tea polyphenols. In general, the anti-carcinogenic activities of EGCG are mediated *via* multiple mechanisms, including the inhibition of mitogen activation protein kinases (MAPK), activator protein-1 and cell transformation[22-24], inhibition of epidermal growth factor receptor (EGFR) phosphorylation[25], induction of cell cycle arrest (G0/G1)[26,27] and apoptosis[28], and inhibition of DNA methyltransferase (DNMT) activity[29].

EGCG also regulates multiple targets and mechanisms in protecting against esophageal cancer. EGCG (40 μmol/L) inhibits phosphorylation of ERK1/2, c-Jun, and cyclooxygenase-2 (COX-2), which are increased in the human esophageal cancer cell lines SKGT-4 and TE-8 as well as in esophageal tissue specimens obtained from patients[30]. *In vivo* analysis using nude mouse xenograft models also confirmed that lower tumor formation and growth are associated with the decreased expression levels of phosphorylated extracellular-signal-regulated kinase (ERK) and COX-2 induced by EGCG treatment(50 μg/kg per day)[30]. Together these suggest that EGCG may protect against esophageal cancer by reducing pro-inflammatory mediators, including ERK, c-Jun

Table 1 Chemopreventive phytochemicals and their actions and targets/mechanisms during the development of esophageal cancer, gastric cancer and colon cancer

	Natural phytochemical	Chemopreventive action	Targets/mechanisms	Ref.
Esophageal cancer	Isothiocyanate	Inhibition of DNA damage	Inhibition of DNA methylation	[17]
		Inhibition of tumorigenesis	Inhibition of cytochrome P450 enzymes activity	[11,18]
	EGCG	Anti-inflammation	Inhibition of phosphorylated ERK1/2, c-Jun and COX-2 expressions	[30]
			Decreased COX-2 expression and PGE_2 production	[32]
		Growth inhibition	Inhibition of EGFR phosphorylation	[31]
		Induction of cell cycle arrest	Inhibition of cyclin D1	[32]
		DNA damage	Inhibition of DNA methyltransferase activity	[29]
	Curcumin	Antioxidant	Induction of SOD-1	[46]
		Anti-inflammation	Inhibition of COX-2	[46]
			Inhibition of NF-κB activity	[50-52]
			Inhibition of IL-8 mRNA expression	[52]
		Apoptosis and cell cycle arrest	Inhibition of Notch signaling	[57]
Gastric cancer	Curcumin	Chemoresistance	Downregulation of NF-κB	[61]
			Induction of apoptotic genes *Bcl-2* and *Bcl-xL*	[61]
		Suppression of cell proliferation and invasion	Downregulation of EGFR-PAK1	[62]
			Reduction of cyclin D1 expression	[62]
	Resveratrol	Inhibition of cell cycle progression	Inhibition of PKC	[72,73]
			MEK1/2-ERK1/2-c-Jun	[74]
		Induction of apoptosis	Reduction in Bcl-2, enhancing *Bax* gene	[76,77]
	Sulforaphane	Protection against oxidative stress	Stimulation of Nrf2	[78-80]
			Enhancement of GST and glutathione levels	[78,81]
		Anti-bacterial activity	Decrease in gastric bacterial colonization	[82]
			Reduction in the expression of TNF-α and IL-1β	[82]
Colon cancer	Curcumin	Growth inhibition	EGFR/IGFR	[102]
			ERK/Egr-1/EGFR	[87,96]
		Suppression of tumorigenesis	AMPK-COX-2	[101]
			Wnt/β-catenin	[102,106]
	Resveratrol	Anti-proliferation Apoptosis	IGF-1, p53	[108]
			PPP-FAK signaling	[109]
		Apoptosis growth inhibition	AMPK	[110]

ERK: Extracellular-signal-regulated kinase; COX-2: Cyclooxygenase-2; PGE_2: Prostaglandin E2; EGFR: Epidermal growth factor receptor; EGCG: Epigallocatechin gallate; SOD: Superoxide dismutase; NF-κB: Nuclear factor κB; IL-8: Interleukin-8; PKC: Protein kinase C; MEK: Mitogen-activated protein kinase; Bcl-2: B-cell lymphoma 2; GST: Glutathione S-transferase; TNF: Tumor necrosis factor; IGFR: Insulin-like growth factor receptor; PPP: Pentose phosphate pathway; FAK: Focal adhesion kinase; AMPK: Adenosine monophosphate-activated potein kinase.

and COX-2 in *in vitro* carcinogenesis and *in vivo* tumorigenesis models, and cancer patients.

EGCG inhibits phosphorylation of EGFR and HER-2/neu in KYSE 150 esophageal squamous cell carcinoma, leading to the inhibition of growth factor receptor and, thus, exerting anti-carcinogenic activity[31]. Another study demonstrated that EGCG (4 mg/kg *i.p.*) attenuates cyclin D1 and *COX-2* gene expression, thereby reducing the production of prostaglandin E2 (PGE_2) in rats treated with NMBA[32]. This suggests that cyclin D1 and COX-2 may act as partial targets of EGCG. EGCG-mediated decrease in PGE_2 production following NMBA treatment was further supported by another study using F344 rats[33]. Lastly, EGCG also inhibits DNA methylation, thereby suppressing the onset of esophageal cancer. Indeed, hypermethylation of DNA is associated with critical events during cancer progression, such as cell cycle regulation, DNA repair, and apoptosis[34-36]. In particular, methylation of CpG by DNMT is known to cause chromosome condensation and transcription repression[37,38]. The importance of DNA demethylation has been emphasized in strategies to develop cancer therapies and was further supported by studies using DNMT inhibitors. Treatment with DNMT inhibitors leads to the inhibition of cancer cell growth, induction of cancer cell apoptosis, and attenuation of tumor volume in mice[39-42]. In fact, Fang *et al*[29] demonstrated that EGCG acts as a potent DNA methylation inhibitor by suppressing DNMT activity, leading to the demethylation of CpG and reactivation of methylation-silenced genes in the human esophageal cancer cell line KYSE 510.

Curcumin

Curcumin is a yellow pigment derived from turmeric, the powdered rhizome of Curcuma longa Linn. Accumulating evidence suggests numerous health benefits of curcumin, including antioxidant, anti-inflammatory, and anti-carcinogenic properties[43,44], which are probably mediated by regulation of multiple intracellular targets[5]. Multiple molecular targets have been identified using various cancer cell lines and xenograft animal models of esophageal cancer.

Curcumin is a well-known antioxidant[43]. Excess amounts of reactive oxygen species (ROS) lead to the initiation, progression, and promotion of various cancers[45]. Therefore, the role of antioxidants is critical dur-

ing the development of cancers. In fact, treatment with curcumin (10-100 μmol/L) reversed suppression of the powerful antioxidant superoxide dismutase (SOD)-1 and induction of *COX-2* gene expression following treatment with bile acid in an esophageal epithelial cell line (HET-1A)[46]. This suggests that the antioxidant capacity of curcumin contributes to the prevention of esophageal cancer by increasing the activity and/or expression levels of antioxidant enzymes, including SOD, and reducing pro-oxidant enzymes, such as COX-2.

In addition to its antioxidant capacity, curcumin exerts its anti-cancer activities *via* its anti-inflammatory activity. Nuclear factor κB (NF-κB) is a well-known pro-inflammatory transcription factor involved in the initiation, promotion and progression of cancers[47]. It is also known that increased NF-κB activity is associated with greater cell proliferation, invasion, angiogenesis, metastasis, suppression of apoptosis, and chemoresistance in various types of cancer[48,49]. Several studies have demonstrated that curcumin inhibits NF-κB activity in esophageal adenocarcinoma[50,51]. Rawat *et al*[52] reported that curcumin (50 μmol/L) protects against bile acid-induced enhanced NF-κB activity in an esophageal cell line (OE33), which in turn reduces the expression levels of NF-κB target genes, including interleukin (IL)-8. In addition, patients with Barrett's esophagus supplemented with curcumin (a 500 mg curcumin tablet daily for 7 d) showed decreased IL-8 mRNA expression, suggesting that curcumin can act as a potential chemopreventive agent against esophageal cancer[52].

Recently, it was demonstrated that curcumin induces cell death (apoptosis) and cell cycle arrest by blocking Notch signaling pathways. Notch signaling was recently found to be upregulated in esophageal cancer and is as a therapeutic target for esophageal cancer due to its critical roles in tumor cell proliferation, apoptosis and stem cell maintenance and renewal[53-56]. Inhibition of Notch signaling in oral squamous carcinoma cells by curcumin also contributes to downregulation of NF-κB, which in turn reduces the expression of target genes of NF-κB, including Bcl-2, cyclin D1, vascular endothelial growth factor, and matrix metalloproteinase-9[57].

PHYTOCHEMICALS IN GASTRIC CANCER

Gastric cancer is the seventh most common cause of cancer-related mortality in the world. Exposure to chemical carcinogens or *Helicobacter pylori* (*H. pylori*) infection causes several events which may lead to the development of gastric cancer[58]. In particular, *H. pylori* infection results in infiltration of neutrophils and macrophages into the gastric mucosa. Infiltration of neutrophils and macrophages leads to the production of free radicals, including superoxide and nitric oxide. ROS-mediated stress responses result in gastric mucosal injury, ulcers, and ultimately gastric cancer[59]. Therefore, agents that have powerful antioxidant potential *via* scavenging ROS or enhancing antioxidant capacity may help to protect against gastric cancer development (Table 1).

Curcumin

It has been suggested that curcumin inhibits *H. pylori* infection in mice by reducing its growth[60]. The mechanisms of cellular growth and potential therapeutic capacity of curcumin have been further investigated by *in vitro* studies using multiple gastric cancer cell lines. Curcumin protects against chemoresistance in human gastric cancer cells by downregulating NF-κB and subsequent NF-κB-mediated anti-apoptotic genes, such as Bcl-2 and Bcl-xL in the human gastric cancer SGC 7901 cell line[61].

In addition, curcumin reduces EGFR expression and the activity of p21-activated kinase (PAK)1, a downstream regulator of EGFR. Curcumin also reduces NF-κB activity, regulated by PAK1, leading to decreases in cell proliferation by reducing the mRNA and protein expression of cyclin D1 and suppresses cell cycle progression from the G1 to S phases. Therefore, curcumin inhibits the proliferation and invasion of various gastric cancer cells[62].

Resveratrol

Resveratrol is a highly abundant polyphenol found in red grapes and red wine. Epidemiological studies have revealed an inverse relationship between red wine consumption and the incidence of cardiovascular disease[63,64]. The cardioprotective effect of red wine was attributed to resveratrol[65]. During the last two decades, extensive research has focused on the antioxidant, anti-inflammatory and anti-carcinogenic health benefits of resveratrol[66]. Specifically, resveratrol was found to have antibacterial effects[67] by inhibiting the growth of multiple *H. pylori* strains[68-70]. Increased expression of IL-8 and increased production of ROS were detected in the gastric mucosa following exposure to *H. pylori*. Furthermore, *H. pylori*-mediated infection increases motility and leads to morphological changes in co-cultured cells, known as the hummingbird phenomenon. Treatment with resveratrol (1-100 μmol/L) significantly attenuated IL-8 secretion, ROS formation, and markedly inhibited morphological changes in cells infected with *H. pylori*[71]. Hence, resveratrol is a candidate therapeutic agent against gastric cancer.

Resveratrol inhibits cell cycle progression of nitrosamine-stimulated KATO-Ⅲ and RF-1 cells by inducing cell cycle arrest in the G0/G1 phase through inhibiting kinase C-mediated mechanisms and induces apoptotic cell death in various gastric adenocarcinoma cell lines[72,73]. Another mechanism by which resveratrol regulates cell proliferation is associated with the MEK1/2-ERK1/2-c-Jun signaling cascade, a critical signaling pathway in the proliferation and growth of human adenocarcinoma gastric cells. Resveratrol was found to suppress the phosphorylation of MEK1/2-ERK1/2, which subsequently inhibits translocation of c-Jun into the nuclear compartment, leading to inhibition of cell proliferation[74].

In addition to the inhibition of cell proliferation, resveratrol (50-200 μmol/L) induces apoptosis in human gastric cancer SGC7901 cells by producing ROS, which can be reversed by treatment of cells with SOD or catalase, leading to the attenuation of resveratrol-mediated

cellular apoptosis[75]. Resveratrol induces apoptosis in esophageal carcinoma (EC-9706) cells, mediated by reducing the expression of Bcl-2 and enhancing that of the pro-apoptotic gene Bax[76]. Resveratrol can induce apoptosis of transplanted tumor cells, probably mediated by downregulation of the anti-apoptotic gene bcl-2 and upregulation of the apoptotic gene Bax by resveratrol in implanted primary human gastric cancer cells in nude mice[77].

Sulforaphane

The natural chemical compound sulforaphane is an isothiocyanate and is abundant in cruciferous vegetables, especially broccoli[59]. Sulforaphane is present as sulforaphane glucosinolates (SGS), which is biologically inactive. SGS is hydrolyzed by the action of myrosinase in the oral cavity and small intestine to produce sulforaphane. Biologically active sulforaphane is ultimately absorbed into the systemic circulation, where it exerts various activities[59]. Although sulforaphane is not itself an antioxidant, it exerts antioxidant activity by stimulating Nrf2-depedent antioxidant enzymes, such as glutathione S-transferase (GST), thereby protecting cells against oxidative stress[78-80]. Compared to other strong antioxidants, such as vitamin C or polyphenols, sulforaphane maintains the activities of antioxidant enzymes, including NAD(P)H: quinone oxidoreductase (NQO1) and GST in the gastric mucosa of Nrf2-/- mice infected with *H. pylori* and fed a high salt diet[59]. This renders sulforaphane a more potent antioxidant substance and mediates its protection of the gastric mucosa against oxidative stress.

Sulforaphane increases detoxification as well as antioxidant enzymes in a Nrf2-dependent manner. Fahey *et al*[78] demonstrated that sulforaphane suppresses benzo-[a]pyrene-evoked forestomach tumors in ICR mice. This is probably mediated by inducing phase 2 detoxification enzymes, including NQO1 and GST, and upregulating antioxidant enzymes, which are abrogated in mice without the *Nrf2* gene[78]. In patients with *H. pylori*-associated gastritis, *H. pylori* eradication increased or restored the activity of GST and glutathione levels in the antral mucosa[81]. This further emphasizes the importance of antioxidants during the development of gastric cancers associated with *H. pylori* infection.

In addition to its antioxidant capacity, sulforaphane exerts chemoprotective effects which are attributed to its *in vitro* anti-bacterial activity[59]. In a clinical study of *H. pylori*-infected patients (n = 48), the group that consumed broccoli (70 g/d; containing 420 μmol/L sulforaphane precursor) for 8 wk showed decreased levels of markers of *H. pylori* colonization (*i.e.*, urease level and *H. pylori* stool antigen) and markers of gastric inflammation (*i.e.*, serum pepsinogens Ⅰ and Ⅱ) compared to the placebo group[82]. An *in vivo* study using C57BL/6 female mice infected with *H. pylori* Sydney strain 1 and maintained on a high-salt (7.5% NaCl) diet confirmed the anti-bacterial activity of sulforaphane. Mice treated with broccoli rich in sulforaphane showed decreased gastric bacterial colonization as well as reduced expression of tumor necrosis factor (TNF)-α and IL-1β in the gastric mucosa, contributing to amelioration of inflammation and, thus, prevention of high salt-induced gastric corpus atrophy[82]. Interestingly, the anti-bacterial and anti-inflammatory activities of sulforaphane were not observed in mice with *Nrf2* gene depletion, suggesting that sulforaphane exerts its effect *via* Nrf2[82].

PHYTOCHEMICALS IN COLORECTAL CANCER

Colorectal cancer is one of the most commonly diagnosed cancers in both males and females[83]. The mortality rate of males with colon and rectal cancer was the third highest for cancers in the United States between 1930 and 2007[84]. Consumption of a high-calorie diet that is high in fat leads to obesity. Many studies have investigated the contribution of obesity to colorectal diseases[85-88]. The colon is one of the first organs to encounter various factors in foods and, thus, the effects of natural bioactive compounds in the diet on colorectal tissue are the subject of extensive investigation.

The Wnt signaling pathway is a primary factor in colorectal cancer. Among several Wnt signaling proteins, β-catenin is a key regulator, which turns on and off cell proliferation proteins. In the normal state, the "destruction" complex comprising axin, APC, and glycogen synthase kinase-3β, phosphorylates β-catenin, which subsequently becomes degraded[89]. However, after activation of the Wnt signaling pathway, the "destruction" complex is suppressed and β-catenin is not degraded by ubiquitination. Accumulated β-catenin then translocates into the nucleus and binds directly to the T-cell factor (TCF)/lymphoid enhancer factor (LEF) family molecules. These interactions stimulate TCF/LEF target genes involved in cellular proliferation, such as c-myc and cyclin D1[90,91].

More importantly, the Wnt signaling pathway is stimulated by obesity[92] and is a secondary factor in the development of colon cancers[93,94]. Obesity is associated with chronic inflammation[85]. In obesity-related cancers, phosphoinositide-3-kinase (PI3K)/Akt, MAPK, and their downstream signaling proteins, including mammalian target of rapamycin (mTOR), are activated as the severity of obesity increases[95]. Overall, it is commonly accepted that suppression of the inflammatory signaling pathway may represent an important strategy for inhibition of both Wnt- and obesity-related colon cancers. Several lines of evidence have reported the anti-colon carcinogenic effects of natural compounds, which act as small molecule inhibitors of the inflammatory signaling pathway. Among them, curcumin and resveratrol are the most significant anti-carcinogenic compounds (Table 1).

Curcumin

Curcumin is the yellow pigment of turmeric and numerous studies using various carcinogenesis models have

shown its chemopreventive effects. One clinical study reported that curcumin has anti-colon carcinogenic effects. Indeed, oral intake of curcumin (4 g for 30 d) decreased the number of aberrant crypto foci in the colon in this Phase Ⅱ a clinical trial of curcumin for the prevention of colorectal neoplasia.

A number of studies have investigated the mechanisms underlying the inhibition of colon cancer development by curcumin. The major targets of the signaling pathways regulated by curcumin are EGFR[96,97], AMPK-COX-2[98], MAPK[99] and Wnt/β-catenin[100]. EGFR is one of four family erbB receptors and is involved in many malignancies, including colorectal cancer[97], by modulating multiple signaling pathways. Specifically, ligand-activated EGFRs are autophosphorylated and activate Ras and other signaling pathways, which in turn increase the expression of EGFR target genes. Indeed, increased levels and function of EGFR are closely associated with the metastatic potential of human colon carcinoma cells. Chen *et al*[96] reported that curcumin inhibits colon cancer cell growth by reducing the ERK/Egr-1/EGFR signaling pathway and decreasing the expression of EGFR. Curcumin was also demonstrated to prevent the emergence of chemoresistant colon cancer cells *via* inhibition of EGFR and insulin-like growth factor (IGF)-1R.

AMPK is a highly conserved kinase in eukaryotes. Although its main function is in maintenance of energy homeostasis, novel roles for AMPK were discovered recently. The AMPK-COX-2 cascade is an important pathway associated with cancer growth. Previous studies demonstrated that a signaling cascade involving AMPK, pAkt and COX-2 is a promising target as it is regulated by curcumin during cancer cell growth. Over 80% of colonic adenomas and carcinomas exhibit mutations in the *APC* gene and constitutive activation of Wnt signaling[101]. Thus, Wnt/β-catenin signaling has been targeted for the development of novel anti-colorectal cancer drugs. Curcumin also inhibits the Wnt/β-catenin signaling pathway[102]. Curcumin impairs Wnt signaling and cell-cell adhesion pathways, subsequently inducing G_2/M phase and apoptosis in colon cancer cells.

Resveratrol

Resveratrol is a naturally occurring phenolic phytochemical, which is present in red grapes. Resveratrol exerts its chemopreventive and chemotherapeutic effects by modulating multiple biological activities. Daily *p.o.* doses of 0.5 g or 10 g resveratrol for 8 d inhibited tumor cell proliferation by 5% with no resveratrol-related adverse effects in patients with resectable colorectal cancer (n = 90)[103]. Another clinical study demonstrated that ingestion of grape powder suppresses the expression of Wnt target genes, including cyclin D1 and axin Ⅱ, in normal colonic mucosa. This suggests that dietary supplementation with resveratrol-containing products is a potential colon cancer preventive strategy and that Wnt/β-catenin is a potential target for resveratrol in normal colonic mucosa[104]. The anti-colonic tumor effects of resveratrol have been investigated in various *in vivo* studies[105-107]. Resveratrol (300 ppm) supplementation reduced levels of markers of DSS-mediated colitis inflammation, such as iNOS, COX-2 and TNF-α, in mice[105]. Resveratrol also inhibited the 1,2-dimethylhydrazine-induced tumor burden per animal, per group and over the three regimens of colon carcinogenesis (initiation, post-initiation and entire period)[107].

Resveratrol suppresses IGF-1-induced human colon cancer cell proliferation by activating p53 signaling pathways. Because the IGF signaling pathway is closely related to obesity-mediated colorectal carcinogenesis[108], resveratrol may be useful for suppressing obesity-induced colorectal cancers. Additionally, resveratrol inhibits human colon cancer cell proliferation and promotes apoptosis by suppressing the pentose phosphate pathway and focal adhesion kinase, a critical protein for cell-extracellular matrix communication. This supports the anti-colon-carcinogenic effect of resveratrol in obese individuals[109].

Furthermore, resveratrol exerts synergistic anti-cancer effects on chemoresistant cancer cells by regulating the AMPK signaling pathway. The HT-29 cell line has been used to develop anti-cancer drugs intended to overcome chemoresistance[110]. Although 100 μmol/L etoposide, an anti-cancer agent, did not inhibit the proliferation of HT-29 cells, pretreatment with resveratrol (50-400 μmol/L) induced cytotoxicity under 100 μmol/L etoposide[110]. Additionally, the phosphorylation level of acetyl-CoA carboxylase (ACC), the downstream molecule of AMPK, was increased by co-treatment with resveratrol and etoposide and increased phospho-ACC and pAMPK inhibition of cell viability. Compound C, an AMPK inhibitor, reduced resveratrol/etoposide-induced cytotoxicity on HT-29 cells[110].

CONCLUSION

Gastroenterological cancers, including those of the esophagus, stomach and colon, are closely associated with lifestyle factors, especially diet. Patients suffering from gastroenterological cancers often cannot be completely cured with regular chemopreventive strategies and, thus, prevention, suppression, and/or delaying the onset of these cancers are critical. A number of natural phytochemicals, including curcumin, resveratrol, isothiocyanates, EGCG, and sulforaphane have been shown to have anti-carcinogenic, anti-inflammatory, and antioxidant activities by targeting small molecules or regulating signaling cascades, thereby protecting against the development of gastroenterological cancers. Although most phytochemicals act as small molecule inhibitors, they often have low bioavailability following oral administration. Indeed, the concentration of resveratrol is lower than its major metabolite resveratrol sulfate glucuronide after daily administration of 0.5 g resveratrol for 8 d to colorectal cancer patients[103]. The majority of the phytochemicals are readily converted to their metabolites in the gastrointestinal tract. These metabolites may have similar or better effects than their parent compounds and, thus, may also represent primary therapeutic agents.

REFERENCES

1 **Block G**, Patterson B, Subar A. Fruit, vegetables, and cancer prevention: a review of the epidemiological evidence. *Nutr Cancer* 1992; **18**: 1-29 [PMID: 1408943 DOI: 10.1080/01635589209514201]

2 **Frankel EN**, Kanner J, German JB, Parks E, Kinsella JE. Inhibition of oxidation of human low-density lipoprotein by phenolic substances in red wine. *Lancet* 1993; **341**: 454-457 [PMID: 8094487 DOI: 10.1016/0140-6736(93)90206-V]

3 **Renaud S**, de Lorgeril M. Wine, alcohol, platelets, and the French paradox for coronary heart disease. *Lancet* 1992; **339**: 1523-1526 [PMID: 1351198 DOI: 10.1016/0140-6736(92)91277-F]

4 **Steinmetz KA**, Potter JD. Vegetables, fruit, and cancer. I. Epidemiology. *Cancer Causes Control* 1991; **2**: 325-357 [PMID: 1834240 DOI: 10.1007/BF00051672]

5 **Lee KW**, Bode AM, Dong Z. Molecular targets of phytochemicals for cancer prevention. *Nat Rev Cancer* 2011; **11**: 211-218 [PMID: 21326325 DOI: 10.1038/nrc3017]

6 **Parkin DM**, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. *CA Cancer J Clin* 2005; **55**: 74-108 [PMID: 15761078 DOI: 10.3322/canjclin.55.2.74]

7 **Jemal A**, Siegel R, Ward E, Murray T, Xu J, Thun MJ. Cancer statistics, 2007. *CA Cancer J Clin* 2005; **57**: 43-66 [PMID: 17237035 DOI: 10.3322/canjclin.57.1.43]

8 **Subramaniam D**, Ponnurangam S, Ramamoorthy P, Standing D, Battafarano RJ, Anant S, Sharma P. Curcumin induces cell death in esophageal cancer cells through modulating Notch signaling. *PLoS One* 2012; **7**: e30590 [PMID: 22363450 DOI: 10.1371/journal.pone.0030590]

9 **Sant M**, Aareleid T, Berrino F, Bielska Lasota M, Carli PM, Faivre J, Grosclaude P, Hédelin G, Matsuda T, Møller H, Möller T, Verdecchia A, Capocaccia R, Gatta G, Micheli A, Santaquilani M, Roazzi P, Lisi D. EUROCARE-3: survival of cancer patients diagnosed 1990-94--results and commentary. *Ann Oncol* 2003; **14** Suppl 5: v61-118 [PMID: 14684501 DOI: 10.1093/annonc/mdg754]

10 **Wynder EL**, BROSS IJ. A study of etiological factors in cancer of the esophagus. *Cancer* 1961; **14**: 389-413 [PMID: 13786981 DOI: 10.1002/1097-0142(196103/04)14]

11 **Stoner GD**, Kresty LA, Carlton PS, Siglin JC, Morse MA. Isothiocyanates and freeze-dried strawberries as inhibitors of esophageal cancer. *Toxicol Sci* 1999; **52**: 95-100 [PMID: 10630596]

12 **Engel LS**, Chow WH, Vaughan TL, Gammon MD, Risch HA, Stanford JL, Schoenberg JB, Mayne ST, Dubrow R, Rotterdam H, West AB, Blaser M, Blot WJ, Gail MH, Fraumeni JF. Population attributable risks of esophageal and gastric cancers. *J Natl Cancer Inst* 2003; **95**: 1404-1413 [PMID: 13130116 DOI: 10.1093/jnci/djg047]

13 **Stoner GD**, Gupta A. Etiology and chemoprevention of esophageal squamous cell carcinoma. *Carcinogenesis* 2001; **22**: 1737-1746 [PMID: 11698334 DOI: 10.1093/carcin/22.11.1737]

14 **VanEtten CH**, Daxenbichler ME, Williams PH, Kwolek WF. Glucosinolates and derived products in cruciferous vegetables. Analysis of the edible part from twenty-two varieties of cabbage. *J Agric Food Chem* 1976; **24**: 452-455 [PMID: 1270657 DOI: 10.1021/jf60205a049]

15 **Eklind KI**, Morse MA, Chung FL. Distribution and metabolism of the natural anticarcinogen phenethyl isothiocyanate in A/J mice. *Carcinogenesis* 1990; **11**: 2033-2036 [PMID: 2225337 DOI: 10.1093/carcin/11.11.2033]

16 **Wilkinson JT**, Morse MA, Kresty LA, Stoner GD. Effect of alkyl chain length on inhibition of N-nitrosomethylbenzylamine-induced esophageal tumorigenesis and DNA methylation by isothiocyanates. *Carcinogenesis* 1995; **16**: 1011-1015 [PMID: 7767958 DOI: 10.1093/carcin/16.5.1011]

17 **Morse MA**, Lu J, Gopalakrishnan R, Peterson LA, D'Ambrosio SM, Wani G, Stoner GD. Mechanism of enhancement of esophageal tumorigenesis by 6-phenylhexyl isothiocyanate. *Cancer Lett* 1997; **112**: 119-125 [PMID: 9029177 DOI: 10.1016/S0304-3835(96)04556-9]

18 **Stoner GD**, Morse MA. Isothiocyanates and plant polyphenols as inhibitors of lung and esophageal cancer. *Cancer Lett* 1997; **114**: 113-119 [PMID: 9103268 DOI: 10.1016/S0304-3835(97)04639-9]

19 **Weisburger JH**. Mechanisms of action of antioxidants as exemplified in vegetables, tomatoes and tea. *Food Chem Toxicol* 1999; **37**: 943-948 [PMID: 10541449 DOI: 10.1016/S0278-6915(99)00086-1]

20 **Wang ZY**, Wang LD, Lee MJ, Ho CT, Huang MT, Conney AH, Yang CS. Inhibition of N-nitrosomethylbenzylamine-induced esophageal tumorigenesis in rats by green and black tea. *Carcinogenesis* 1995; **16**: 2143-2148 [PMID: 7554067 DOI: 10.1093/carcin/16.9.2143]

21 **Chen J**. The effects of Chinese tea on the occurrence of esophageal tumors induced by N-nitrosomethylbenzylamine in rats. *Prev Med* 1992; **21**: 385-391 [PMID: 1615000 DOI: 10.1016/0091-7435(92)90046-K]

22 **Chung JY**, Huang C, Meng X, Dong Z, Yang CS. Inhibition of activator protein 1 activity and cell growth by purified green tea and black tea polyphenols in H-ras-transformed cells: structure-activity relationship and mechanisms involved. *Cancer Res* 1999; **59**: 4610-4617 [PMID: 10493515]

23 **Chung JY**, Park JO, Phyu H, Dong Z, Yang CS. Mechanisms of inhibition of the Ras-MAP kinase signaling pathway in 30.7b Ras 12 cells by tea polyphenols (-)-epigallocatechin-3-gallate and theaflavin-3,3'-digallate. *FASEB J* 2001; **15**: 2022-2024 [PMID: 11511526 DOI: 10.1096/fj.01-0031fje01-0031fje]

24 **Dong Z**, Ma W, Huang C, Yang CS. Inhibition of tumor promoter-induced activator protein 1 activation and cell transformation by tea polyphenols, (-)-epigallocatechin gallate, and theaflavins. *Cancer Res* 1997; **57**: 4414-4419 [PMID: 9331105]

25 **Shimizu M**, Deguchi A, Lim JT, Moriwaki H, Kopelovich L, Weinstein IB. (-)-Epigallocatechin gallate and polyphenon E inhibit growth and activation of the epidermal growth factor receptor and human epidermal growth factor receptor-2 signaling pathways in human colon cancer cells. *Clin Cancer Res* 2005; **11**: 2735-2746 [PMID: 15814656 DOI: 10.1158/1078-0432.CCR-04-2014]

26 **Ahmad N**, Cheng P, Mukhtar H. Cell cycle dysregulation by green tea polyphenol epigallocatechin-3-gallate. *Biochem Biophys Res Commun* 2000; **275**: 328-334 [PMID: 10964666 DOI: 10.1006/bbrc.2000.3297]

27 **Gupta S**, Hussain T, Mukhtar H. Molecular pathway for (-)-epigallocatechin-3-gallate-induced cell cycle arrest and apoptosis of human prostate carcinoma cells. *Arch Biochem Biophys* 2003; **410**: 177-185 [PMID: 12559991 DOI: 10.1016/S0003-9861(02)00668-9]

28 **Ahmad N**, Feyes DK, Nieminen AL, Agarwal R, Mukhtar H. Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells. *J Natl Cancer Inst* 1997; **89**: 1881-1886 [PMID: 9414176 DOI: 10.1093/jnci/89.24.1881]

29 **Fang MZ**, Wang Y, Ai N, Hou Z, Sun Y, Lu H, Welsh W, Yang CS. Tea polyphenol (-)-epigallocatechin-3-gallate inhibits DNA methyltransferase and reactivates methylation-silenced genes in cancer cell lines. *Cancer Res* 2003; **63**: 7563-7570 [PMID: 14633667]

30 **Ye F**, Zhang GH, Guan BX, Xu XC. Suppression of esophageal cancer cell growth using curcumin, (-)-epigallocatechin-3-gallate and lovastatin. *World J Gastroenterol* 2012; **18**: 126-135 [PMID: 22253518 DOI: 10.3748/wjg.v18.i2.126]

31 **Hou Z**, Sang S, You H, Lee MJ, Hong J, Chin KV, Yang CS. Mechanism of action of (-)-epigallocatechin-3-gallate: auto-oxidation-dependent inactivation of epidermal growth factor receptor and direct effects on growth inhibition in human esophageal cancer KYSE 150 cells. *Cancer Res* 2005; **65**:

8049-8056 [PMID: 16140980]
32 **Li ZG**, Shimada Y, Sato F, Maeda M, Itami A, Kaganoi J, Komoto I, Kawabe A, Imamura M. Inhibitory effects of epigallocatechin-3-gallate on N-nitrosomethylbenzylamine-induced esophageal tumorigenesis in F344 rats. *Int J Oncol* 2002; **21**: 1275-1283 [PMID: 12429978]
33 **Li ZG**, Shimada Y, Sato F, Maeda M, Itami A, Kaganoi J, Komoto I, Kawabe A, Imamura M. Promotion effects of hot water on N-nitrosomethylbenzylamine-induced esophageal tumorigenesis in F344 rats. *Oncol Rep* 2003; **10**: 421-426 [PMID: 12579283]
34 **Esteller M**. CpG island hypermethylation and tumor suppressor genes: a booming present, a brighter future. *Oncogene* 2002; **21**: 5427-5440 [PMID: 12154405 DOI: 10.1038/sj.onc.1205600]
35 **Jones PA**, Baylin SB. The fundamental role of epigenetic events in cancer. *Nat Rev Genet* 2002; **3**: 415-428 [PMID: 12042769 DOI: 10.1038/nrg816nrg816]
36 **Lichtenstein AV**, Kisseljova NP. DNA methylation and carcinogenesis. *Biochemistry* (Mosc) 2001; **66**: 235-255 [PMID: 11333147]
37 **Nguyen CT**, Gonzales FA, Jones PA. Altered chromatin structure associated with methylation-induced gene silencing in cancer cells: correlation of accessibility, methylation, MeCP2 binding and acetylation. *Nucleic Acids Res* 2001; **29**: 4598-4606 [PMID: 11713309 DOI: 10.1093/nar/29.22.4598]
38 **Rice JC**, Massey-Brown KS, Futscher BW. Aberrant methylation of the BRCA1 CpG island promoter is associated with decreased BRCA1 mRNA in sporadic breast cancer cells. *Oncogene* 1998; **17**: 1807-1812 [PMID: 9778046 DOI: 10.1038/sj.onc.1202086]
39 **Bender CM**, Pao MM, Jones PA. Inhibition of DNA methylation by 5-aza-2′-deoxycytidine suppresses the growth of human tumor cell lines. *Cancer Res* 1998; **58**: 95-101 [PMID: 9426064]
40 **Cheng JC**, Matsen CB, Gonzales FA, Ye W, Greer S, Marquez VE, Jones PA, Selker EU. Inhibition of DNA methylation and reactivation of silenced genes by zebularine. *J Natl Cancer Inst* 2003; **95**: 399-409 [PMID: 12618505 DOI: 10.1093/jnci/95.5.399]
41 **Christman JK**. 5-Azacytidine and 5-aza-2′-deoxycytidine as inhibitors of DNA methylation: mechanistic studies and their implications for cancer therapy. *Oncogene* 2002; **21**: 5483-5495 [PMID: 12154409 DOI: 10.1038/sj.onc.1205699]
42 **Zhou L**, Cheng X, Connolly BA, Dickman MJ, Hurd PJ, Hornby DP. Zebularine: a novel DNA methylation inhibitor that forms a covalent complex with DNA methyltransferases. *J Mol Biol* 2002; **321**: 591-599 [PMID: 12206775 DOI: 10.1016/S0022-2836(02)00676-9]
43 **Esatbeyoglu T**, Huebbe P, Ernst IM, Chin D, Wagner AE, Rimbach G. Curcumin--from molecule to biological function. *Angew Chem Int Ed Engl* 2012; **51**: 5308-5332 [PMID: 22566109 DOI: 10.1002/anie.201107724]
44 **Goel A**, Aggarwal BB. Curcumin, the golden spice from Indian saffron, is a chemosensitizer and radiosensitizer for tumors and chemoprotector and radioprotector for normal organs. *Nutr Cancer* 2010; **62**: 919-930 [PMID: 20924967 DOI: 10.1080/01635581.2010.509835]
45 **Valko M**, Rhodes CJ, Moncol J, Izakovic M, Mazur M. Free radicals, metals and antioxidants in oxidative stress-induced cancer. *Chem Biol Interact* 2006; **160**: 1-40 [PMID: 16430879 DOI: 10.1016/j.cbi.2005.12.009]
46 **Bower MR**, Aiyer HS, Li Y, Martin RC. Chemoprotective effects of curcumin in esophageal epithelial cells exposed to bile acids. *World J Gastroenterol* 2010; **16**: 4152-4158 [PMID: 20806431 DOI: 10.3748/wjg.v16.i33.4152]
47 **Rayet B**, Gélinas C. Aberrant rel/nfkb genes and activity in human cancer. *Oncogene* 1999; **18**: 6938-6947 [PMID: 10602468 DOI: 10.1038/sj.onc.1203221]
48 **Arlt A**, Gehrz A, Müerköster S, Vorndamm J, Kruse ML, Fölsch UR, Schäfer H. Role of NF-kappaB and Akt/PI3K in the resistance of pancreatic carcinoma cell lines against gemcitabine-induced cell death. *Oncogene* 2003; **22**: 3243-3251 [PMID: 12761494 DOI: 10.1038/sj.onc.1206390]
49 **Kunnumakkara AB**, Guha S, Krishnan S, Diagaradjane P, Gelovani J, Aggarwal BB. Curcumin potentiates antitumor activity of gemcitabine in an orthotopic model of pancreatic cancer through suppression of proliferation, angiogenesis, and inhibition of nuclear factor-kappaB-regulated gene products. *Cancer Res* 2007; **67**: 3853-3861 [PMID: 17440100 DOI: 10.1158/0008-5472.CAN-06-4257]
50 **Hartojo W**, Silvers AL, Thomas DG, Seder CW, Lin L, Rao H, Wang Z, Greenson JK, Giordano TJ, Orringer MB, Rehemtulla A, Bhojani MS, Beer DG, Chang AC. Curcumin promotes apoptosis, increases chemosensitivity, and inhibits nuclear factor kappaB in esophageal adenocarcinoma. *Transl Oncol* 2010; **3**: 99-108 [PMID: 20360934]
51 **Tian F**, Song M, Xu PR, Liu HT, Xue LX. [Curcumin promotes apoptosis of esophageal squamous carcinoma cell lines through inhibition of NF-kappaB signaling pathway]. *Ai Zheng* 2008; **27**: 566-570 [PMID: 18570726]
52 **Rawat N**, Alhamdani A, McAdam E, Cronin J, Eltahir Z, Lewis P, Griffiths P, Baxter JN, Jenkins GJ. Curcumin abrogates bile-induced NF-κB activity and DNA damage in vitro and suppresses NF-κB activity whilst promoting apoptosis in vivo, suggesting chemopreventative potential in Barrett's oesophagus. *Clin Transl Oncol* 2012; **14**: 302-311 [PMID: 22484638 DOI: 10.1007/s12094-012-0799-x]
53 **Masuda S**. Dysfunctional transforming growth factor-β signaling with constitutively active notch signaling in Barrett's esophageal adenocarcinoma. *Cancer* 2012; **118**: 1956-1957; author reply 1957-1958 [PMID: 21898377 DOI: 10.1002/cncr.26444]
54 **Mendelson J**, Song S, Li Y, Maru DM, Mishra B, Davila M, Hofstetter WL, Mishra L. Dysfunctional transforming growth factor-β signaling with constitutively active Notch signaling in Barrett's esophageal adenocarcinoma. *Cancer* 2011; **117**: 3691-3702 [PMID: 21305538 DOI: 10.1002/cncr.25861]
55 **Peters JH**, Avisar N. The molecular pathogenesis of Barrett's esophagus: common signaling pathways in embryogenesis metaplasia and neoplasia. *J Gastrointest Surg* 2010; **14** Suppl 1: S81-S87 [PMID: 19760304 DOI: 10.1007/s11605-009-1011-7]
56 **Woo SM**, Kim J, Han HW, Chae JI, Son MY, Cho S, Chung HM, Han YM, Kang YK. Notch signaling is required for maintaining stem-cell features of neuroprogenitor cells derived from human embryonic stem cells. *BMC Neurosci* 2009; **10**: 97 [PMID: 19682396 DOI: 10.1186/1471-2202-10-97]
57 **Liao S**, Xia J, Chen Z, Zhang S, Ahmad A, Miele L, Sarkar FH, Wang Z. Inhibitory effect of curcumin on oral carcinoma CAL-27 cells via suppression of Notch-1 and NF-κB signaling pathways. *J Cell Biochem* 2011; **112**: 1055-1065 [PMID: 21308734 DOI: 10.1002/jcb.23019]
58 **Kuipers EJ**. Review article: exploring the link between Helicobacter pylori and gastric cancer. *Aliment Pharmacol Ther* 1999; **13** Suppl 1: 3-11 [PMID: 10209681 DOI: 10.1046/j.1365-2036.1999.00002.x]
59 **Yanaka A**. Sulforaphane enhances protection and repair of gastric mucosa against oxidative stress in vitro, and demonstrates anti-inflammatory effects on Helicobacter pylori-infected gastric mucosae in mice and human subjects. *Curr Pharm Des* 2011; **17**: 1532-1540 [PMID: 21548875 DOI: 10.2174/138161211796196945]
60 **De R**, Kundu P, Swarnakar S, Ramamurthy T, Chowdhury A, Nair GB, Mukhopadhyay AK. Antimicrobial activity of curcumin against Helicobacter pylori isolates from India and during infections in mice. *Antimicrob Agents Chemother* 2009; **53**: 1592-1597 [PMID: 19204190 DOI: 10.1128/AAC.01242-08]
61 **Yu LL**, Wu JG, Dai N, Yu HG, Si JM. Curcumin reverses

chemoresistance of human gastric cancer cells by down-regulating the NF-κB transcription factor. *Oncol Rep* 2011; **26**: 1197-1203 [PMID: 21811763 DOI: 10.3892/or.2011.1410]
62 **Cai XZ**, Wang J, Li XD, Wang GL, Liu FN, Cheng MS, Li F. Curcumin suppresses proliferation and invasion in human gastric cancer cells by downregulation of PAK1 activity and cyclin D1 expression. *Cancer Biol Ther* 2009; **8**: 1360-1368 [PMID: 19448398 DOI: 10.4161/cbt.8.14.8720]
63 **Kopp P**. Resveratrol, a phytoestrogen found in red wine. A possible explanation for the conundrum of the 'French paradox'? *Eur J Endocrinol* 1998; **138**: 619-620 [PMID: 9678525 DOI: 10.1530/eje.0.1380619]
64 **Sun AY**, Simonyi A, Sun GY. The "French Paradox" and beyond: neuroprotective effects of polyphenols. *Free Radic Biol Med* 2002; **32**: 314-318 [PMID: 11841921 DOI: 10.1016/S0891-5849(01)00803-6]
65 **Hung LM**, Chen JK, Huang SS, Lee RS, Su MJ. Cardioprotective effect of resveratrol, a natural antioxidant derived from grapes. *Cardiovasc Res* 2000; **47**: 549-555 [PMID: 10963727 DOI: 10.1016/S0008-6363(00)00102-4]
66 **Catalgol B**, Batirel S, Taga Y, Ozer NK. Resveratrol: French paradox revisited. *Front Pharmacol* 2012; **3**: 141 [PMID: 22822401 DOI: 10.3389/fphar.2012.00141]
67 **Chan MM**. Antimicrobial effect of resveratrol on dermatophytes and bacterial pathogens of the skin. *Biochem Pharmacol* 2002; **63**: 99-104 [PMID: 11841782 DOI: 10.1016/S0006-2952(01)00886-3]
68 **Daroch F**, Hoeneisen M, González CL, Kawaguchi F, Salgado F, Solar H, García A. In vitro antibacterial activity of Chilean red wines against Helicobacter pylori. *Microbios* 2001; **104**: 79-85 [PMID: 11297014]
69 **Mahady GB**, Pendland SL. Resveratrol inhibits the growth of Helicobacter pylori in vitro. *Am J Gastroenterol* 2000; **95**: 1849 [PMID: 10926010]
70 **Mahady GB**, Pendland SL, Chadwick LR. Resveratrol and red wine extracts inhibit the growth of CagA+ strains of Helicobacter pylori in vitro. *Am J Gastroenterol* 2003; **98**: 1440-1441 [PMID: 12818294 DOI: 10.1111/j.1572-0241.2003.07513.x]
71 **Zaidi SF**, Ahmed K, Yamamoto T, Kondo T, Usmanghani K, Kadowaki M, Sugiyama T. Effect of resveratrol on Helicobacter pylori-induced interleukin-8 secretion, reactive oxygen species generation and morphological changes in human gastric epithelial cells. *Biol Pharm Bull* 2009; **32**: 1931-1935 [PMID: 19881312 DOI: 10.1248/bpb.32.1931]
72 **Atten MJ**, Attar BM, Milson T, Holian O. Resveratrol-induced inactivation of human gastric adenocarcinoma cells through a protein kinase C-mediated mechanism. *Biochem Pharmacol* 2001; **62**: 1423-1432 [PMID: 11709203 DOI: 10.1016/S0006-2952(01)00788-2]
73 **Atten MJ**, Godoy-Romero E, Attar BM, Milson T, Zopel M, Holian O. Resveratrol regulates cellular PKC alpha and delta to inhibit growth and induce apoptosis in gastric cancer cells. *Invest New Drugs* 2005; **23**: 111-119 [PMID: 15744586 DOI: 10.1007/s10637-005-5855-8]
74 **Aquilano K**, Baldelli S, Rotilio G, Ciriolo MR. trans-Resveratrol inhibits H2O2-induced adenocarcinoma gastric cells proliferation via inactivation of MEK1/2-ERK1/2-c-Jun signalling axis. *Biochem Pharmacol* 2009; **77**: 337-347 [PMID: 19038233 DOI: 10.1016/j.bcp.2008.10.034]
75 **Wang Z**, Li W, Meng X, Jia B. Resveratrol induces gastric cancer cell apoptosis via reactive oxygen species, but independent of sirtuin1. *Clin Exp Pharmacol Physiol* 2012; **39**: 227-232 [PMID: 22211760 DOI: 10.1111/j.1440-1681.2011.05660.x]
76 **Zhou HB**, Yan Y, Sun YN, Zhu JR. Resveratrol induces apoptosis in human esophageal carcinoma cells. *World J Gastroenterol* 2003; **9**: 408-411 [PMID: 12632486]
77 **Zhou HB**, Chen JJ, Wang WX, Cai JT, Du Q. Anticancer activity of resveratrol on implanted human primary gastric carcinoma cells in nude mice. *World J Gastroenterol* 2005; **11**: 280-284 [PMID: 15633232]
78 **Fahey JW**, Haristoy X, Dolan PM, Kensler TW, Scholtus I, Stephenson KK, Talalay P, Lozniewski A. Sulforaphane inhibits extracellular, intracellular, and antibiotic-resistant strains of Helicobacter pylori and prevents benzo[a]pyrene-induced stomach tumors. *Proc Natl Acad Sci USA* 2002; **99**: 7610-7615 [PMID: 12032331 DOI: 10.1073/pnas.112203099]
79 **Fahey JW**, Zhang Y, Talalay P. Broccoli sprouts: an exceptionally rich source of inducers of enzymes that protect against chemical carcinogens. *Proc Natl Acad Sci USA* 1997; **94**: 10367-10372 [PMID: 9294217 DOI: 10.1073/pnas.94.19.10367]
80 **Ramos-Gomez M**, Kwak MK, Dolan PM, Itoh K, Yamamoto M, Talalay P, Kensler TW. Sensitivity to carcinogenesis is increased and chemoprotective efficacy of enzyme inducers is lost in nrf2 transcription factor-deficient mice. *Proc Natl Acad Sci USA* 2001; **98**: 3410-3415 [PMID: 11248092 DOI: 10.1073/pnas.051618798]
81 **Oijen AH**, Verhulst ML, Roelofs HM, Peters WH, de Boer WA, Jansen JB. Eradication of Helicobacter pylori restores glutathione S-transferase activity and glutathione levels in antral mucosa. *Jpn J Cancer Res* 2001; **92**: 1329-1334 [PMID: 11749699 DOI: 10.1111/j.1349-7006.2001.tb02157.x]
82 **Yanaka A**, Fahey JW, Fukumoto A, Nakayama M, Inoue S, Zhang S, Tauchi M, Suzuki H, Hyodo I, Yamamoto M. Dietary sulforaphane-rich broccoli sprouts reduce colonization and attenuate gastritis in Helicobacter pylori-infected mice and humans. *Cancer Prev Res* (Phila) 2009; **2**: 353-360 [PMID: 19349290 DOI: 10.1158/1940-6207.CAPR-08-0192]
83 **Basch E**, Jia X, Heller G, Barz A, Sit L, Fruscione M, Appawu M, Iasonos A, Atkinson T, Goldfarb S, Culkin A, Kris MG, Schrag D. Adverse symptom event reporting by patients vs clinicians: relationships with clinical outcomes. *J Natl Cancer Inst* 2009; **101**: 1624-1632 [PMID: 19920223 DOI: 10.1093/jnci/djp386]
84 **Siegel R**, Ward E, Brawley O, Jemal A. Cancer statistics, 2011: the impact of eliminating socioeconomic and racial disparities on premature cancer deaths. *CA Cancer J Clin* 2011; **61**: 212-236 [PMID: 21685461 DOI: 10.3322/caac.20121]
85 **Padidar S**, Farquharson AJ, Williams LM, Kearney R, Arthur JR, Drew JE. High-fat diet alters gene expression in the liver and colon: links to increased development of aberrant crypt foci. *Dig Dis Sci* 2012; **57**: 1866-1874 [PMID: 22373862 DOI: 10.1007/s10620-012-2092-9]
86 **Park H**, Kim M, Kwon GT, Lim do Y, Yu R, Sung MK, Lee KW, Daily JW, Park JH. A high-fat diet increases angiogenesis, solid tumor growth, and lung metastasis of CT26 colon cancer cells in obesity-resistant BALB/c mice. *Mol Carcinog* 2012; **51**: 869-880 [PMID: 21919080 DOI: 10.1002/mc.20856]
87 **Sung MK**, Yeon JY, Park SY, Park JH, Choi MS. Obesity-induced metabolic stresses in breast and colon cancer. *Ann NY Acad Sci* 2011; **1229**: 61-68 [PMID: 21793840 DOI: 10.1111/j.1749-6632.2011.06094.x]
88 **Tang FY**, Pai MH, Chiang EP. Consumption of high-fat diet induces tumor progression and epithelial-mesenchymal transition of colorectal cancer in a mouse xenograft model. *J Nutr Biochem* 2012; **23**: 1302-1313 [PMID: 22221675 DOI: 10.1016/j.jnutbio.2011.07.011]
89 **Herbst A**, Kolligs FT. Wnt signaling as a therapeutic target for cancer. *Methods Mol Biol* 2007; **361**: 63-91 [PMID: 17172707]
90 **He TC**, Sparks AB, Rago C, Hermeking H, Zawel L, da Costa LT, Morin PJ, Vogelstein B, Kinzler KW. Identification of c-MYC as a target of the APC pathway. *Science* 1998; **281**: 1509-1512 [PMID: 9727977 DOI: 10.1126/science.281.5382.1509]
91 **Tetsu O**, McCormick F. Beta-catenin regulates expression of cyclin D1 in colon carcinoma cells. *Nature* 1999; **398**: 422-426 [PMID: 10201372 DOI: 10.1038/18884]
92 **Liu Z**, Brooks RS, Ciappio ED, Kim SJ, Crott JW, Bennett G, Greenberg AS, Mason JB. Diet-induced obesity elevates colonic TNF-α in mice and is accompanied by an activation of Wnt signaling: a mechanism for obesity-associated

colorectal cancer. *J Nutr Biochem* 2012; **23**: 1207-1213 [PMID: 22209007 DOI: 10.1016/j.jnutbio.2011.07.002]

93 **Campbell PT**, Jacobs ET, Ulrich CM, Figueiredo JC, Poynter JN, McLaughlin JR, Haile RW, Jacobs EJ, Newcomb PA, Potter JD, Le Marchand L, Green RC, Parfrey P, Younghusband HB, Cotterchio M, Gallinger S, Jenkins MA, Hopper JL, Baron JA, Thibodeau SN, Lindor NM, Limburg PJ, Martínez ME. Case-control study of overweight, obesity, and colorectal cancer risk, overall and by tumor microsatellite instability status. *J Natl Cancer Inst* 2010; **102**: 391-400 [PMID: 20208017 DOI: 10.1093/jnci/djq011]

94 **Key TJ**, Spencer EA, Reeves GK. Symposium 1: Overnutrition: consequences and solutions. Obesity and cancer risk. *Proc Nutr Soc* 2010; **69**: 86-90 [PMID: 19954565 DOI: 10.1017/S0029665109991698]

95 **Chen J**. Multiple signal pathways in obesity-associated cancer. *Obes Rev* 2011; **12**: 1063-1070 [PMID: 22093240 DOI: 10.1111/j.1467-789X.2011.00917.x]

96 **Chen A**, Xu J, Johnson AC. Curcumin inhibits human colon cancer cell growth by suppressing gene expression of epidermal growth factor receptor through reducing the activity of the transcription factor Egr-1. *Oncogene* 2006; **25**: 278-287 [PMID: 16170359 DOI: 10.1038/sj.onc.1209019]

97 **Patel BB**, Gupta D, Elliott AA, Sengupta V, Yu Y, Majumdar AP. Curcumin targets FOLFOX-surviving colon cancer cells via inhibition of EGFRs and IGF-1R. *Anticancer Res* 2010; **30**: 319-325 [PMID: 20332435]

98 **Lee YK**, Park SY, Kim YM, Park OJ. Regulatory effect of the AMPK-COX-2 signaling pathway in curcumin-induced apoptosis in HT-29 colon cancer cells. *Ann NY Acad Sci* 2009; **1171**: 489-494 [PMID: 19723094 DOI: 10.1111/j.1749-6632.2009.04699.x]

99 **Binion DG**, Otterson MF, Rafiee P. Curcumin inhibits VEGF-mediated angiogenesis in human intestinal microvascular endothelial cells through COX-2 and MAPK inhibition. *Gut* 2008; **57**: 1509-1517 [PMID: 18596194 DOI: 10.1136/gut.2008.152496]

100 **Ryu MJ**, Cho M, Song JY, Yun YS, Choi IW, Kim DE, Park BS, Oh S. Natural derivatives of curcumin attenuate the Wnt/beta-catenin pathway through down-regulation of the transcriptional coactivator p300. *Biochem Biophys Res Commun* 2008; **377**: 1304-1308 [PMID: 19000900]

101 **Powell SM**, Zilz N, Beazer-Barclay Y, Bryan TM, Hamilton SR, Thibodeau SN, Vogelstein B, Kinzler KW. APC mutations occur early during colorectal tumorigenesis. *Nature* 1992; **359**: 235-237 [PMID: 1528264 DOI: 10.1038/359235a0]

102 **Jaiswal AS**, Marlow BP, Gupta N, Narayan S. Beta-catenin-mediated transactivation and cell-cell adhesion pathways are important in curcumin (diferuylmethane)-induced growth arrest and apoptosis in colon cancer cells. *Oncogene* 2002; **21**: 8414-8427 [PMID: 12466962 DOI: 10.1038/sj.onc.1205947]

103 **Patel KR**, Brown VA, Jones DJ, Britton RG, Hemingway D, Miller AS, West KP, Booth TD, Perloff M, Crowell JA, Brenner DE, Steward WP, Gescher AJ, Brown K. Clinical pharmacology of resveratrol and its metabolites in colorectal cancer patients. *Cancer Res* 2010; **70**: 7392-7399 [PMID: 20841478 DOI: 10.1158/0008-5472.CAN-10-20270008-5472.CAN-10-2027]

104 **Nguyen AV**, Martinez M, Stamos MJ, Moyer MP, Planutis K, Hope C, Holcombe RF. Results of a phase I pilot clinical trial examining the effect of plant-derived resveratrol and grape powder on Wnt pathway target gene expression in colonic mucosa and colon cancer. *Cancer Manag Res* 2009; **1**: 25-37 [PMID: 21188121]

105 **Cui X**, Jin Y, Hofseth AB, Pena E, Habiger J, Chumanevich A, Poudyal D, Nagarkatti M, Nagarkatti PS, Singh UP, Hofseth LJ. Resveratrol suppresses colitis and colon cancer associated with colitis. *Cancer Prev Res* (Phila) 2010; **3**: 549-559 [PMID: 20332304 DOI: 10.1158/1940-6207.CAPR-09-0117]

106 **Sengottuvelan M**, Deeptha K, Nalini N. Resveratrol ameliorates DNA damage, prooxidant and antioxidant imbalance in 1,2-dimethylhydrazine induced rat colon carcinogenesis. *Chem Biol Interact* 2009; **181**: 193-201 [PMID: 19523937 DOI: 10.1016/j.cbi.2009.06.004]

107 **Sengottuvelan M**, Nalini N. Dietary supplementation of resveratrol suppresses colonic tumour incidence in 1,2-dimethylhydrazine-treated rats by modulating biotransforming enzymes and aberrant crypt foci development. *Br J Nutr* 2006; **96**: 145-153 [PMID: 16870003]

108 **LeRoith D**, Roberts CT. The insulin-like growth factor system and cancer. *Cancer Lett* 2003; **195**: 127-137 [PMID: 12767520]

109 **Vanamala J**, Radhakrishnan S, Reddivari L, Bhat VB, Ptitsyn A. Resveratrol suppresses human colon cancer cell proliferation and induces apoptosis via targeting the pentose phosphate and the talin-FAK signaling pathways-A proteomic approach. *Proteome Sci* 2011; **9**: 49 [PMID: 21849056]

110 **Hwang JT**, Kwak DW, Lin SK, Kim HM, Kim YM, Park OJ. Resveratrol induces apoptosis in chemoresistant cancer cells via modulation of AMPK signaling pathway. *Ann NY Acad Sci* 2007; **1095**: 441-448 [PMID: 17404056]

P- Reviewer Tanabe S **S- Editor** Song XX
L- Editor Hughes D **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i7.994

World J Gastroenterol 2013 February 21; 19(7): 994-1004
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

TOPIC HIGHLIGHT

Jung Eun Lee, ScD, Assistant Professor, *Series Editor*

Nutritional modulators of ulcerative colitis: Clinical efficacies and mechanistic view

Mi-Kyung Sung, Mi-Young Park

Mi-Kyung Sung, Department of Food and Nutrition, Sookmyung Women's University, Seoul 140-742, South Korea
Mi-Young Park, Department of Pharmacy, the Catholic University of Korea, Bucheon 420-743, South Korea
Author contributions: Sung MK and Park MY contributed equally to this paper.
Supported by Mid-career Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology (2012R1A2A2A01046228)
Correspondence to: Mi-Kyung Sung, PhD, Department of Food and Nutrition, Sookmyung Women's University, 100, Cheongparo 47-gil, Yongsan-gu, Seoul 140-742, South Korea. mksung@sm.ac.kr
Telephone: +82-2-7109395 Fax: +82-2-7109453
Received: September 19, 2012 Revised: November 17, 2012
Accepted: December 22, 2012
Published online: February 21, 2013

Abstract

Ulcerative colitis (UC) is an inflammation-associated disease of the colon and rectum. The onset and progress of the disease are directly influenced by the nature of the intestinal microflora, the intestinal barrier function, and the immunological responses of the host. The epithelial invasion of pathogenic bacteria due to excess contact and/or barrier dysfunction is related to inflammation mediated by intestinal immune responses. Although the etiology of UC is not clearly understood, recent studies have shown a rising incidence of UC worldwide, and this phenomenon is more prominent in Asian countries and in Asian immigrants in Western countries. The increased prevalence of UC also contributes to an increased risk of developing colorectal cancer. Environmental factors, including changes in dietary habits, have been suggested as major risk factors of UC. A systematic review showed a negative association between UC risk and vegetable intake, whereas total fat, omega-6 fatty acids and meat intake were positively associated with an increased risk of UC. Individual dietary factors and energy balance have been suggested as having important roles in inducing changes in the microbial population and intestinal barrier integrity and in regulating inflammatory immune responses, directly or indirectly. Excess energy intake is now known to increase pathogenic microbial populations. Likewise, the application of appropriate probiotics may reverse the pathogenic progression of the disease. In the meantime, dietary anti-inflammatory compounds, including omega-3 fatty acids and other phytochemicals, may directly suppress inflammatory responses in the course of UC development. In this review, the increased prevalence of UC and its management are interpreted from the standpoint of nutritional modulation to regulate the intestinal microflora population, intestinal epithelium permeability, and inflammatory responses.

© 2013 Baishideng. All rights reserved.

Key words: Ulcerative colitis; Intestinal microflora; Immunity; Inflammation; Clinical; Obesity; Probiotics; Omega-3 fatty acids; Antioxidants

Sung MK, Park MY. Nutritional modulators of ulcerative colitis: Clinical efficacies and mechanistic view. *World J Gastroenterol* 2013; 19(7): 994-1004 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i7/994.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i7.994

INTRODUCTION

Ulcerative colitis (UC) is a major type of inflammatory bowel disease (IBD) characterized by chronic inflammation in the colon and rectum. It progresses by extensive epithelial apoptosis and ulceration due to chronic inflammation induced by T-helper (Th) 2 cytokines[1]. Recent studies have also indicated that a balance between proinflammatory Th17 cells and immunsuppressive Treg cells play a crucial role in the development of

UC[2]. Although the etiology of UC has not been clearly determined, environmental factors are thought to stimulate overt immune responses to bacterial components in individuals with high genetic susceptibility. A recent report on the incidence of IBD in Asia indicated that the prevalence of UC is growing rapidly in Japan, Hong Kong, and South Korea[3-5], countries where IBD used to be rare. The most recent reports on UC prevalence in Japan and South Korea provided figures of 63.6 and 30.9 per 100 000 people, respectively[4,5]. UC reportedly affects 0.24% of the United States population[6], and the prevalence in Northern European countries ranges from 40 to 240 per 100 000 people[7].

Lifestyle changes, as well as increased awareness of the disease and improved diagnosis, may have contributed to the increased incidence. Dietary habits in Asian countries have changed, resulting in a Western-style diet with fewer plant-based and more processed foods. A recent systematic review of 19 studies reported a negative association between UC risk and vegetable intake, whereas total fat, omega-6 fatty acids, and meat intake were positively associated with increased UC risk[8]. Information is limited, however, on the role of individual dietary components in UC development, and most nutritional modulation studies have focused on delaying relapses of UC, efforts that involve secondary rather than primary prevention. The cumulative incidence of relapse in UC are 30%, 72%, and 88% after 1, 5, and 10 years following the initial diagnosis[9]. Patients younger than 40 years have been shown to present with more severe disease at the time of diagnosis compared with older patients[10]. Importantly, the increased incidence of UC may be closely related to an increase in the prevalence of colorectal cancer (CRC). A subset study population with UC from the Kaiser Permanente Medical Care Program was analyzed for CRC incidence and mortality, and the standardized mortality ratio for CRC among UC patients was 2.0 (95%CI 1.3-2.7)[11]. Chronic inflammation mediates a wide range of signaling cascades that possibly facilitate colorectal carcinogenesis, and UC remission may reduce the risk of CRC. In this review, the pathophysiology of UC is summarized to allow understanding of the molecular mechanisms involved in the action of dietary components. Major dietary components reported to regulate, directly or indirectly, inflammatory responses in UC are discussed, with a focus on human studies where available. Genetic factors and therapeutic measures, while important to consider, fall outside the scope of this review.

INTESTINAL BARRIER FUNCTIONS AND INFLAMMATION IN UC

The human large intestine contains a concentration of approximately 10^{11}-10^{12} microorganisms per gram of luminal content. These microbes can be either beneficial or harmful to the intestinal epithelium. Under normal circumstances, multiple mechanisms protect the intestinal epithelium from microbial invasion, but environmental stimuli in combination with genetic factors can facilitate overgrowth of harmful microflora and induce abnormal immune responses that disrupt the mucosal barrier, causing inflammation (Figure 1).

The luminal side of the intestinal membrane is in constant contact with intestinal microflora. The most evident characteristic of pathogenic bacteria are their invasiveness and induction of inflammatory responses in the intestinal epithelium[12]. The host system, in other words, is able to recognize and differentiate pathogens from the commensals. To protect the host from pathogenic bacteria, the intestinal epithelium is equipped with multiple defense systems. Intestinal epithelial cells (IECs), which are layered by glycoproteins such as mucin, form the first line of immune defense. Tight junction (TJ) proteins seal the space between IECs. The layer below the IECs is the sub-epithelial dome, containing antigen-presenting cells, and underneath are Payer's patches, where B-cell follicles and T-cells reside.

Bacterial invasion is recognized by receptors called pattern recognition receptors (PRRs) in the IEC membrane, and the primary PRRs are toll-like receptors (TLRs). There are 10 different classes of TLRs expressed throughout the whole human gastrointestinal tract. The recognition of bacteria is carried out by means of communication between PRRs and microbial components such as lipopolysaccharide (LPS), and this is followed by immune responses to destroy the invading pathogens. Microbiota and viral-associated ligands use different types of TLRs depending on molecular patterns. For example, TLR2 recognizes lipopeptides, TLR 3 recognizes viral-derived dsRNA, and TLR4 recognizes LPSs[13]. Constant immune responses provoke chronic inflammation, which is mediated by pro-inflammatory cytokines and chemokines. The inflammation not only contributes to clinical features of inflammatory disease, but also exacerbates the penetration of pathogenic bacteria by increasing the membrane permeability, creating a vicious cycle. The ability of the intestinal epithelium to distinguish commensal from pathogenic bacteria is important because the intestine requires a symbiotic relationship with commensals without immune responses. Several explanations exist for the intestinal epithelium's ability to discriminate harmful bacteria from commensals[14]. In brief, pathogenic bacteria possesses virulence factors to stimulate the innate immune responses while commensals mutate their molecular patterns, escaping recognition by TLRs, and attenuate the nuclear factor (NF)-κB pathway. Also, the hyporesponsiveness to commensals has been explained by the anti-inflammatory nature of the gut mucosa including reduced expression of PRRs and reduced inflammatory nature of intestinal immune cells.

DIETARY FACTORS MODULATING INTESTINAL BARRIER FUNCTIONS IN UC

Excess energy (obesity)

Obesity is the most convincing risk factor in the devel-

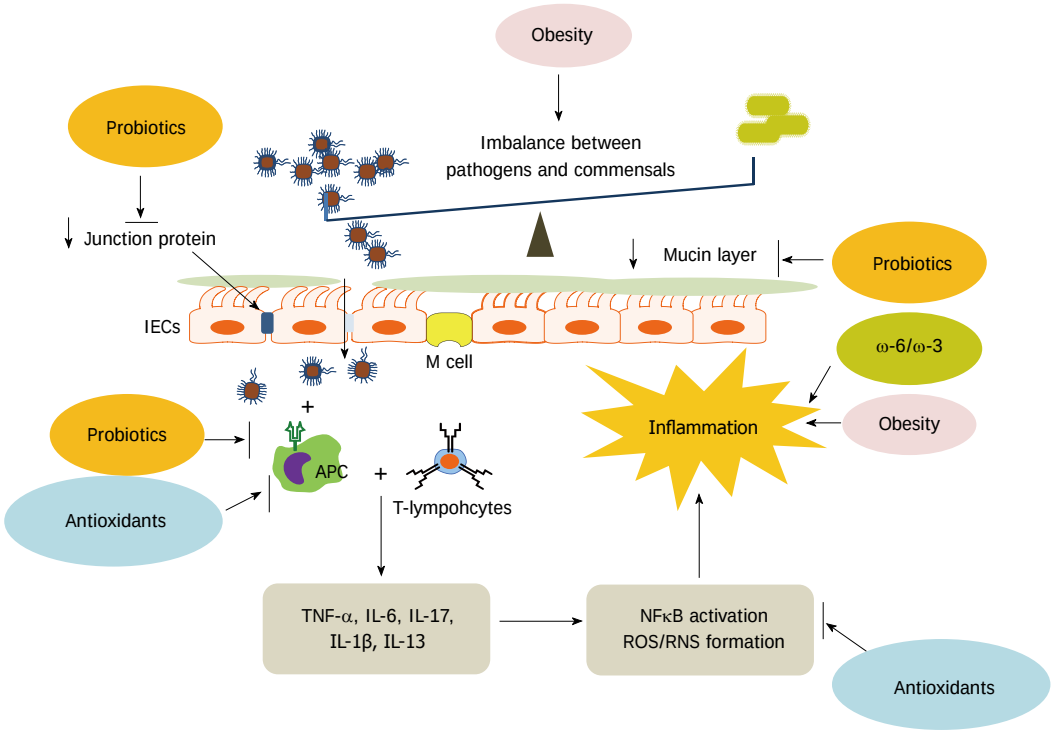


Figure 1 Disrupted intestinal homeostasis in ulcerative colitis and the role of nutritional factors. Ulcerative colitis is a chronic inflammatory disease of the colon and rectum. Inflammatory responses are induced by the penetration of excessive pathogenic bacteria due to the increased population of pathogenic bacteria, the loss of junction proteins and thin mucin layer. Once pathogens are recognized by antigen-presenting cells (APC), T-lymphocytes produce pro-inflammatory cytokines activating inflammation-inducing nuclear transcription factor, nuclear factor (NF)-κB and generating reactive oxygen species (ROS) and reactive nitrogen species (RNS) which result in the inflamed intestine. Obesity is known to cause imbalances between pathogens and commensals as well as chronic inflammation. The increased ω-6 to ω-3 fatty acid ratio in the diet also accelerates inflammatory responses. Probiotics supplementation helps to maintain gut health by retaining tight junctions and mucin layer. Probiotics and antioxidants suppress immoderate immune responses, and ROS-induced inflammatory responses are moderated by antioxidants. TNF-α: Tumor necrosis factor-α; IL-1β: Interleukin-1β; IECs: Intestinal epithelial cells.

opment of many noncommunicable diseases, including type 2 diabetes, cardiovascular diseases, non-alcoholic fatty liver disease, and selected types of cancer. World Health Organization statistics stated that physical inactivity and being overweight or obese contributed, respectively, to 5% and 6% of deaths[15]. The estimated prevalence of overweight [body mass index (BMI) $\geqslant$ 25 kg/m^2] and obese (BMI $\geqslant$ 30 kg/m^2) males and females aged 15 or older was $\geqslant$ 80% in the United States, $\geqslant$ 65% in Canada, Mexico, Argentina, Australia, the United Kingdom, and Germany, and $\geqslant$ 35% in all other countries, except for several African countries, India, and South East Asian countries[16].

Obesity and abdominal obesity have been specifically identified as the most convincing risk factors for the development of CRC[17]. In many studies, obesity-related inflammatory biomarkers were shown to be associated with disease activity. Among adipokines, resistin was considered to have a positive relationship with disease activity[18,19]. In a multicenter registry of children with IBD, 1 in 3 children with UC were overweight or obese, which is comparable to the rate in the general population[20]. However, prior IBD-related surgery was positively associated with being overweight or obese, which implies that obesity may provoke a more severe disease course. CRP was found to be a marker predictive of disease progression in a population-based prospective study[21]. CRP concentrations above 23 mg/L at diagnosis showed an odds ratio (OR) of 4.8 (95%CI, 1.5-15.1) in the prediction of surgery in UC patients. These results suggest that systemic inflammation worsens disease activity, and therefore the suppression of inflammatory events may be critical for the management of UC.

Mechanistic explanations have not been provided regarding the relationship between obesity and intestinal inflammation in patients. However, a number of rodent model studies have indicated that intestinal inflammation is mediated through obesity-related factors. Leptin was shown to act as a critical mediator in colitis development[22]. Leptin-deficient (*ob*/*ob*) mice were shown to be resistant to chemically-induced colitis, indicating that leptin is a critical regulator. Adiponectin, on the other hand, was shown to be a negative regulator in the development of dextran sulfate sodium (DSS)-induced colitis through the suppression of pro-inflammatory cytokine and chemokine production[23]. In a recent study, the effects of high-fat diet-induced obesity and chemically-induced colitis were compared in the development of UC[24]. A high-fat diet alone did not induce characteristic histopathological features of UC, indicating UC development is accelerated through inflammatory infiltration in the colon tissue of chemically-induced colitis. It is also interesting to note that the inflamed intestines of mice with chemically-induced colitis exhibited higher

inflammatory activities in the mesenteric fat compared with obese mice, which implies that intestinal inflammation may precede systemic inflammation[25]. These results suggest that obesity may not be an independent risk factor for UC development, although it may aggravate disease progression.

There are several reports showing evidence of obesity-associated changes in the intestinal microbiota. However, there is no clear explanation if this is due to adiposity or dietary composition[26]. Therefore, the altered microbial population is either a cause or a consequences of obesity. A recent report suggested that diets high in saturated fat promote taurine-conjugated bile acid formation, altering microbial composition towards the overgrowth of a sulfite-reducing pathobiont, *Bilophilla wadsworthia*. This accelerated the development of colitis in interleukin (IL)-10 -/- mice[27], providing evidence of a direct contribution from increased dietary fat. High-saturated fat diets altered microbial composition, resulting in a significantly higher ratio of *Firmicutes* to *Bacteroidetes*[28]. A decreased proportion of *Bacteroidetes*[29] was also observed in IBD, suggesting a possible mediating effect of these bacteria in obesity-related pathogenic changes in the intestinal epithelium. Others have reported that changes in the gut microbiota directly contribute to the development of obesity and related metabolic disturbances[30,31], which requires further investigation.

Fatty acids

The intakes of pro-inflammatory omega-6 fatty acids and anti-inflammatory omega-3 fatty acids have been suggested as important regulators in UC disease activity. A recent systematic review concluded that available randomized controlled trials do not indicate that omega-3 fatty acids are useful to alleviate IBD[32].

Epidemiological studies have been conducted to investigate the association between UC and nutrients. A total of 139 UC patients were identified as a subgroup of the population participating in a large prospective cohort study, the European Prospective Investigation into Cancer and Nutrition (EPIC)[33]. Results indicated that there is a marginally significant association between UC and an increasing percentage intake of energy from total polyunsaturated fatty acids (OR, 1.19; 95%CI, 0.99-1.14, $P = 0.07$). In another EPIC sub-cohort report, the highest quartile of intake of linoleic acid was positively associated with UC risk (OR, 2.49; 95%CI, 1.23-5.07)[34]. A nested United Kingdom cohort study analyzed the effect of total and specific dietary omega-3 fatty acids intake on the risk of UC[35]. Docosahexaenoic acid (DHA) was found to have a statistically significant protective OR of 0.43 (95%CI, 0.22-0.86), while total omega-3 fatty acids and eicosapentaenoic acid (EPA) showed marginally negative values.

Few studies have reported fatty acid composition markers of UC in human biospecimens. In accordance with the above mentioned epidemiological evidence, one study showed that the erythrocyte membrane content of linoleic acid, the most abundant dietary polyunsaturated acid present in plant seed oil was significantly higher in IBD patients (29 UC and 20 CD patients) compared with control subjects ($n = 31$)[36]. In the same study, UC patients showed a significantly higher ratio of arachidonic acid (AA) to EPA compared with control subjects. Leukotrienes (LTs) are pro-inflammatory lipid mediators involved in the progression of UC. The 5-lipoxygenase pathway (5-LOX) catalyzes the formation of LTs from AA, and the deletion of the gene encoding 5-LOX in an animal model prevented the development of chemically-induced colitis[37]. In a prospective case-control study, UC patients had significantly higher urinary LTE4 excretion compared with controls[38]. A subpopulation of the EPIC prospective cohort study showed that subjects in the highest quartile for adipose tissue AA concentration had a relative risk for UC of 4.16 (95%CI, 1.38-2.27)[39].

Experimental animal model studies have investigated the effects on UC development of omega-3 fatty acid supplementation or an increased ratio of omega-3 to omega-6 fatty acid in the diet[41-43]. Possible mechanistic explanations include: (1) the restoration of membrane TJ protein expression and distribution; or (2) the activation of peroxisome proliferator-activated receptor-γ, possibly inhibiting NFκB transcriptional activity. However, Varnalidis *et al*[42] suggested that the ameliorating effects of omega-3 fatty acids are accompanied by increased colonic neutrophil infiltration, and needs further clarification. Matsunaga *et al*[41] reported that feeding fish oil (8% w/w) exacerbates DSS-induced colitis in mice by suppressing the expression of adiponectin in myofibroblasts of the intestinal epithelium.

Apart from omega-6 and omega-3 fatty acids, short chain fatty acids have been suggested to suppress intestinal inflammation through G-protein-coupled receptor 43 (GPR-43)[44]. Acetate supplementation suppressed DSS-induced colonic inflammation in wild-type mice, while GPR-43 $^{-/-}$ mice did not respond to acetate. These results explain the role that intestinal microbiota play in producing short chain fatty acids from dietary fiber in the maintenance of a healthy gut.

The efficacy and safety of fish oil in the remission of IBD [Crohn's disease (CD) or UC] have been investigated in clinical trials. A recent meta-analysis retrieved 9 randomized, placebo-controlled trial of fish oil administered for at least 6 mo[45]. A total of 1.8-6.2 g/d of DHA + EPA or total omega-3 fatty acids were provided during the study period, and the primary outcome was relapse rate. A meta-analysis indicated that there was no significant reduction in the relative risk of UC, while the pooled relative risk of CD relapse was 0.77 (95%CI, 0.61-0.98). A clinical trial based on n-3 polyunsaturated diet therapy (n-6 to n-3 fatty acid ratio of approximately 1) reported that subjects exhibiting disease remission after a n-3 PUFA diet had a higher ratio of n-3/n-6 fatty acids in the red cell membrane compared with the relapse group[46].

Probiotics

Probiotics are live, nonpathogenic microorganisms that

contribute to the improvement of epithelial and mucosal barrier function through various mechanisms, including reduced intestinal pH, inhibition of pathogenic bacteria, and modulation of the intestinal mucosa immune responses[47-49]. Although there is a growing body of evidence from *in vivo* studies on the beneficial effects of probiotics in UC, reliable clinical trials are limited in number. The aim of this chapter is to review randomized, double-blind, placebo-controlled clinical trials which suggest that probiotics are effective in UC.

The probiotic *Escherichia Coli* strain Nissle 1917 (EcN) is non-pathogenic and one of the best characterized strains used as a probiotic drug[50]. The clinical effectiveness of EcN in adult UC patients seems to be promising[51]. Patients with moderate distal active UC were assigned to treatment with either 10, 20, or 40 mL of EcN enema (n = 23, 23, 24) containing 10^8 CFU/mL or placebo once a day for at least 2 wk. If there was no disease activity index (DAI) improvement after 2 wk, patients were classified as non-responders and discontinued therapy. Those classified as responders could receive the treatment for 4 or 8 wk. Although the number of responders was not significantly higher in the EcN group than in the placebo group, the efficacy of rectal EcN treatment was dose-dependent and significant in the per-protocol analysis. This Phase Ⅱ study showed that EcN is an effective and well-tolerated alternative or supplementary treatment in mild-to-moderate distal UC patients.

The induction of remission in patients with mild-to-moderate UC was also demonstrated with VSL3™ treatment. VSL3 is a high-concentration probiotic mixture, which combines 8 different bacterial species including *Bifidobacterium* (*B.*) *longum, B. breve, B. infantis, Lactobacillus* (*L.*) *casei, L. plantarum, L. acidophilus, L. Bulgaricus,* and *Streptococcus thermophilus*[52]. In a multicenter, randomized, double-blind, placebo-controlled trial, subjects received either VSL3 (n = 77) containing 3.6×10^{12} CFU or placebo (n = 70) twice per day for 12 wk[53]. VSL3 led to a 50% improvement in DAI at 6 wk and clinical remission was shown in 33 patients given VSL3 (42.9%) compared with 11 patients given placebo (15.7%) at 12 wk.

A single-center, randomized, double-blind, placebo-controlled trial of the efficacy of symbiotic treatment was performed using 18 patients with active UC[54]. Participants were given either 2×10^{11} *B. longum and* 6 g of prebiotic fructo-oligosaccharide/inulin mix (n = 9) or placebo (n = 9) twice a day for 4 wk. The results showed that consumption of symbiotic treatment over 4 wk significantly ameliorated mucosal mRNA levels of human beta-defensins 2, 3, and 4, which are strongly upregulated in active UC. In addition, mucosal mRNA levels of tumor necrosis factor-α (TNF-α) and IL-1α were significantly decreased in the symbiotic group, with remission of sigmoidoscopy scores. This small trial provided the potential efficacy of symbiotic therapy for patients with active UC. The effect of *L. acidophilus* La-5 and *B. lactis* BB-12 (Probio-Tec AB-25) was investigated in a randomized, double-blind, placebo-controlled trial[55]. Patients with left-side UC were given either Probio-Tec AB-25 (n = 20) or placebo (n = 12) for 52 wk, and clinical, endoscopic, and histological parameters were analyzed at entry and in case of relapse. In this study, there was no statistical difference in remission rate and the median time to relapse between the two groups after 1 year of treatment. The authors concluded that Probio-Tec AB-25 was well tolerated by study participants and that a failure to prove efficacy in remission rate may be due to the small sample size.

The use of probiotics as maintenance therapy in UC patients seems promising. Two randomized, double blind, double-dummy clinical trials to determine the efficacy of EcN were conducted. In the preliminary study, 120 patients with inactive UC were treated with either an oral preparation of EcN containing 25×10^9 bacteria daily or mesalazine 1500 mg daily for 12 wk. The start and end DAI scores demonstrated no significant difference between the two groups. The relapse rate was 16.0% in EcN group and 11.3% among those taking mesalazine[56]. The same authors investigated the effectiveness of EcN in 327 patients with UC. Subjects received either probiotics containing 5×10^{10} bacteria or mesalazine 1500 mg daily (n = 162, 165) for 12 mo. According to the per protocol analysis, 36.4% of patients in the EcN group experienced a relapse, compared with 33.9% of patients in the mesalazine group. The investigators concluded that EcN is efficient and safe in maintaining remission, and equivalent to the standard mesalazine[57]. Another study also confirmed that the administration of EcN was as effective as mesalazine in the maintenance of remission in patients with UC[58]. Patients with active UC were randomized to EcN treatment of 5×10^{10} probiotics/d (n = 57) or mesalazine treatment (1200 mg/d, n = 59) for 12 mo. In the intention-to-treat analysis, the health conditions were improved in 39 (68.4%) subjects in the EcN group and 44 (74.5%) subjects in the mesalazine group. The mean time of remission was 42 d (median 37 d) in the EcN group and 44 d (mean 42 d) in the mesalazine group. These results support the conclusion that EcN treatment has an equivalent effect to mesalazine in maintaining remission of UC.

The efficacy of VSL3 on induction and maintenance of remission in patients with active UC was studied in a long-term, randomized, double-blind, placebo-controlled trial[59]. In this study, subjects were children (mean age: 9.8 years; range: 1.7-16.1 years) and they were received either VSL3 containing 4.5-18 $\times 10^{11}$ bacteria/d (n = 14) or placebo (n = 15) for 1 year. All of the 29 patients were also treated with mesalazine during this trial period. Three of the 14 (21.4%) subjects in the VSL3 group and 11 of the 15 (73.3%) subjects in the placebo group relapsed within 1 year of follow-up. At 6 mo, 12 mo, or at time of relapse, endoscopic and histological scores were significantly lower in the VSL3 group compared with the patients in the placebo group. No adverse effect related to VSL3 was observed. Although the sample size of this

study was small, it provided evidence for the efficacy and safety of VSL3 in pediatric UC patients receiving conventional IBD therapy.

Another study evaluated the efficacy of VSL3 supplementation in patients with mild-to-moderate UC who were already being treated with 5-aminosalicylic acid (5-ASA) and/or immunosuppressants[60]. The patients were randomly treated with either VSL3 (n = 65) including 3600 × 10^9 CFU/d or placebo (n = 66) for 8 wk. After 8 wk, decreases in DAI scores of 50% or more were significantly higher in the VSL3 group than in the placebo group. However, there was no significant difference in stool frequency, physician rating of disease, and endoscopic scores between the two groups. Although the mechanism(s) of VSL3 treatment in IBD therapy is unclear, these two randomized, double-blind, placebo-controlled clinical trials showed the potential synergistic activity of probiotics.

A large number of animal model studies have reported protective effects of probiotics, and these results are summarized elsewhere[61]. Most of the studies used DSS mouse[62-64], trinitrobenzene sulfonic acid (TNBS) mouse[65-67] and IL-$10^{-/-}$ mouse[68-70]. The most frequently used probiotics were VSL3, *Lactobacillus, Bifidobacterium*, and *Saccharomyces*. The proposed mechanisms of action were as follows: (1) reduction of inflammatory cytokine and chemokine levels; (2) downregulation of TLR signaling followed by immune suppression; (3) increase in epithelium gap junction protein expressions; (4) prevention of pathogen growth and/or attachment; (5) increase in mucin production; (6) decrease in leukotriene B4 production; and (7) increase in short-chain fatty acid production. The reduced level of inflammatory cytokines and chemokines were thought to be mediated through signal transducer and activator of transcriptions-3 signaling[71,72] and/or NF-κB activation[73,74].

Although the specific mechanisms of action of probiotics are unknown, their therapeutic effects may be related to modulation of antigen-presenting cells. Local T cell immunity is an important factor involved in the specific intestinal immune system and its activation is programmed by dendritic cells (DCs) that help maintain mucosal tolerance and contribute to the development of chronic intestinal inflammation as key initiators of innate and adaptive immune responses in the gut[75-77]. However, the immunological mechanisms of certain probiotics are still poorly understood while several studies have demonstrated that anti-inflammatory activity of probiotics is due to modulation of DC and regulatory T cell (Treg) phenotype function. It was reported that the therapeutic effect of VSL3 was due to regulation mucosal CD4+ T cell responses in a colitis-associated CRC model[78]. VSL3 administration in UC patients significantly decreased TLR-2 expression on colonic DC[79]. Also, VSL3 supplementation increased IL-10 production and decreased IL-12p40 production by colonic DC[76]. VSL3 administration induced a significant expansion of mucosal Treg cells in UC patients[80]. *L. rhamnosus* induced maturation of monocyte-derived DC and induced both lower IL-12 and IL-18 production and development of T cells without a typical Th phenotype[81]. A probiotic mixture containing *L. helveticus* and *L. rhamnosus* increased follicular Treg cells in *Citrobacter rodentium*-induced colitis[82]. *L. casei* inhibited TNF-induced secretion of the T-cell chemokine interferon-inducible protein 10 (IP-10) in IECs by blocking IP-10 protein secretion and IP-10-mediated T-cell transmigration[83]. *L. casei* lysate DN-114 001 treatment increased the numbers of CD4(+)FoxP3(+)Treg cells in mesenteric lymph nodes, decreased the production of TNF-α, interferon-γ, and IL-10 in Peyer's patches and large intestine, and changed the gut microbiota composition in a DSS-induced colitis model[84]. *L*-peptidoglycan purified from Ls33 also ameliorated TNBS-induced colitis by development of CD103(+) DCs and CD4(+)Foxp3(+) Treg cells[85]. Thomas *et al*[86] showed that *Saccharomyces bourladii*, a probiotic yeast preparation, reduced IL-6 and TNF-α and decreased the expression of co-stimulated surface markers CD40, CD80, and the migration marker CD197 (CCR7) on LPS-stimulated human 92 induced apoptosis of antigen-stimulated T cells. These data suggest that each strain exhibits a different ability to modulate DC and Treg functions and explain various potential mechanism(s) of bacterial strains.

Current evidence suggests that probiotics are good complementary and alternative medicine candidates to maintain remission and prevent relapse of UC. However, the clinical efficacy results are limited, and strain-specific mechanistic explanations are insufficient. Additional large-scale clinical trials are required to shed light on optimal doses and treatment periods.

Antioxidant vitamins and phytochemicals

Oxidative stress is known as a potential etiological and/or triggering factor in the initiation and preservation of UC[47,87-89]. Inflammation augments oxidative stress by activating reactive oxygen and/or reactive nitrogen, generating enzymes such as NAD(P)H oxidase, inducible nitric oxide synthase, and myeloperoxidase[89,90]. A group of antioxidants has been used to ameliorate the clinical condition of UC patients. In addition, several studies have shown that patients with UC often have antioxidant nutrient deficiencies at the time of diagnosis[91-93]. Although the important role of antioxidants in UC has been seen in several studies, few clinical trials have been conducted. A randomized, controlled trial evaluated the efficacy of a nutritionally balanced oral supplement including fish oil, fructo-oligosaccharides, gum arabic, vitamin E, vitamin C, and selenium in 121 patients with mild-to-moderate UC[93]. Patients consumed either 510.3 g of the oral supplement or placebo each day for 6 mo. Compared with the placebo group, both intent-to-treat and completer patients given the oral supplement showed a significant decrease in the dose of prednisone required to control clinical symptoms over 6 mo. Mirbagheri *et al*[94] performed an open-label, preliminary trial for the efficacy of D-α-tocopherol using 14 patients who were receiving concomitant therapy with 5-ASA and/or immunosuppressants. Patients received a D-α-

Table 1 Action targets of major beneficial dietary components in ulcerative colitis

	Omega-3 fatty acids	Probiotics	Anti-oxidants	Aloe
Beneficial bacteria growth		+		
Short chain fatty acid production		+		
Mucin repletion		+		+
Junction protein restoration		+		
Suppression of inflammatory eicosanoids	+			
Blockade of inflammatory signals	+		+	+
Suppression of immune response	+	+	+	
Reduction in oxidative stress			+	

tocopherol enema (8000 U/d) for 12 wk. At the end of 12 wk, the DAI score had statistically decreased from the beginning of the study, all 14 patients responded clinically to the therapy, and remission was induced in 9 patients (64%) without adverse events.

In recent years, several studies have investigated the efficacy of curcumin, a polyphenolic antioxidant in *Curcuma longa L.*, in experimental models of UC. These studies have showed a strong antioxidant effect, as well as an anti-inflammatory effect, of curcumin in this model. A small, open-label, pilot clinical trial of the effect of curcumin in patients with IBD (5 UC, 5 CD) receiving IBD medication (5-ASA or corticosteroids) was conducted[95]. The patients were given curcumin 1100 mg/d for 1 mo and 1650 mg/d for an additional 2 mo. All 5 patients with UC had significant improvement in their medication, as follows: two patients stopped taking 5-ASA, two reduced 5-ASA dosage, and one stopped corticosteroids entirely. This encouraging pilot study showed the strong potential efficacy of curcumin in UC patients.

In a multicenter, randomized, double-blind, placebo-controlled trial, the efficacy of curcumin in 89 patients with quiescent UC was evaluated[96]. In addition to their usual medication (sulfasalazine or mesalamine), participants received either curcumin (2 g/d) or placebo (n = 45, 44) for 6 mo. DAI and endoscopic scores were determined at entry, every 2 mo (DAI), at the conclusion of the 6-mo trial, and after a 6-mo follow-up. The relapse rate was significantly lower in the curcumin group than in the placebo group and the recurrence rate was significantly reduced in the curcumin group compared with the placebo group. Both the DAI and endoscopic score were also improved in the curcumin group without any adverse effects. Although only two clinical trials have been performed, the potential therapeutic capability of curcumin in UC patients has been shown, possibly resulting from its antioxidant and anti-inflammatory effects. However, large-scale, randomized, double-blind, placebo-controlled clinical trials are required to achieve convincing evidence for the routine use of antioxidants in patients with UC.

Many studies have reported that antioxidant supplementation has therapeutic efficacy in animal models, and these results were summarized in a recent review[97]. Mechanisms of action include: (1) direct scavenging of reactive oxygen species; (2) suppression of proinflammatory protein expression by downregulating related enzymes or transcription factors; and (3) alteration of leukocyte cell surface molecules.

UC is associated with increased gastrointestinal permeability[98,99]. Although the causes of increased permeability are not completely understood, an inflammation-associated increase in permeability has been shown to result in bacterial translocation into the lamina propria, which exacerbates the loss of barrier function. We have previously reported that aloe anthraquinones and chromone ameliorate colonic inflammatory responses in a DSS-induced UC model; aloesin, an aloe chromone, showed the most drastic results[100]. Additionally, we studied whether aloesin modulates mucosal permeability and found that this phytochemical recovered the gene expression levels of apical junctional complex proteins, which regulate gut permeability and barrier function (unpublished data). These results suggest that the anti-inflammatory property of aloesin is partly based on regulation of gut permeability, although human studies of clinical use are required.

Over the last few decades, phytochemical-based complementary and alternative treatments have emerged as relatively effective and safe therapeutic strategies for UC. Although a significant number of animal studies have established the potential beneficial effects on UC of phytochemicals and bioactive components of other natural sources, the exact mechanisms and/or molecular targets of their anti-inflammatory actions are not fully understood. Further studies are needed to evaluate their safety, targets, phytochemical-drug interactions, and pharmacokinetic information. Understanding these issues through well-designed clinical trials will enable us to choose reliable complementary and alternative therapies for patients with UC.

CONCLUSION

Because the increased prevalence of UC is potentially due to changes in dietary habits, especially in Eastern countries, it is now considered a lifestyle-related disease. It has been proposed that UC onset and progress are modulated by dietary factors such as excess energy intake, saturated fat intake, fatty acid ratio in the diet, and antioxidant intake. However, well-controlled human intervention trials are required to clarify the effect of nutritional factors on UC development. As understanding of the mechanisms involved in UC improves, a target-oriented search for compounds possessing therapeutic or complementary value becomes possible (Table 1). Nutritional modulation that aims to not only prevent the onset and relapse of the disease but also maintain remission will contribute to the successful management of UC.

REFERENCES

1 **Strober W**, Fuss IJ. Proinflammatory cytokines in the patho-

genesis of inflammatory bowel diseases. *Gastroenterology* 2011; **140**: 1756-1767 [PMID: 21530742 DOI: 10.1053/j.gastro.2011.02.016]
2 **Di Sabatino A**, Biancheri P, Rovedatti L, Macdonald TT, Corazza GR. Recent advances in understanding ulcerative colitis. *Intern Emerg Med* 2012; **7**: 103-111 [PMID: 22068230 DOI: 10.1007/s11739-011-0719-z]
3 **Prideaux L**, Kamm MA, De Cruz PP, Chan FK, Ng SC. Inflammatory bowel disease in Asia: a systematic review. *J Gastroenterol Hepatol* 2012; **27**: 1266-1280 [PMID: 22497584 DOI: 10.1111/j.1440-1746.2012.07150.x]
4 **Asakura K**, Nishiwaki Y, Inoue N, Hibi T, Watanabe M, Takebayashi T. Prevalence of ulcerative colitis and Crohn's disease in Japan. *J Gastroenterol* 2009; **44**: 659-665 [PMID: 19424654 DOI: 10.1007/s00535-009-0057-3]
5 **Yang SK**, Yun S, Kim JH, Park JY, Kim HY, Kim YH, Chang DK, Kim JS, Song IS, Park JB, Park ER, Kim KJ, Moon G, Yang SH. Epidemiology of inflammatory bowel disease in the Songpa-Kangdong district, Seoul, Korea, 1986-2005: a KASID study. *Inflamm Bowel Dis* 2008; **14**: 542-549 [PMID: 17941073 DOI: 10.1002/ibd.20310]
6 **Kappelman MD**, Rifas-Shiman SL, Kleinman K, Ollendorf D, Bousvaros A, Grand RJ, Finkelstein JA. The prevalence and geographic distribution of Crohn's disease and ulcerative colitis in the United States. *Clin Gastroenterol Hepatol* 2007; **5**: 1424-1429 [PMID: 17904915 DOI: 10.1016/j.cgh.2007.07.012]
7 **Cosnes J**, Gower-Rousseau C, Seksik P, Cortot A. Epidemiology and natural history of inflammatory bowel diseases. *Gastroenterology* 2011; **140**: 1785-1794 [PMID: 21530745 DOI: 10.1053/j.gastro.2011.01.055]
8 **Hou JK**, Abraham B, El-Serag H. Dietary intake and risk of developing inflammatory bowel disease: a systematic review of the literature. *Am J Gastroenterol* 2011; **106**: 563-573 [PMID: 21468064 DOI: 10.1038/ajg.2011.44]
9 **Park JB**, Yang SK, Byeon JS, Park ER, Moon G, Myung SJ, Park WK, Yoon SG, Kim HS, Lee JG, Kim JH, Il Min Y, Kim KY. Familial occurrence of inflammatory bowel disease in Korea. *Inflamm Bowel Dis* 2006; **12**: 1146-1151 [PMID: 17119389 DOI: 10.1097/01.mib.0000235094.01608.59]
10 **Lee JH**, Cheon JH, Moon CM, Park JJ, Hong SP, Kim TI, Kim WH. Do patients with ulcerative colitis diagnosed at a young age have more severe disease activity than patients diagnosed when older? *Digestion* 2010; **81**: 237-243 [PMID: 20110709 DOI: 10.1159/000253850]
11 **Herrinton LJ**, Liu L, Levin TR, Allison JE, Lewis JD, Velayos F. Incidence and mortality of colorectal adenocarcinoma in persons with inflammatory bowel disease from 1998 to 2010. *Gastroenterology* 2012; **143**: 382-389 [PMID: 22609382 DOI: 10.1053/j.gastro.2012.04.054]
12 **Sansonetti PJ**. War and peace at the intestinal epithelial surface: an integrated view of bacterial commensalism versus bacterial pathogenicity. *J Pediatr Gastroenterol Nutr* 2008; **46** Suppl 1: E6-E7 [PMID: 18354328 DOI: 10.1097/01.mpg.0000313819.96520.27]
13 **Cario E**. Toll-like receptors in inflammatory bowel diseases: a decade later. *Inflamm Bowel Dis* 2010; **16**: 1583-1597 [PMID: 20803699 DOI: 10.1002/ibd.21282]
14 **Srinivasan N**. Telling apart friend from foe: discriminating between commensals and pathogens at mucosal sites. *Innate Immun* 2010; **16**: 391-404 [PMID: 20083498 DOI: 10.1177/1753425909357577]
15 **World Health Organization.** Global health risks: mortality and burden of disease attributable to selected major risks. 2009. Available from: URL: http: //www.who.int/healthinfo/global_burden_disease/GlobalHealthRisks_report_full.pdf.
16 WHO Global Infobase. Available from: URL: http: //apps.who.int/infobase
17 **World Cancer Research Fund/American Institute for Cancer Research.** Food, Nutrition, Physical Activity and the Prevention of Cancer: a Global Perspective. 2007
18 **Valentini L**, Wirth EK, Schweizer U, Hengstermann S, Schaper L, Koernicke T, Dietz E, Norman K, Buning C, Winklhofer-Roob BM, Lochs H, Ockenga J. Circulating adipokines and the protective effects of hyperinsulinemia in inflammatory bowel disease. *Nutrition* 2009; **25**: 172-181 [PMID: 18849144 DOI: 10.1016/j.nut.2008.07.020]
19 **Konrad A**, Lehrke M, Schachinger V, Seibold F, Stark R, Ochsenkühn T, Parhofer KG, Göke B, Broedl UC. Resistin is an inflammatory marker of inflammatory bowel disease in humans. *Eur J Gastroenterol Hepatol* 2007; **19**: 1070-1074 [PMID: 17998831 DOI: 10.1097/MEG.0b013e3282f16251]
20 **Long MD**, Crandall WV, Leibowitz IH, Duffy L, del Rosario F, Kim SC, Integlia MJ, Berman J, Grunow J, Colletti RB, Schoen BT, Patel AS, Baron H, Israel E, Russell G, Ali S, Herfarth HH, Martin C, Kappelman MD. Prevalence and epidemiology of overweight and obesity in children with inflammatory bowel disease. *Inflamm Bowel Dis* 2011; **17**: 2162-2168 [PMID: 21910178 DOI: 10.1002/ibd.21585]
21 **Henriksen M**, Jahnsen J, Lygren I, Stray N, Sauar J, Vatn MH, Moum B. C-reactive protein: a predictive factor and marker of inflammation in inflammatory bowel disease. Results from a prospective population-based study. *Gut* 2008; **57**: 1518-1523 [PMID: 18566104 DOI: 10.1136/gut.2007.146357]
22 **Siegmund B**, Lehr HA, Fantuzzi G. Leptin: a pivotal mediator of intestinal inflammation in mice. *Gastroenterology* 2002; **122**: 2011-2025 [PMID: 12055606 DOI: 10.1053/gast.2002.33631]
23 **Nishihara T**, Matsuda M, Araki H, Oshima K, Kihara S, Funahashi T, Shimomura I. Effect of adiponectin on murine colitis induced by dextran sulfate sodium. *Gastroenterology* 2006; **131**: 853-861 [PMID: 16952554 DOI: 10.1053/j.gastro.2006.06.015]
24 **Teixeira LG**, Leonel AJ, Aguilar EC, Batista NV, Alves AC, Coimbra CC, Ferreira AV, de Faria AM, Cara DC, Alvarez Leite JI. The combination of high-fat diet-induced obesity and chronic ulcerative colitis reciprocally exacerbates adipose tissue and colon inflammation. *Lipids Health Dis* 2011; **10**: 204 [PMID: 22073943 DOI: 10.1186/1476-511X-10-204]
25 **Li H**, Lelliott C, Håkansson P, Ploj K, Tuneld A, Verolin-Johansson M, Benthem L, Carlsson B, Storlien L, Michaëlsson E. Intestinal, adipose, and liver inflammation in diet-induced obese mice. *Metabolism* 2008; **57**: 1704-1710 [PMID: 19013294 DOI: 10.1016/j.metabol.2008.07.029]
26 **Bäckhed F**. Programming of host metabolism by the gut microbiota. *Ann Nutr Metab* 2011; **58** Suppl 2: 44-52 [PMID: 21846980 DOI: 10.1159/000328042]
27 **Devkota S**, Wang Y, Musch MW, Leone V, Fehlner-Peach H, Nadimpalli A, Antonopoulos DA, Jabri B, Chang EB. Dietary-fat-induced taurocholic acid promotes pathobiont expansion and colitis in Il10-/- mice. *Nature* 2012; **487**: 104-108 [PMID: 22722865 DOI: 10.1038/nature11225]
28 **Lam YY**, Ha CW, Campbell CR, Mitchell AJ, Dinudom A, Oscarsson J, Cook DI, Hunt NH, Caterson ID, Holmes AJ, Storlien LH. Increased gut permeability and microbiota change associate with mesenteric fat inflammation and metabolic dysfunction in diet-induced obese mice. *PLoS One* 2012; **7**: e34233 [PMID: 22457829]
29 **Spor A**, Koren O, Ley R. Unravelling the effects of the environment and host genotype on the gut microbiome. *Nat Rev Microbiol* 2011; **9**: 279-290 [PMID: 21407244 DOI: 10.1038/nrmicro2540]
30 **Greiner T**, Bäckhed F. Effects of the gut microbiota on obesity and glucose homeostasis. *Trends Endocrinol Metab* 2011; **22**: 117-123 [PMID: 21353592 DOI: 10.1016/j.tem.2011.01.002]
31 **Turnbaugh PJ**, Ley RE, Mahowald MA, Magrini V, Mardis ER, Gordon JI. An obesity-associated gut microbiome with increased capacity for energy harvest. *Nature* 2006; **444**: 1027-1031 [PMID: 17183312 DOI: 10.1038/nature05414]
32 **Cabré E**, Mañosa M, Gassull MA. Omega-3 fatty acids and inflammatory bowel diseases - a systematic review. *Br J*

Nutr 2012; **107** Suppl 2: S240-S252 [PMID: 22591898 DOI: 10.1017/S0007114512001626]
33 **Hart AR**, Luben R, Olsen A, Tjonneland A, Linseisen J, Nagel G, Berglund G, Lindgren S, Grip O, Key T, Appleby P, Bergmann MM, Boeing H, Hallmans G, Danielsson A, Palmqvist R, Sjodin H, Hagglund G, Overvad K, Palli D, Masala G, Riboli E, Kennedy H, Welch A, Khaw KT, Day N, Bingham S. Diet in the aetiology of ulcerative colitis: a European prospective cohort study. *Digestion* 2008; **77**: 57-64 [PMID: 18349539 DOI: 10.1159/000121412]
34 **Tjonneland A**, Overvad K, Bergmann MM, Nagel G, Linseisen J, Hallmans G, Palmqvist R, Sjodin H, Hagglund G, Berglund G, Lindgren S, Grip O, Palli D, Day NE, Khaw KT, Bingham S, Riboli E, Kennedy H, Hart A. Linoleic acid, a dietary n-6 polyunsaturated fatty acid, and the aetiology of ulcerative colitis: a nested case-control study within a European prospective cohort study. *Gut* 2009; **58**: 1606-1611 [PMID: 19628674 DOI: 10.1136/gut.2008.169078]
35 **John S**, Luben R, Shrestha SS, Welch A, Khaw KT, Hart AR. Dietary n-3 polyunsaturated fatty acids and the aetiology of ulcerative colitis: a UK prospective cohort study. *Eur J Gastroenterol Hepatol* 2010; **22**: 602-606 [PMID: 20216220]
36 **Ueda Y**, Kawakami Y, Kunii D, Okada H, Azuma M, Le DS, Yamamoto S. Elevated concentrations of linoleic acid in erythrocyte membrane phospholipids in patients with inflammatory bowel disease. *Nutr Res* 2008; **28**: 239-244 [PMID: 19083414 DOI: 10.1016/j.nutres.2008.02.005]
37 **Cuzzocrea S**, Rossi A, Mazzon E, Di Paola R, Genovese T, Muià C, Caputi AP, Sautebin L. 5-Lipoxygenase modulates colitis through the regulation of adhesion molecule expression and neutrophil migration. *Lab Invest* 2005; **85**: 808-822 [PMID: 15821759 DOI: 10.1038/labinvest.3700276]
38 **Stanke-Labesque F**, Pofelski J, Moreau-Gaudry A, Bessard G, Bonaz B. Urinary leukotriene E4 excretion: a biomarker of inflammatory bowel disease activity. *Inflamm Bowel Dis* 2008; **14**: 769-774 [PMID: 18286646 DOI: 10.1002/ibd.20403]
39 **de Silva PS**, Olsen A, Christensen J, Schmidt EB, Overvaad K, Tjonneland A, Hart AR. An association between dietary arachidonic acid, measured in adipose tissue, and ulcerative colitis. *Gastroenterology* 2010; **139**: 1912-1917 [PMID: 20950616 DOI: 10.1053/j.gastro.2010.07.065]
40 **Li Q**, Zhang Q, Zhang M, Wang C, Zhu Z, Li N, Li J. Effect of n-3 polyunsaturated fatty acids on membrane microdomain localization of tight junction proteins in experimental colitis. *FEBS J* 2008; **275**: 411-420 [PMID: 18167140 DOI: 10.1111/j.1742-4658.2007.06210.x]
41 **Matsunaga H**, Hokari R, Kurihara C, Okada Y, Takebayashi K, Okudaira K, Watanabe C, Komoto S, Nakamura M, Tsuzuki Y, Kawaguchi A, Nagao S, Itoh K, Miura S. Omega-3 fatty acids exacerbate DSS-induced colitis through decreased adiponectin in colonic subepithelial myofibroblasts. *Inflamm Bowel Dis* 2008; **14**: 1348-1357 [PMID: 18484673 DOI: 10.1002/ibd.20491]
42 **Varnalidis I**, Ioannidis O, Karamanavi E, Ampas Z, Poutahidis T, Taitzoglou I, Paraskevas G, Botsios D. Omega 3 fatty acids supplementation has an ameliorative effect in experimental ulcerative colitis despite increased colonic neutrophil infiltration. *Rev Esp Enferm Dig* 2011; **103**: 511-518 [PMID: 22054266]
43 **Grimstad T**, Bjørndal B, Cacabelos D, Aasprong OG, Janssen EA, Omdal R, Svardal A, Hausken T, Bohov P, Portero-Otin M, Pamplona R, Berge RK. Dietary supplementation of krill oil attenuates inflammation and oxidative stress in experimental ulcerative colitis in rats. *Scand J Gastroenterol* 2012; **47**: 49-58 [PMID: 22126533 DOI: 10.3109/00365521.2011.634025]
44 **Maslowski KM**, Vieira AT, Ng A, Kranich J, Sierro F, Yu D, Schilter HC, Rolph MS, Mackay F, Artis D, Xavier RJ, Teixeira MM, Mackay CR. Regulation of inflammatory responses by gut microbiota and chemoattractant receptor GPR43. *Nature* 2009; **461**: 1282-1286 [PMID: 19865172 DOI: 10.1038/nature08530]
45 **Turner D**, Shah PS, Steinhart AH, Zlotkin S, Griffiths AM. Maintenance of remission in inflammatory bowel disease using omega-3 fatty acids (fish oil): a systematic review and meta-analyses. *Inflamm Bowel Dis* 2011; **17**: 336-345 [PMID: 20564531 DOI: 10.1002/ibd.21374]
46 **Uchiyama K**, Nakamura M, Odahara S, Koido S, Katahira K, Shiraishi H, Ohkusa T, Fujise K, Tajiri H. N-3 polyunsaturated fatty acid diet therapy for patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2010; **16**: 1696-1707 [PMID: 20222122 DOI: 10.1002/ibd.21251]
47 **Ioannidis O**, Varnalidis I, Paraskevas G, Botsios D. Nutritional modulation of the inflammatory bowel response. *Digestion* 2011; **84**: 89-101 [PMID: 21494040 DOI: 10.1159/000323456]
48 **Triantafillidis JK**, Merikas E, Georgopoulos F. Current and emerging drugs for the treatment of inflammatory bowel disease. *Drug Des Devel Ther* 2011; **5**: 185-210 [PMID: 21552489 DOI: 10.2147/DDDT.S11290]
49 **Khan MW**, Kale AA, Bere P, Vajjala S, Gounaris E, Pakanati KC. Microbes, intestinal inflammation and probiotics. *Expert Rev Gastroenterol Hepatol* 2012; **6**: 81-94 [PMID: 22149584 DOI: 10.1586/egh.11.94]
50 **Trebichavsky I**, Splichal I, Rada V, Splichalova A. Modulation of natural immunity in the gut by Escherichia coli strain Nissle 1917. *Nutr Rev* 2010; **68**: 459-464 [PMID: 20646223 DOI: 10.1111/j.1753-4887.2010.00305.x]
51 **Matthes H**, Krummenerl T, Giensch M, Wolff C, Schulze J. Clinical trial: probiotic treatment of acute distal ulcerative colitis with rectally administered Escherichia coli Nissle 1917 (EcN). *BMC Complement Altern Med* 2010; **10**: 13 [PMID: 20398311 DOI: 10.1186/1472-6882-10-13]
52 **Chapman TM**, Plosker GL, Figgitt DP. VSL3 probiotic mixture: a review of its use in chronic inflammatory bowel diseases. *Drugs* 2006; **66**: 1371-1387 [PMID: 16903771 DOI: 10.2165/00003495-200666100-00006]
53 **Sood A**, Midha V, Makharia GK, Ahuja V, Singal D, Goswami P, Tandon RK. The probiotic preparation, VSL3 induces remission in patients with mild-to-moderately active ulcerative colitis. *Clin Gastroenterol Hepatol* 2009; **7**: 1202-129, 1209.e1 [PMID: 19631292 DOI: 10.1016/j.cgh.2009.07.016]
54 **Furrie E**, Macfarlane S, Kennedy A, Cummings JH, Walsh SV, O'neil DA, Macfarlane GT. Synbiotic therapy (Bifidobacterium longum/Synergy 1) initiates resolution of inflammation in patients with active ulcerative colitis: a randomised controlled pilot trial. *Gut* 2005; **54**: 242-249 [PMID: 15647189 DOI: 10.1136/gut.2004.044834]
55 **Wildt S**, Nordgaard I, Hansen U, Brockmann E, Rumessen JJ. A randomised double-blind placebo-controlled trial with Lactobacillus acidophilus La-5 and Bifidobacterium animalis subsp. lactis BB-12 for maintenance of remission in ulcerative colitis. *J Crohns Colitis* 2011; **5**: 115-121 [PMID: 21453880 DOI: 10.1016/j.crohns.2010.11.004]
56 **Kruis W**, Schütz E, Fric P, Fixa B, Judmaier G, Stolte M. Double-blind comparison of an oral Escherichia coli preparation and mesalazine in maintaining remission of ulcerative colitis. *Aliment Pharmacol Ther* 1997; **11**: 853-858 [PMID: 9354192 DOI: 10.1046/j.1365-2036.1997.00225.x]
57 **Kruis W**, Fric P, Pokrotnieks J, Lukás M, Fixa B, Kascák M, Kamm MA, Weismueller J, Beglinger C, Stolte M, Wolff C, Schulze J. Maintaining remission of ulcerative colitis with the probiotic Escherichia coli Nissle 1917 is as effective as with standard mesalazine. *Gut* 2004; **53**: 1617-1623 [PMID: 15479682 DOI: 10.1136/gut.2003.037747]
58 **Rembacken BJ**, Snelling AM, Hawkey PM, Chalmers DM, Axon AT. Non-pathogenic Escherichia coli versus mesalazine for the treatment of ulcerative colitis: a randomised trial. *Lancet* 1999; **354**: 635-639 [PMID: 10466665 DOI: 10.1016/S0140-6736(98)06343-0]
59 **Miele E**, Pascarella F, Giannetti E, Quaglietta L, Baldassano

RN, Staiano A. Effect of a probiotic preparation (VSL3) on induction and maintenance of remission in children with ulcerative colitis. *Am J Gastroenterol* 2009; **104**: 437-443 [PMID: 19174792 DOI: 10.1038/ajg.2008.118]

60 **Tursi A**, Brandimarte G, Papa A, Giglio A, Elisei W, Giorgetti GM, Forti G, Morini S, Hassan C, Pistoia MA, Modeo ME, Rodino' S, D'Amico T, Sebkova L, Sacca' N, Di Giulio E, Luzza F, Imeneo M, Larussa T, Di Rosa S, Annese V, Danese S, Gasbarrini A. Treatment of relapsing mild-to-moderate ulcerative colitis with the probiotic VSL3 as adjunctive to a standard pharmaceutical treatment: a double-blind, randomized, placebo-controlled study. *Am J Gastroenterol* 2010; **105**: 2218-2227 [PMID: 20517305 DOI: 10.1038/ajg.2010.218]

61 **Nanau RM**, Neuman MG. Nutritional and probiotic supplementation in colitis models. *Dig Dis Sci* 2012; **57**: 2786-2810 [PMID: 22736018 DOI: 10.1007/s10620-012-2284-3]

62 **Wong CC**, Zhang L, Li ZJ, Wu WK, Ren SX, Chen YC, Ng TB, Cho CH. Protective effects of cathelicidin-encoding Lactococcus lactis in murine ulcerative colitis. *J Gastroenterol Hepatol* 2012; **27**: 1205-1212 [PMID: 22507188 DOI: 10.1111/j.1440-1746.2012.07158.x]

63 **Hudcovic T**, Kolinska J, Klepetar J, Stepankova R, Rezanka T, Srutkova D, Schwarzer M, Erban V, Du Z, Wells JM, Hrncir T, Tlaskalova-Hogenova H, Kozakova H. Protective effect of Clostridium tyrobutyricum in acute dextran sodium sulphate-induced colitis: differential regulation of tumour necrosis factor-α and interleukin-18 in BALB/c and severe combined immunodeficiency mice. *Clin Exp Immunol* 2012; **167**: 356-365 [PMID: 22236013 DOI: 10.1111/j.1365-2249.2011.04498.x]

64 **Atkins HL**, Geier MS, Prisciandaro LD, Pattanaik AK, Forder RE, Turner MS, Howarth GS. Effects of a Lactobacillus reuteri BR11 mutant deficient in the cystine-transport system in a rat model of inflammatory bowel disease. *Dig Dis Sci* 2012; **57**: 713-719 [PMID: 22038505 DOI: 10.1007/s10620-011-1943-0]

65 **Mariman R**, Kremer B, van Erk M, Lagerweij T, Koning F, Nagelkerken L. Gene expression profiling identifies mechanisms of protection to recurrent trinitrobenzene sulfonic acid colitis mediated by probiotics. *Inflamm Bowel Dis* 2012; **18**: 1424-1433 [PMID: 22162025 DOI: 10.1002/ibd.22849]

66 **Mencarelli A**, Distrutti E, Renga B, D'Amore C, Cipriani S, Palladino G, Donini A, Ricci P, Fiorucci S. Probiotics modulate intestinal expression of nuclear receptor and provide counter-regulatory signals to inflammation-driven adipose tissue activation. *PLoS One* 2011; **6**: e22978 [PMID: 21829567 DOI: 10.1371/journal.pone.0022978]

67 **Uronis JM**, Arthur JC, Keku T, Fodor A, Carroll IM, Cruz ML, Appleyard CB, Jobin C. Gut microbial diversity is reduced by the probiotic VSL3 and correlates with decreased TNBS-induced colitis. *Inflamm Bowel Dis* 2011; **17**: 289-297 [PMID: 20564535 DOI: 10.1002/ibd.21366]

68 **Chen HQ**, Yang J, Zhang M, Zhou YK, Shen TY, Chu ZX, Zhang M, Hang XM, Jiang YQ, Qin HL. Lactobacillus plantarum ameliorates colonic epithelial barrier dysfunction by modulating the apical junctional complex and PepT1 in IL-10 knockout mice. *Am J Physiol Gastrointest Liver Physiol* 2010; **299**: G1287-G1297 [PMID: 20884889 DOI: 10.1152/ajpgi.00196.2010]

69 **Chu ZX**, Chen HQ, Ma YL, Zhou YK, Zhang M, Zhang P, Qin HL. Lactobacillus plantarum prevents the upregulation of adhesion molecule expression in an experimental colitis model. *Dig Dis Sci* 2010; **55**: 2505-2513 [PMID: 19960256 DOI: 10.1007/s10620-009-1063-2]

70 **Ohkusa T**, Yoshida T, Sato N, Watanabe S, Tajiri H, Okayasu I. Commensal bacteria can enter colonic epithelial cells and induce proinflammatory cytokine secretion: a possible pathogenic mechanism of ulcerative colitis. *J Med Microbiol* 2009; **58**: 535-545 [PMID: 19369513 DOI: 10.1099/jmm.0.005801-0]

71 **Yeganegi M**, Leung CG, Martins A, Kim SO, Reid G, Challis JR, Bocking AD. Lactobacillus rhamnosus GR-1 stimulates colony-stimulating factor 3 (granulocyte) (CSF3) output in placental trophoblast cells in a fetal sex-dependent manner. *Biol Reprod* 2011; **84**: 18-25 [PMID: 20811016 DOI: 10.1095/biolreprod.110.085167]

72 **Matsumoto S**, Hara T, Hori T, Mitsuyama K, Nagaoka M, Tomiyasu N, Suzuki A, Sata M. Probiotic Lactobacillus-induced improvement in murine chronic inflammatory bowel disease is associated with the down-regulation of pro-inflammatory cytokines in lamina propria mononuclear cells. *Clin Exp Immunol* 2005; **140**: 417-426 [PMID: 15932502 DOI: 10.1111/j.1365-2249.2005.02790.x]

73 **Kim CH**, Kim HG, Kim JY, Kim NR, Jung BJ, Jeong JH, Chung DK. Probiotic genomic DNA reduces the production of pro-inflammatory cytokine tumor necrosis factor-alpha. *FEMS Microbiol Lett* 2012; **328**: 13-19 [PMID: 22126103 DOI: 10.1111/j.1574-6968.2011.02470.x]

74 **Thomas CM**, Versalovic J. Probiotics-host communication: Modulation of signaling pathways in the intestine. *Gut Microbes* 2011; **1**: 148-163 [PMID: 20672012 DOI: 10.4161/gmic.1.3.11712]

75 **Niess JH**, Reinecker HC. Dendritic cells in the recognition of intestinal microbiota. *Cell Microbiol* 2006; **8**: 558-564 [PMID: 16548882 DOI: 10.1111/j.1462-5822.2006.00694.x]

76 **Hespel C**, Moser M. Role of inflammatory dendritic cells in innate and adaptive immunity. *Eur J Immunol* 2012; **42**: 2535-2543 [PMID: 23042650 DOI: 10.1002/eji.201242480]

77 **Kayama H**, Takeda K. Regulation of intestinal homeostasis by innate and adaptive immunity. *Int Immunol* 2012; **24**: 673-680 [PMID: 22962437 DOI: 10.1093/intimm/dxs094]

78 **Bassaganya-Riera J**, Viladomiu M, Pedragosa M, De Simone C, Hontecillas R. Immunoregulatory mechanisms underlying prevention of colitis-associated colorectal cancer by probiotic bacteria. *PLoS One* 2012; **7**: e34676 [PMID: 22511958 DOI: 10.1371/journal.pone.0034676]

79 **Ng SC**, Plamondon S, Kamm MA, Hart AL, Al-Hassi HO, Guenther T, Stagg AJ, Knight SC. Immunosuppressive effects via human intestinal dendritic cells of probiotic bacteria and steroids in the treatment of acute ulcerative colitis. *Inflamm Bowel Dis* 2010; **16**: 1286-1298 [PMID: 20155842 DOI: 10.1002/ibd.21222]

80 **Pronio A**, Montesani C, Butteroni C, Vecchione S, Mumolo G, Vestri A, Vitolo D, Boirivant M. Probiotic administration in patients with ileal pouch-anal anastomosis for ulcerative colitis is associated with expansion of mucosal regulatory cells. *Inflamm Bowel Dis* 2008; **14**: 662-668 [PMID: 18240282 DOI: 10.1002/ibd.20369]

81 **Braat H**, de Jong EC, van den Brande JM, Kapsenberg ML, Peppelenbosch MP, van Tol EA, van Deventer SJ. Dichotomy between Lactobacillus rhamnosus and Klebsiella pneumoniae on dendritic cell phenotype and function. *J Mol Med* (Berl) 2004; **82**: 197-205 [PMID: 14673529 DOI: 10.1007/s00109-003-0509-9]

82 **Rodrigues DM**, Sousa AJ, Johnson-Henry KC, Sherman PM, Gareau MG. Probiotics are effective for the prevention and treatment of Citrobacter rodentium-induced colitis in mice. *J Infect Dis* 2012; **206**: 99-109 [PMID: 22430833 DOI: 10.1093/infdis/jis177]

83 **Hoermannsperger G**, Clavel T, Hoffmann M, Reiff C, Kelly D, Loh G, Blaut M, Hölzlwimmer G, Laschinger M, Haller D. Post-translational inhibition of IP-10 secretion in IEC by probiotic bacteria: impact on chronic inflammation. *PLoS One* 2009; **4**: e4365 [PMID: 19197385 DOI: 10.1371/journal.pone.0004365]

84 **Zakostelska Z**, Kverka M, Klimesova K, Rossmann P, Mrazek J, Kopecny J, Hornova M, Srutkova D, Hudcovic T, Ridl J, Tlaskalova-Hogenova H. Lysate of probiotic Lactobacillus casei DN-114 001 ameliorates colitis by strengthening the gut barrier function and changing the gut microenvi-

ronment. *PLoS One* 2011; **6**: e27961 [PMID: 22132181 DOI: 10.1371/journal.pone.0027961]
85 **Macho Fernandez E**, Valenti V, Rockel C, Hermann C, Pot B, Boneca IG, Grangette C. Anti-inflammatory capacity of selected lactobacilli in experimental colitis is driven by NOD2-mediated recognition of a specific peptidoglycan-derived muropeptide. *Gut* 2011; **60**: 1050-1059 [PMID: 21471573 DOI: 10.1136/gut.2010.232918]
86 **Thomas S,** Przesdzing I, Metzke D, Schmitz J, Radbruch A, Baumgart DC. Saccharomyces boulardii inhibits lipopolysaccharide-induced activation of human dendritic cells and T cell proliferation. *Clin Exp Immunol* 2009; **156:** 78-87 [PMID: 19161443 DOI: 10.1111/j.1365-2249.2009.03878.x]
87 **Jena G**, Trivedi PP, Sandala B. Oxidative stress in ulcerative colitis: an old concept but a new concern. *Free Radic Res* 2012; **46**: 1339-1345 [PMID: 22856328 DOI: 10.3109/10715762.2012.717692]
88 **Kim YJ**, Kim EH, Hahm KB. Oxidative stress in inflammation-based gastrointestinal tract diseases: challenges and opportunities. *J Gastroenterol Hepatol* 2012; **27**: 1004-1010 [PMID: 22413852 DOI: 10.1111/j.1440-1746.2012.07108.x]
89 **Zhu H**, Li YR. Oxidative stress and redox signaling mechanisms of inflammatory bowel disease: updated experimental and clinical evidence. *Exp Biol Med* (Maywood) 2012; **237**: 474-480 [PMID: 22442342 DOI: 10.1258/ebm.2011.011358]
90 **Yasukawa K**, Tokuda H, Tun X, Utsumi H, Yamada K. The detrimental effect of nitric oxide on tissue is associated with inflammatory events in the vascular endothelium and neutrophils in mice with dextran sodium sulfate-induced colitis. *Free Radic Res* 2012; **46**: 1427-1436 [PMID: 22998024 DOI: 10.3109/10715762.2012.732698]
91 **Razack R**, Seidner DL. Nutrition in inflammatory bowel disease. *Curr Opin Gastroenterol* 2007; **23**: 400-405 [PMID: 17545776 DOI: 10.1097/MOG.0b013e3281ddb2a3]
92 **Marx G**, Seidman EG. Inflammatory bowel disease in pediatric patients. *Curr Opin Gastroenterol* 1999; **15**: 322-325 [PMID: 17023965 DOI: 10.1097/00001574-199907000-00008]
93 **Seidner DL**, Lashner BA, Brzezinski A, Banks PL, Goldblum J, Fiocchi C, Katz J, Lichtenstein GR, Anton PA, Kam LY, Garleb KA, Demichele SJ. An oral supplement enriched with fish oil, soluble fiber, and antioxidants for corticosteroid sparing in ulcerative colitis: a randomized, controlled trial. *Clin Gastroenterol Hepatol* 2005; **3**: 358-369 [PMID: 15822041 DOI: 10.1016/S1542-3565(04)00672-X]
94 **Mirbagheri SA**, Nezami BG, Assa S, Hajimahmoodi M. Rectal administration of d-alpha tocopherol for active ulcerative colitis: a preliminary report. *World J Gastroenterol* 2008; **14**: 5990-5995 [PMID: 18932276 DOI: 10.3748/wjg.14.5990]
95 **Holt PR**, Katz S, Kirshoff R. Curcumin therapy in inflammatory bowel disease: a pilot study. *Dig Dis Sci* 2005; **50**: 2191-2193 [PMID: 16240238 DOI: 10.1007/s10620-005-3032-8]
96 **Hanai H**, Iida T, Takeuchi K, Watanabe F, Maruyama Y, Andoh A, Tsujikawa T, Fujiyama Y, Mitsuyama K, Sata M, Yamada M, Iwaoka Y, Kanke K, Hiraishi H, Hirayama K, Arai H, Yoshii S, Uchijima M, Nagata T, Koide Y. Curcumin maintenance therapy for ulcerative colitis: randomized, multicenter, double-blind, placebo-controlled trial. *Clin Gastroenterol Hepatol* 2006; **4**: 1502-1506 [PMID: 17101300 DOI: 10.1016/j.cgh.2006.08.008]
97 **Singh UP**, Singh NP, Busbee B, Guan H, Singh B, Price RL, Taub DD, Mishra MK, Nagarkatti M, Nagarkatti PS. Alternative medicines as emerging therapies for inflammatory bowel diseases. *Int Rev Immunol* 2012; **31**: 66-84 [PMID: 22251008 DOI: 10.3109/08830185.2011.642909]
98 **Büning C**, Geissler N, Prager M, Sturm A, Baumgart DC, Büttner J, Bühner S, Haas V, Lochs H. Increased small intestinal permeability in ulcerative colitis: rather genetic than environmental and a risk factor for extensive disease? *Inflamm Bowel Dis* 2012; **18**: 1932-1939 [PMID: 22344959 DOI: 10.1002/ibd.22909]
99 **Toedter G**, Li K, Sague S, Ma K, Marano C, Macoritto M, Park J, Deehan R, Matthews A, Wu GD, Lewis JD, Arijs I, Rutgeerts P, Baribaud F. Genes associated with intestinal permeability in ulcerative colitis: changes in expression following infliximab therapy. *Inflamm Bowel Dis* 2012; **18**: 1399-1410 [PMID: 22223479 DOI: 10.1002/ibd.22853]
100 **Park MY**, Kwon HJ, Sung MK. Dietary aloin, aloesin, or aloe-gel exerts anti-inflammatory activity in a rat colitis model. *Life Sci* 2011; **88**: 486-492 [PMID: 21277867 DOI: 10.1016/j.lfs.2011.01.010]

P- Reviewers Meyer N, Yamakawa M, Odes S
S- Editor Song XX **L- Editor** Cant MR **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i7.1005

World J Gastroenterol 2013 February 21; 19(7): 1005-1010
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

TOPIC HIGHLIGHT

Jung Eun Lee, ScD, Assistant Professor, *Series Editor*

Vitamin B6 and colorectal cancer: Current evidence and future directions

Xue-Hong Zhang, Jing Ma, Stephanie A Smith-Warner, Jung Eun Lee, Edward Giovannucci

Xue-Hong Zhang, Jing Ma, Edward Giovannucci, Channing Division of Network Medicine, Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA 02115, United States
Stephanie A Smith-Warner, Edward Giovannucci, Department of Nutrition, and Department of Epidemiology, Harvard School of Public Health, Boston, MA 02115, United States
Jung Eun Lee, Department of Food and Nutrition, Sookmyung Women's University, Seoul 140742, South Korea
Author contributions: All authors contributed to conception of the study; ZhangXH drafted the article; Ma J, Smith-Warner SA, Lee JE and Giovannucci E revised it critically for important intellectual content; all authors approved the final version of this article.
Correspondence to: Edward Giovannucci, MD, Professor of Nutrition and Epidemiology, Department of Epidemiology, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02115, United States. egiovann@hsph.harvard.edu
Telephone: +1-617-4324648 Fax: +1-617-4322435
Received: September 19, 2012 Revised: December 25, 2012
Accepted: January 11, 2013
Published online: February 21, 2013

Abstract

Colorectal cancer remains the third most common cancer in both women and men worldwide. Identifying modifiable dietary factors is crucial in developing primary prevention strategies. Vitamin B6 is involved in more than 100 coenzyme reactions, and may influence colorectal cancer risk in multiple ways including through its role in one-carbon metabolism related DNA synthesis and methylation and by reducing inflammation, cell proliferation, and oxidative stress. Observational studies of dietary or dietary plus supplementary intake of vitamin B6 and colorectal cancer risk have been inconsistent with most studies reporting non-significant positive or inverse associations. However, published studies of plasma pyridoxal 5'-phosphate (the active form of vitamin B6) levels consistently support an approximately 30%-50% reduction in risk of colorectal cancer comparing high with low concentrations. The reasons for the discrepancy in the results between dietary-based and plasma-based studies remain unresolved. Other unresolved questions include the effects of vitamin B6 intake in early life (*i.e.*, childhood or adolescence) and of suboptimal vitamin B6 status on colorectal cancer risk, whether the associations with vitamin B6 differ across molecular subtypes of colorectal cancer, and whether the vitamin B6-colorectal cancer association is modified by genetic variants of one-carbon metabolism.

© 2013 Baishideng. All rights reserved.

Key words: Vitamin B6; Plasma pyridoxal 5'-phosphate; Colorectal cancer; Adenoma; Incidence; Case-control study; Cohort study; Randomized controlled trial; Epidemiology

Zhang XH, Ma J, Smith-Warner SA, Lee JE, Giovannucci E. Vitamin B6 and colorectal cancer: Current evidence and future directions. *World J Gastroenterol* 2013; 19(7): 1005-1010 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i7/1005.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i7.1005

INTRODUCTION

Colorectal cancer is the third most common cancer worldwide[1]. In the United States, colorectal cancer will account for approximately 143 460 new cases in 2012[2]. The wide variation in age-adjusted incidence rates for colorectal cancer between countries suggests a role of environmental factors such as diet in colorectal carcinogenesis. However, as summarized in several recent reviews[3-5], the effect of dietary factors on colorectal cancer remains largely inconclusive. For example, based on a comprehensive review of published studies, the 2011 World Cancer Research Foundation and American Institute for Cancer Research report on diet and colorectal

cancer identified that there was convincing evidence only for red meat, processed meat, and alcoholic drinks (in men) as risk factors and for dietary fiber as a protective factor for colorectal cancer[3]. The effect of other dietary factors such as vitamin B6 on colorectal cancer remains to be elucidated. Vitamin B6, a one-carbon metabolism related nutrient, may have a potential role in colorectal carcinogenesis. The current epidemiologic studies examining vitamin B6 and risk of sporadic colorectal cancer and adenomas are briefly summarized here. Although the possible effect of vitamin B6 may differ by molecular subtype of colorectal cancer or be mediated by genetic variants in one-carbon metabolism, few data are available to date[6-11] and thus are not reviewed here.

Vitamin B6 is a water-soluble vitamin that participates in more than 100 coenzyme reactions involved in the metabolism of protein, carbohydrates, and lipids[12]. In the United States, the major food sources for vitamin B6 include fortified cereals, starchy vegetables, beef, and poultry[12]. The recommended daily allowance (RDA) for vitamin B6 intake is 1.7 mg/d for men and 1.5 mg/d for women aged 51 years or older[12] although some subgroups of the population including smokers, blacks, seniors, and current and former oral contraceptive users require higher intakes[13]. Plasma pyridoxal 5'-phosphate (PLP) is the active form of vitamin B6 and is most commonly used to measure vitamin B6 status. A PLP level of more than 20 nmol/L is an indicator of adequate vitamin B6 status in adults[12]. The 2003-2006 national health and nutrition examination survey found that 11% of vitamin B6 supplement users (approximately 20%-30% of United States adults[14]) and 24% of people in the United States who do not take supplements containing vitamin B6 have suboptimal plasma PLP concentrations (< 20 nmol/L)[13].

VITAMIN B6 AND POTENTIAL MECHANISMS RELATED TO COLORECTAL CARCINOGENESIS

Vitamin B6 may influence colorectal carcinogenesis through its role in DNA synthesis and methylation[15], both of which are potentially involved in colorectal carcinogenesis. In addition, animal models have demonstrated that supplemental vitamin B6 suppressed cell proliferation and reduced the number of tumors in the colon[16,17]. Moreover, vitamin B6 has been shown to inhibit angiogenesis[18], suppress nitric oxide[16], and reduce oxidative stress[19], all of which are associated with preventing carcinogenesis. Further, low vitamin B6 status may link to chronic inflammation[20], a potential risk factor for colorectal cancer[21], based on significantly lower PLP concentrations observed among patients with inflammatory bowel diseases[22] and rheumatoid arthritis[23,24] compared to generally healthy populations. Other evidence of the link between vitamin B6 and inflammation includes the inverse relation of plasma PLP with cardiovascular disease[25,26], C-reactive protein[23,27], and tumor necrosis factor alpha[24].

OBSERVATIONAL STUDIES OF VITAMIN B6 INTAKE AND COLORECTAL CANCER RISK

Despite potential mechanisms supporting the hypothesis that vitamin B6 may reduce colorectal cancer risk, epidemiologic evidence examining vitamin B6 intake and colorectal cancer risk has been inconclusive. At least 9 case-control studies have examined the relation between vitamin B6 intake and colorectal cancer risk. The majority of the case-control studies reported a modest significant inverse association for comparisons of the highest with the lowest vitamin B6 intake categories. For example, a quantitative review of six case-control studies published in 2008 reported a summary multivariable relative risk (RR) of 0.67 (95%CI: 0.60-0.75) comparing high with low vitamin B6 intake[28]. However, there was borderline significant heterogeneity among these case-control studies (*P* value for heterogeneity = 0.09) with risk estimates ranging from 0.51 to 1.00. In addition, a meta-analysis of nine cohort studies that included eleven risk estimates (3 studies analyzed men and women separately) found no substantial effect of vitamin B6 intake on colorectal cancer risk (high *vs* low intake categories, summary RR = 0.90, 95%CI: 0.75-1.07). However, there was statistically significant heterogeneity in the results from the cohort studies (*P* value for heterogeneity = 0.01) with 6 cohort studies reporting 18%-39% lower risks of colorectal cancer comparing the highest *vs* lowest categories (the associations in three studies were statistically significant) and 5 studies reporting nonsignificant positive associations[29]. In the meta-analysis, only two cohort studies had evaluated associations with total vitamin B6 intake and a nonsignificant association was observed (summary RR = 0.90, 95%CI: 0.73-1.11)[29]. The only subsequently published study of two cohorts examined potential latency effects of vitamin B6 intake on colorectal cancer risk and found no difference for intakes measured 0-4 years before diagnosis compared to intakes measured 12-16 years before diagnosis[30]. Of note, the study populations in these studies were relatively well nourished, with a low prevalence (*i.e.*, 5%-10%) of individuals below the RDA levels of vitamin B6 intake, limiting the ability to test the potential effect of suboptimal vitamin B6 status on colorectal cancer risk.

NESTED CASE-CONTROL STUDIES OF PLASMA PLP CONCENTRATIONS AND COLORECTAL CANCER RISK

Four out of five nested case-control studies conducted to date have shown that higher pre-diagnostic plasma PLP concentrations were statistically significantly associated with a 30%-50% lower risk of colorectal cancer[29]. The first study was from the United States (*n* = 188 cases, highest *vs* lowest quartile, RR = 0.48, 95%CI: 0.25-0.92, *P* value for trend = 0.03)[31]. The second study conducted

in Finland found that men in the highest quartile of PLP concentrations had non-significant lower risk of colorectal cancer (n = 275 cases, RR = 0.61, 95%CI: 0.32-1.14, P value for trend = 0.08)[32]. Similar magnitudes of inverse associations were also observed in subsequent analyses using data from the Multiethnic Cohort study (n = 223 cases, RR = 0.52, 95%CI: 0.29-0.92, P value for trend = 0.03)[33] and the Physicians' Heath Study (n = 197 cases, RR = 0.49, 95%CI: 0.26-0.92, P value for trend = 0.01)[34]. Likewise, in the largest and most recent analysis to date (n = 1365 cases), the RR comparing the highest to lowest quintile was 0.68 (95%CI: 0.53-0.87, P value for trend < 0.001) in the European Prospective Investigation into Cancer and Nutrition cohort[9]. As shown in the meta-analysis of these studies[29], the pooled RR of colorectal cancer for the highest *vs* lowest categories of PLP levels was 0.52 (95%CI: 0.38-0.71).

CLINICAL TRIALS OF VITAMIN B6 SUPPLEMENT AND COLORECTAL CANCER

The effect of treatment with vitamin B6 supplements (40 mg/d) on colorectal cancer incidence and mortality has been evaluated, to the best of our knowledge, in only two randomized double-blind, placebo-controlled trials, the Norwegian Vitamin Trial[35] and the Western Norway B Vitamin Intervention Trial[36]. These studies were not primarily designed to examine cancer outcomes and included 6837 participants with ischemic heart disease after a median of 39 mo of treatment and an additional 38 mo of post-trial observational follow-up[37]. In both trials, participants were randomized into one of four groups: (1) folic acid (0.8 mg/d), vitamin B12 (0.4 mg/d), and vitamin B6 (40 mg/d); (2) folic acid (0.8 mg/d) and vitamin B12 (0.4 mg/d); (3) vitamin B6 alone (40 mg/d); or (4) placebo. The pooled analysis of data from these two trials showed no benefit of vitamin B6 supplementation on incident colorectal cancer or fatal colorectal cancer. Of note, only a limited number of colorectal cancer cases and deaths were included in the analysis. A total of 26 participants (1.5%) who received vitamin B6 and 22 (1.3%) participants in the placebo group were diagnosed with incident colorectal cancer during the trial (vitamin B6 *vs* non-vitamin B6 group, RR = 1.18, 95%CI: 0.69-2.00)[37]. Furthermore, there were only 5 deaths due to colorectal cancer in the vitamin B6 group and 7 in the placebo group (vitamin B6 *vs* non-vitamin B6 group, RR = 0.51, 95%CI: 0.17-1.55)[37].

VITAMIN B6 INTAKE, PLASMA PLP CONCENTRATIONS AND COLORECTAL ADENOMAS

The evidence for the association between vitamin B6 intake or plasma PLP concentrations and risk of colorectal adenoma, precursors of colorectal cancer, is less consistent than observed for colorectal cancer, with only a small number of studies published[10,11,31,38]. Specifically, the first study found a suggestive inverse association between plasma PLP concentration and advanced (≥ 1 cm in size, or villous or tubulovillous) distal colorectal adenoma (n = 408 cases, RR = 0.65, 95%CI: 0.37-1.11, P value for trend = 0.08), but a weaker association with low risk of (small and tubulous) adenoma (n = 210 cases, RR = 0.85, 95%CI: 0.52-1.38, P value for trend = 0.52)[31]. The other cohort study showed that high plasma levels of PLP were inversely associated with risk of colorectal adenoma (n = 210 cases, highest *vs* lowest tertile, RR = 0.44, 95%CI: 0.26-0.74, P value for trend = 0.002)[10]. Among 2 studies that evaluated the effect of vitamin B6 and adenoma recurrence, the first study from the Wheat Bran Fiber intervention trial showed a lower odds of adenoma recurrence for higher vitamin B6 intake (n = 495 recurrences, highest *vs* lowest quartile, OR = 0.65, 95%CI: 0.45-0.94, P value for trend = 0.03)[38]. The Aspirin/Folate Polyp Prevention Study, a trial of folic acid supplementation, found a borderline significant inverse association with plasma PLP concentrations and risk of adenoma recurrence (n = 430 recurrences, highest *vs* lowest quartile, RR = 0.78, 95%CI: 0.61-1.00, P value for trend = 0.08)[11].

DISCUSSION

Overall, based on a meta-analysis of nine cohort studies a substantial effect of vitamin B6 intake in adulthood and colorectal cancer risk was not evident although the study-specific results were inconsistent. In contrast, all five studies of circulating PLP levels found that participants with higher plasma PLP levels had a 30%-50% lower risk of colorectal cancer with the associations being statistically significant in four of the studies. Of note, only two nested case-control studies[31,33] have examined associations with both vitamin B6 intake and PLP levels and colorectal cancer risk and in these two studies inverse associations of similar magnitude were observed for vitamin B6 intake and plasma PLP concentrations and colorectal cancer risk. Several issues related to examining associations between vitamin B6 and colorectal cancer risk are discussed below.

Confounding by other factors?

Vitamin B6 intake is an important determinant of PLP levels[39]. However, individuals with high vitamin B6 intake tend to have healthy behaviors such as higher physical activity, less smoking, and higher intakes of folate, calcium, and vitamin D compared to individuals with lower vitamin B6 intake[30,40-42]. Because being physically active, not smoking, and having higher intakes of folate, calcium and vitamin D may reduce the risk of colorectal cancer[3,5,43,44], the inverse associations observed with higher PLP concentrations might be simply due to the correlations between vitamin B6 intake and these healthy behaviors. Although previous studies have adjusted for these potential confounding factors[29,30], residual confounding may still exist. Further, it is challenging to tease

out the independent effect of vitamin B6 from certain other nutrients such as folate, calcium, and vitamin D given that their intakes are positively correlated, particularly for intakes from food and supplemental sources combined. In addition, variation in plasma PLP could possibly reflect metabolic states such as chronic inflammation[22-24,27,45], a risk factor for colorectal cancer[21]. Studies have shown that patients with inflammatory bowel diseases[22] and rheumatoid arthritis[23,24] have significantly lower plasma PLP concentrations than healthy individuals. Although the data are not in full agreement[46], plasma PLP concentrations also have been found to be inversely correlated with C-reactive protein levels[23,27], and tumor necrosis factor alpha levels[24], both of which are markers of inflammation and possible risk factors for colorectal cancer[47]. Possibly, lower PLP levels could reflect a patho-physiologic state such as inflammation, which may be associated with higher risk of colorectal cancer, but increasing plasma PLP concentrations through increased intake may not necessarily lead to lower colorectal cancer risk. However, one study found that the inverse association with higher plasma PLP concentrations did not change even after adjustment for plasma concentrations of homocysteine, C-reactive protein, tumor necrosis factor alpha, and interleukin-6[34]. Nonetheless, it is unclear whether plasma PLP levels *per se* or the healthy behaviors or physiologic states associated with plasma PLP levels conferred the benefits observed in the studies of PLP concentrations and colorectal cancer risk.

Measurement error in assessment of vitamin B6?

Measurement error may have occurred in estimated vitamin B6 intake assessed using food frequency questionnaires (FFQs). However, the relatively high correlations (ranged from 0.4 to 0.8) observed between vitamin B6 intake assessed by FFQs and intake assessed using a reference methods (*i.e.*, dietary records)[6,30,40-42,48,49] reduce the possibility of missing a strong association between vitamin B6 intake and colorectal cancer risk in cohort studies. Moreover, total vitamin B6 intake (from food and supplemental sources combined) appears to be a good predictor of plasma PLP concentrations. For example, among representative random samples from the Nurses' Health Study (n = 381 women) and the Health Professionals Follow-up Study (n = 345 men) who had provided blood samples and served as controls in a nested case-control study of colorectal cancer[30], an approximately 3-fold difference was observed in mean plasma PLP concentrations comparing the top *vs* bottom quintiles of total vitamin B6 intake. Specifically, for the top and bottom quintile categories of total vitamin B6 intake, the mean plasma PLP concentrations were 98.3 nmol/L and 38.9 nmol/L in women and 183.2 nmol/L and 66.0 nmol/L in men. In addition, the Spearman correlation coefficients between total vitamin B6 intake and plasma PLP concentrations were 0.52 in women and 0.54 in men[30]. Thus, the ability of FFQs to predict an almost 3-fold difference in plasma PLP levels argues against measurement error in vitamin B6 intake masking detection of a strong association between vitamin B6 intake and colorectal cancer risk. Misclassification of vitamin B6 status also may occur in studies of PLP levels and colorectal cancer risk given that the studies published to date have only used one blood sample to measure PLP levels which may not reflect long term vitamin B6 status.

Interaction with other factors?

Inconsistent results might also result from differences in distributions of potential effect modifiers of the association between vitamin B6 and colorectal cancer risk. Given that alcohol consumption may decrease vitamin B6 levels[50,51], any effect of vitamin B6 on colorectal cancer risk might therefore be stronger among heavy drinkers. To date, results have been conflicting among the six studies we identified. A non-significant interaction between vitamin B6 and total alcohol consumption including wine, beer, spirits was observed in three studies of vitamin B6 intakes[30,52] and one study of plasma PLP levels[31]. In contrast, a stronger inverse association with plasma PLP concentrations[9] or vitamin B6 intake[41] has been reported among alcohol drinkers compared to nondrinkers. In addition, if vitamin B6 influences the development of colorectal cancer through the one-carbon metabolism pathway, the potential benefit of vitamin B6 might be stronger among individuals with low intake of other one-carbon metabolism related nutrients such as folate, riboflavin, methionine, and vitamin B12. However, current studies are limited and found no evident pattern[30,31]. With respect to adenomas, studies are limited and results have also been mixed. Results from the Multiethnic Cohort Study showed a non-significant interaction between plasma PLP and alcohol consumption[10] and the Aspirin/Folate Polyp Prevention Study found that the inverse association with plasma PLP was evident only among nondrinkers (P value for interaction = 0.03)[11].

Timing of intake is important?

Because most cohort studies conducted to date have only a single assessment of vitamin B6 intake[29,30], it is uncertain when in the natural history vitamin B6 intake may influence colorectal cancer risk. However, a recent study that specifically evaluated the timing using time lagged analyses found no clear pattern[30]. In contrast, when potential latency effects of total folate intake (another one-carbon metabolism related nutrient) were examined, only total folate intake measured 12-16 years prior to diagnosis of colorectal cancer was significantly associated with a lower risk of colorectal cancer, consistent with the only prior study examining the timing of folate intake on colorectal cancer risk[53]. When total folate and total vitamin B6 intakes measured 12-16 years prior to diagnosis were simultaneously included in the multivariate model, a statistically significant inverse association continued to be observed for total folate intake while a weak, nonsignificant association was observed for total vitamin B6 intake. However, it is unknown whether vitamin B6 intake in childhood, adolescence, or early adulthood might be important in colorectal carcinogenesis because dietary

habits experienced early in development may play an important role in adult disease[54,55]. The previous observational studies that we identified have focused on vitamin B6 intake in adulthood[30].

CONCLUSION

In contrast to the inverse associations observed in most studies of plasma PLP concentrations, the results from cohort studies of vitamin B6 intake in adulthood and colorectal cancer risk in relatively nourished populations have been inconsistent. The reason for this discrepancy between dietary-based and plasma-based studies remains unresolved and a better understanding is needed of the determinants of plasma PLP concentrations. Future studies should focus on early life intake (*i.e.*, childhood or adolescence), the effects of suboptimal vitamin B6 status, molecular subtypes of colorectal cancer, and effect modification by genetic variants of one-carbon metabolism.

REFERENCES

1 **Ferlay J**, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; **127**: 2893-2917 [PMID: 21351269 DOI: 10.1002/ijc.25516]

2 **Siegel R**, Naishadham D, Jemal A. Cancer statistics, 2012. *CA Cancer J Clin* 2012; **62**: 10-29 [PMID: 22237781 DOI: 10.3322/caac.20138]

3 **World Cancer Research Fund,** American Institute for Cancer Research. Continuous Update Project Report Summary: Food, Nutrition, Physical Activity, and the Prevention of Colorectal Cancer. 2011

4 **World Cancer Research Fund,** American Institute for Cancer Research Expert Panel. Food, Nutrition, Physical Activity, and the Prevention of Cancer: a Global Perspective. 2007

5 **Chan AT**, Giovannucci EL. Primary prevention of colorectal cancer. *Gastroenterology* 2010; **138**: 2029-2043.e10 [PMID: 20420944]

6 **Schernhammer ES**, Giovannuccci E, Fuchs CS, Ogino S. A prospective study of dietary folate and vitamin B and colon cancer according to microsatellite instability and KRAS mutational status. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 2895-2898 [PMID: 18843035 DOI: 10.1158/1055-9965.EPI-08-0638]

7 **Schernhammer ES**, Giovannucci E, Kawasaki T, Rosner B, Fuchs CS, Ogino S. Dietary folate, alcohol and B vitamins in relation to LINE-1 hypomethylation in colon cancer. *Gut* 2010; **59**: 794-799 [PMID: 19828464 DOI: 10.1136/gut.2009.183707]

8 **Schernhammer ES**, Ogino S, Fuchs CS. Folate and vitamin B6 intake and risk of colon cancer in relation to p53 expression. *Gastroenterology* 2008; **135**: 770-780 [PMID: 18619459 DOI: 10.1053/j.gastro.2008.06.033]

9 **Eussen SJ**, Vollset SE, Hustad S, Midttun Ø, Meyer K, Fredriksen A, Ueland PM, Jenab M, Slimani N, Boffetta P, Overvad K, Thorlacius-Ussing O, Tjønneland A, Olsen A, Clavel-Chapelon F, Boutron-Ruault MC, Morois S, Weikert C, Pischon T, Linseisen J, Kaaks R, Trichopoulou A, Zilis D, Katsoulis M, Palli D, Pala V, Vineis P, Tumino R, Panico S, Peeters PH, Bueno-de-Mesquita HB, van Duijnhoven FJ, Skeie G, Muñoz X, Martínez C, Dorronsoro M, Ardanaz E, Navarro C, Rodríguez L, VanGuelpen B, Palmqvist R, Manjer J, Ericson U, Bingham S, Khaw KT, Norat T, Riboli E. Plasma vitamins B2, B6, and B12, and related genetic variants as predictors of colorectal cancer risk. *Cancer Epidemiol Biomarkers Prev* 2010; **19**: 2549-2561 [PMID: 20813848 DOI: 10.1158/1055-9965.epi-10-0407]

10 **Le Marchand L**, Wang H, Selhub J, Vogt TM, Yokochi L, Decker R. Association of plasma vitamin B6 with risk of colorectal adenoma in a multiethnic case-control study. *Cancer Causes Control* 2011; **22**: 929-936 [PMID: 21437631 DOI: 10.1007/s10552-011-9759-y]

11 **Figueiredo JC**, Levine AJ, Grau MV, Midttun O, Ueland PM, Ahnen DJ, Barry EL, Tsang S, Munroe D, Ali I, Haile RW, Sandler RS, Baron JA. Vitamins B2, B6, and B12 and risk of new colorectal adenomas in a randomized trial of aspirin use and folic acid supplementation. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 2136-2145 [PMID: 18708408 DOI: 10.1158/1055-9965.epi-07-2895]

12 **Institute of Medicine.** Dietary Reference Intakes: Thiamin R, Niacin, Vitamin B6, Folate, Vitamin B12, Pantothenic Acid, Biotin, and Choline. Washington, DC: National Academy Press, 1998

13 **Morris MS**, Picciano MF, Jacques PF, Selhub J. Plasma pyridoxal 5'-phosphate in the US population: the National Health and Nutrition Examination Survey, 2003-2004. *Am J Clin Nutr* 2008; **87**: 1446-1454 [PMID: 18469270]

14 **Bailey RL**, Gahche JJ, Lentino CV, Dwyer JT, Engel JS, Thomas PR, Betz JM, Sempos CT, Picciano MF. Dietary supplement use in the United States, 2003-2006. *J Nutr* 2011; **141**: 261-266 [PMID: 21178089 DOI: 10.3945/jn.110.133025]

15 **Selhub J**. Folate, vitamin B12 and vitamin B6 and one carbon metabolism. *J Nutr Health Aging* 2002; **6**: 39-42 [PMID: 11813080]

16 **Komatsu S**, Watanabe H, Oka T, Tsuge H, Kat N. Dietary vitamin B6 suppresses colon tumorigenesis, 8-hydroxyguanosine, 4-hydroxynonenal, and inducible nitric oxide synthase protein in azoxymethane-treated mice. *J Nutr Sci Vitaminol* (Tokyo) 2002; **48**: 65-68 [PMID: 12026192]

17 **Matsubara K**, Komatsu S, Oka T, Kato N. Vitamin B6-mediated suppression of colon tumorigenesis, cell proliferation, and angiogenesis (review). *J Nutr Biochem* 2003; **14**: 246-250 [PMID: 12832027]

18 **Matsubara K**, Mori M, Matsuura Y, Kato N. Pyridoxal 5'-phosphate and pyridoxal inhibit angiogenesis in serum-free rat aortic ring assay. *Int J Mol Med* 2001; **8**: 505-508 [PMID: 11605018]

19 **Jain SK**, Lim G. Pyridoxine and pyridoxamine inhibits superoxide radicals and prevents lipid peroxidation, protein glycosylation, and (Na+ + K+)-ATPase activity reduction in high glucose-treated human erythrocytes. *Free Radic Biol Med* 2001; **30**: 232-237 [PMID: 11165869]

20 **Shen J**, Lai CQ, Mattei J, Ordovas JM, Tucker KL. Association of vitamin B-6 status with inflammation, oxidative stress, and chronic inflammatory conditions: the Boston Puerto Rican Health Study. *Am J Clin Nutr* 2010; **91**: 337-342 [PMID: 19955400 DOI: 10.3945/ajcn.2009.28571]

21 **Boland CR**, Luciani MG, Gasche C, Goel A. Infection, inflammation, and gastrointestinal cancer. *Gut* 2005; **54**: 1321-1331 [PMID: 16099799 DOI: 10.1136/gut.2004.060079]

22 **Saibeni S**, Cattaneo M, Vecchi M, Zighetti ML, Lecchi A, Lombardi R, Meucci G, Spina L, de Franchis R. Low vitamin B(6) plasma levels, a risk factor for thrombosis, in inflammatory bowel disease: role of inflammation and correlation with acute phase reactants. *Am J Gastroenterol* 2003; **98**: 112-117 [PMID: 12526945 DOI: 10.1111/j.1572-0241.2003.07160.x]

23 **Chiang EP**, Bagley PJ, Selhub J, Nadeau M, Roubenoff R. Abnormal vitamin B(6) status is associated with severity of symptoms in patients with rheumatoid arthritis. *Am J Med* 2003; **114**: 283-287 [PMID: 12681455]

24 **Roubenoff R**, Roubenoff RA, Selhub J, Nadeau MR, Cannon JG, Freeman LM, Dinarello CA, Rosenberg IH. Abnormal vitamin B6 status in rheumatoid cachexia. Association with spontaneous tumor necrosis factor alpha production and markers of inflammation. *Arthritis Rheum* 1995; **38**: 105-109 [PMID: 7818558]

25 **Folsom AR**, Nieto FJ, McGovern PG, Tsai MY, Malinow MR, Eckfeldt JH, Hess DL, Davis CE. Prospective study of coronary heart disease incidence in relation to fasting total ho-

mocysteine, related genetic polymorphisms, and B vitamins: the Atherosclerosis Risk in Communities (ARIC) study. *Circulation* 1998; **98**: 204-210 [PMID: 9697819]
26 **Robinson K**, Arheart K, Refsum H, Brattström L, Boers G, Ueland P, Rubba P, Palma-Reis R, Meleady R, Daly L, Witteman J, Graham I. Low circulating folate and vitamin B6 concentrations: risk factors for stroke, peripheral vascular disease, and coronary artery disease. European COMAC Group. *Circulation* 1998; **97**: 437-443 [PMID: 9490237]
27 **Friso S**, Jacques PF, Wilson PW, Rosenberg IH, Selhub J. Low circulating vitamin B(6) is associated with elevation of the inflammation marker C-reactive protein independently of plasma homocysteine levels. *Circulation* 2001; **103**: 2788-2791 [PMID: 11401933]
28 **Theodoratou E**, Farrington SM, Tenesa A, McNeill G, Cetnarskyj R, Barnetson RA, Porteous ME, Dunlop MG, Campbell H. Dietary vitamin B6 intake and the risk of colorectal cancer. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 171-182 [PMID: 18199722]
29 **Larsson SC**, Orsini N, Wolk A. Vitamin B6 and risk of colorectal cancer: a meta-analysis of prospective studies. *JAMA* 2010; **303**: 1077-1083 [PMID: 20233826]
30 **Zhang X**, Lee JE, Ma J, Je Y, Wu K, Willett WC, Fuchs CS, Giovannucci EL. Prospective cohort studies of vitamin B-6 intake and colorectal cancer incidence: modification by time? *Am J Clin Nutr* 2012; **96**: 874-881 [PMID: 22875713 DOI: 10.3945/ajcn.112.037267]
31 **Wei EK**, Giovannucci E, Selhub J, Fuchs CS, Hankinson SE, Ma J. Plasma vitamin B6 and the risk of colorectal cancer and adenoma in women. *J Natl Cancer Inst* 2005; **97**: 684-692 [PMID: 15870439 DOI: 10.1093/jnci/dji116]
32 **Weinstein SJ**, Albanes D, Selhub J, Graubard B, Lim U, Taylor PR, Virtamo J, Stolzenberg-Solomon R. One-carbon metabolism biomarkers and risk of colon and rectal cancers. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 3233-3240 [PMID: 18990766 DOI: 10.1158/1055-9965.EPI-08-0459]
33 **Le Marchand L**, White KK, Nomura AM, Wilkens LR, Selhub JS, Tiirikainen M, Goodman MT, Murphy SP, Henderson BE, Kolonel LN. Plasma levels of B vitamins and colorectal cancer risk: the multiethnic cohort study. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 2195-2201 [PMID: 19661077 DOI: 10.1158/1055-9965.EPI-09-0141]
34 **Lee JE**, Li H, Giovannucci E, Lee IM, Selhub J, Stampfer M, Ma J. Prospective study of plasma vitamin B6 and risk of colorectal cancer in men. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 1197-1202 [PMID: 19336555 DOI: 10.1158/1055-9965.EPI-08-1001]
35 **Bønaa KH**, Njølstad I, Ueland PM, Schirmer H, Tverdal A, Steigen T, Wang H, Nordrehaug JE, Arnesen E, Rasmussen K. Homocysteine lowering and cardiovascular events after acute myocardial infarction. *N Engl J Med* 2006; **354**: 1578-1588 [PMID: 16531614 DOI: 10.1056/NEJMoa055227]
36 **Ebbing M**, Bleie Ø, Ueland PM, Nordrehaug JE, Nilsen DW, Vollset SE, Refsum H, Pedersen EK, Nygård O. Mortality and cardiovascular events in patients treated with homocysteine-lowering B vitamins after coronary angiography: a randomized controlled trial. *JAMA* 2008; **300**: 795-804 [PMID: 18714059 DOI: 10.1001/jama.300.7.795]
37 **Ebbing M**, Bønaa KH, Nygård O, Arnesen E, Ueland PM, Nordrehaug JE, Rasmussen K, Njølstad I, Refsum H, Nilsen DW, Tverdal A, Meyer K, Vollset SE. Cancer incidence and mortality after treatment with folic acid and vitamin B12. *JAMA* 2009; **302**: 2119-2126 [PMID: 19920236 DOI: 10.1001/jama.2009.1622]
38 **Martínez ME**, Henning SM, Alberts DS. Folate and colorectal neoplasia: relation between plasma and dietary markers of folate and adenoma recurrence. *Am J Clin Nutr* 2004; **79**: 691-697 [PMID: 15051616]
39 **Brussaard JH**, Löwik MR, van den Berg H, Brants HA, Bemelmans W. Dietary and other determinants of vitamin B6 parameters. *Eur J Clin Nutr* 1997; **51** Suppl 3: S39-S45 [PMID: 9598767]
40 **Zhang SM**, Moore SC, Lin J, Cook NR, Manson JE, Lee IM, Buring JE. Folate, vitamin B6, multivitamin supplements, and colorectal cancer risk in women. *Am J Epidemiol* 2006; **163**: 108-115 [PMID: 16339055 DOI: 10.1093/aje/kwj016]
41 **Larsson SC**, Giovannucci E, Wolk A. Vitamin B6 intake, alcohol consumption, and colorectal cancer: a longitudinal population-based cohort of women. *Gastroenterology* 2005; **128**: 1830-1837 [PMID: 15940618]
42 **Ishihara J**, Otani T, Inoue M, Iwasaki M, Sasazuki S, Tsugane S. Low intake of vitamin B-6 is associated with increased risk of colorectal cancer in Japanese men. *J Nutr* 2007; **137**: 1808-1814 [PMID: 17585035]
43 **Zhang X**, Giovannucci E. Calcium, vitamin D and colorectal cancer chemoprevention. *Best Pract Res Clin Gastroenterol* 2011; **25**: 485-494 [PMID: 22122765 DOI: 10.1016/j.bpg.2011.10.001]
44 **Martínez ME**. Primary prevention of colorectal cancer: lifestyle, nutrition, exercise. *Recent Results Cancer Res* 2005; **166**: 177-211 [PMID: 15648191]
45 **Chiang EP**, Smith DE, Selhub J, Dallal G, Wang YC, Roubenoff R. Inflammation causes tissue-specific depletion of vitamin B6. *Arthritis Res Ther* 2005; **7**: R1254-R1262 [PMID: 16277678 DOI: 10.1186/ar1821]
46 **Folsom AR**, Desvarieux M, Nieto FJ, Boland LL, Ballantyne CM, Chambless LE. B vitamin status and inflammatory markers. *Atherosclerosis* 2003; **169**: 169-174 [PMID: 12860264]
47 **Chan AT**, Ogino S, Giovannucci EL, Fuchs CS. Inflammatory markers are associated with risk of colorectal cancer and chemopreventive response to anti-inflammatory drugs. *Gastroenterology* 2011; **140**: 799-808 [PMID: 21115010]
48 **Shu XO**, Yang G, Jin F, Liu D, Kushi L, Wen W, Gao YT, Zheng W. Validity and reproducibility of the food frequency questionnaire used in the Shanghai Women's Health Study. *Eur J Clin Nutr* 2004; **58**: 17-23 [PMID: 14679362 DOI: 10.1038/sj.ejcn.1601738]
49 **Harnack L**, Jacobs DR, Nicodemus K, Lazovich D, Anderson K, Folsom AR. Relationship of folate, vitamin B-6, vitamin B-12, and methionine intake to incidence of colorectal cancers. *Nutr Cancer* 2002; **43**: 152-158 [PMID: 12588695]
50 **Lumeng L**, Li TK. Vitamin B6 metabolism in chronic alcohol abuse. Pyridoxal phosphate levels in plasma and the effects of acetaldehyde on pyridoxal phosphate synthesis and degradation in human erythrocytes. *J Clin Invest* 1974; **53**: 693-704 [PMID: 4359937 DOI: 10.1172/JCI107607]
51 **Giovannucci E**. Alcohol, one-carbon metabolism, and colorectal cancer: recent insights from molecular studies. *J Nutr* 2004; **134**: 2475S-2481S [PMID: 15333745]
52 **Slattery ML**, Schaffer D, Edwards SL, Ma KN, Potter JD. Are dietary factors involved in DNA methylation associated with colon cancer? *Nutr Cancer* 1997; **28**: 52-62 [PMID: 9200151]
53 **Lee JE**, Willett WC, Fuchs CS, Smith-Warner SA, Wu K, Ma J, Giovannucci E. Folate intake and risk of colorectal cancer and adenoma: modification by time. *Am J Clin Nutr* 2011; **93**: 817-825 [PMID: 21270374]
54 **Barker DJ**. The fetal and infant origins of adult disease. *BMJ* 1990; **301**: 1111 [PMID: 2252919]
55 **Trichopoulos D**. Hypothesis: does breast cancer originate in utero? *Lancet* 1990; **335**: 939-940 [PMID: 1970028]

P- Reviewers Bujanda L, Mayol J
S- Editor Huang XZ **L- Editor** A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i7.1011

World J Gastroenterol 2013 February 21; 19(7): 1011-1019
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

TOPIC HIGHLIGHT

Jung Eun Lee, ScD, Assistant Professor, *Series Editor*

Dietary flavonoid intake and risk of stomach and colorectal cancer

Hae Dong Woo, Jeongseon Kim

Hae Dong Woo, Jeongseon Kim, Molecular Epidemiology Branch, Division of Cancer Epidemiology and Prevention, Research Institute, National Cancer Center, Gyeonggi-do 410-769, South Korea

Author contributions: Woo HD and Kim J conducted the literature review; Woo HD analyzed the data and wrote the manuscript; Kim J interpreted the data, and critically reviewed and edited the manuscript.

Correspondence to: Jeongseon Kim, PhD, Molecular Epidemiology Branch, Division of Cancer Epidemiology and Prevention, Research Institute, National Cancer Center, 323 Ilsan-ro, Ilsandong-gu, Goyang-si, Gyeonggi-do 410-769, South Korea. jskim@ncc.re.kr
Telephone: +82-31-9202570 Fax: +82-31-9202579
Received: September 19, 2012 Revised: January 22, 2013
Accepted: February 5, 2013
Published online: February 21, 2013

Abstract

Stomach and colorectal cancers are common cancers and leading causes of cancer deaths. Because the alimentary tract can interact directly with dietary components, stomach and colorectal cancer may be closely related to dietary intake. We systematically searched published literature written in English *via* PubMed by searching for terms related to stomach and colorectal cancer risk and dietary flavonoids up to June 30, 2012. Twenty-three studies out of 209 identified articles were finally selected for the analysis. Log point effect estimates and the corresponding standard errors were calculated using covariate-adjusted point effect estimates and 95%CIs from the selected studies. Total dietary flavonoid intake was not associated with a reduced risk of colorectal or stomach cancer [odds ratio (OR) (95%CI) = 1.00 (0.90-1.11) and 1.07 (0.70-1.61), respectively]. Among flavonoid subclasses, the intake of flavonols, flavan-3-ols, anthocyanidins, and proanthocyanidins showed a significant inverse association with colorectal cancer risk [OR (95%CI) = 0.71 (0.63-0.81), 0.88 (0.79-0.97), 0.68 (0.56-0.82), and 0.72 (0.61-0.85), respectively]. A significant association was found only between flavonols and stomach cancer risk based on a limited number of selected studies [OR (95%CI) = 0.68 (0.46-0.99)]. In the summary estimates from case-control studies, all flavonoid subclasses except flavones and flavanones were inversely associated with colorectal cancer risk, whereas neither total flavonoids nor any subclasses of flavonoids were associated with colorectal cancer risk in the summary estimates based on the cohort studies. The significant association between flavonoid subclasses and cancer risk might be closely related to bias derived from the case-control design. There was no clear evidence that dietary flavonoids are associated with reduced risk of stomach and colorectal cancer.

© 2013 Baishideng. All rights reserved.

Key words: Flavonoids; Flavonols; Flavones; Flavanones; Flavan-3-ols; Anthocyanidins; Proanthocyanidins; Cancer risk; Meta-analysis

Woo HD, Kim J. Dietary flavonoid intake and risk of stomach and colorectal cancer. *World J Gastroenterol* 2013; 19(7): 1011-1019 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i7/1011.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i7.1011

INTRODUCTION

Stomach and colorectal cancer are common cancers and leading causes of cancer deaths[1]. Because the alimentary tract can interact directly with dietary components, stomach and colorectal cancer may be closely related to dietary intake. The consumption of meat, especially of red and processed meats that produce N-nitrosamine, was positively associated with stomach and colorectal cancer risk in a previous meta-analyses[2-4]. However, fruit and vegetable intakes were inversely associated with

colorectal cancer risk in people with high intakes of red and processed meat[5], and inverse association between vitamin C intake and stomach cancer risk was stronger in high red and processed meat intake group[6]. High fruit and vegetable intakes are associated with beneficial health effects, and these effects have been partly attributed to the high flavonoid content of these foods. Flavonoids are polyphenolic compounds that are abundant in fruits and vegetables. The beneficial health effects of flavonoids have been attributed to their free radical scavenging properties. In addition to their antioxidant properties, flavonoids have antiviral, antiallergic, antiinflammatory, and antitumor activities[7,8]. Flavonoids are generally classified as flavonols, flavones, flavanones, flavanols, isoflavones or anthocyanidins based on their chemical structure[9-11]. The antioxidant activities of the dietary flavonoids and their subclasses vary due to the differences in their chemical structures. Flavonols, the most common flavonoids in foods, include kaempferol, myricetin, quercetin, and isorhamnetin. Among them, quercetin is abundant in onions and apples, and has been studied extensively due to its high bioavailability and strong antioxidant effects. Flavones, such as apigenin and luteolin, are abundant in green leafy spices (*e.g.*, parsley, thyme, and celery), and the flavanones naringenin, hesperetin, and eriodictyol are found in citrus fruits. Flavanols, often referred to as flavan-3-ols, include catechin, gallocatechin, and epicatechin, which are abundant in teas, red wine, and apples. Isoflavones, especially genistein, are found in soy foods and have been studied widely due to their antitumor properties. Anthocyanidins are found in cherries, strawberries, and red wine.

A reduced risk of cardiovascular disease associated with flavonoid intake has been observed in many epidemiological studies[12,13]. Flavonoids have been suggested to reduce the risk of cardiovascular disease by modulating various mechanisms[14]. However, the association between cancer risk and dietary flavonoid intake has less supportive evidence from epidemiological studies, and the results have been inconsistent. To date, meta-analyses have focused mainly on dietary flavonoids and cardiovascular disease[12,13] or tea flavonoids and lung cancer[15,16]. Thus, we performed a meta-analysis of summary data to calculate the effect estimates of dietary flavonoid intake, including individual subclasses of flavonoids, on stomach and colorectal cancer risk.

DIETARY FLAVONOID INTAKE AND RISK OF STOMACH AND COLORECTAL CANCER

English-language studies were systemically searched using PubMed with the phrase "cancer risk and (stomach or gastric or colorectal or colon or bowel or rectal) and (flavonoid or flavonol or quercetin or kaempferol or myricetin or isorhamnetin or flavone or luteolin or apigenin or flavanone or eriodictyol or hesperetin or naringenin or flavan-3-ol or catechin or epicatechin or theaflavin or anthocyanidin or cyanidin or delphinidin or malvidin or pelargonidin or peonidin or petunidin or proanthocyanidin or isoflavone)" up to June 30, 2012. The inclusion criteria were as follows: (1) original articles with a case-control or cohort design; (2) articles reporting intake of either total flavonoids or subclasses of flavonoids and stomach or colorectal cancer; (3) articles with at least 3 categories of dietary flavonoid intake; and (4) studies reporting adjusted odds ratios (OR) or relative risks (RR) with 95%CIs for the risk of stomach or colorectal cancer in subjects with the highest dietary flavonoid intake compared with those with the lowest intake of dietary flavonoids.

The retrieved studies were reviewed independently by two investigators (Woo HD and Kim J). Data on authors, publication year, types of flavonoids, cancer sites, country in which the study was performed, number of cases and controls, categories of flavonoids or subclasses of flavonoids, subclasses included in the calculation of total flavonoids, and adjusted OR/RR and 95%CI were collected for the meta-analysis. The multivariate-adjusted values for OR/RR and 95%CI were selected for the meta-analysis to reduce the effects of potential confounding variables. If there was additional adjustment for fruit and vegetable intake, the adjusted values were selected.

All statistical analyses were performed using the STATA software package (version 10; Stata Corp, College Station, TX, United States). Log point effect estimates and the corresponding standard errors were calculated using the covariate-adjusted point effect estimates and 95%CI from selected studies and weighted by the inverse variance to calculate the summary estimates[17]. The heterogeneity across studies was measured using the Q-test based on the χ^2 statistic. Heterogeneity was considered statistically significant when $P < 0.1$ for the Q-test and was quantified using the I^2 test as described by Higgins *et al*[18]. Based on the heterogeneity of the included studies, fixed or random effects models were selected to calculate the pooled effect measures. Sensitivity analyses were performed to test the robustness of the results of the combined effects.

A total of 209 studies were identified, and 160 studies were excluded based on the titles and abstracts. The full texts of the remaining 49 studies were reviewed, and 25 studies were excluded for the following reasons: 7 studies were not related to dietary flavonoid intake or any of the flavonoid subclasses; 5 studies presented only recurrence or survival analysis data; 5 studies were not related to stomach or colorectal cancer: 4 studies did not report cancer risk; 2 studies were review articles; and 2 studies reported plasma concentrations instead of dietary flavonoid intake.

Sensitivity tests were conducted for the remaining 24 studies of total flavonoids and each subclass of flavonoids. One study showing substantial influence on the summary estimates in the sensitivity tests was excluded[19]. Thus, 23 studies, comprising 13 case-control

studies and 10 cohort studies, were finally selected to estimate the overall effects of total dietary flavonoid or flavonoid subclass intake on stomach and colorectal cancer risk (Table 1)[20-42]. Begg's funnel plot and Egger's test were used to evaluate the publication bias in studies of total flavonoids and each subclass of flavonoids. Egger's test showed no significant bias.

The summary estimates for the risk of cancer in subjects with the highest dietary flavonoid intake, compared with that in subjects with the lowest flavonoid intake, are presented in Table 2. The heterogeneity was low in the overall results, but heterogeneity was found in several summary estimates of the studies that included mostly case-control design. Total dietary flavonoids were not associated with a reduced risk of colorectal or stomach cancer [OR (95%CI) = 1.00 (0.90-1.11) and 1.07 (0.70-1.61), respectively]. However, flavonol, flavan-3-ol, anthocyanidin, and proanthocyanidin intakes showed a significant inverse association with colorectal cancer risk among flavonoid subclasses [OR (95%CI) = 0.71 (0.63-0.81), 0.88 (0.79-0.97), 0.68 (0.56-0.82), and 0.72 (0.61-0.85), respectively]. A significant association was found only for flavonol intake and stomach cancer risk in a limited number of selected studies [OR (95%CI) = 0.68 (0.46-0.99)].

Subgroup analyses by study design or sex were conducted for each subclass of flavonoids. In case-control studies, all flavonoid subclasses except flavones and flavanones were inversely associated with colorectal cancer risk. However, neither total flavonoids nor any subclasses of flavonoids were associated with colorectal cancer risk in the cohort studies. Colorectal cancer showed a statistically significant association for the summary estimate of flavonols in both female and male subjects [OR (95%CI) = 0.84 (0.75-0.93) and 0.87 (0.79-0.96), respectively] and isoflavones in male subjects [OR (95%CI) = 0.90 (0.83-0.99)]. A reduced risk of colorectal cancer was not observed for the subclasses of flavonols.

The preventative effects of flavonoids on stomach and colorectal cancer risk were estimated by pooling the estimates based on the published observational studies. Total dietary flavonoid intake was not associated with a reduced risk of stomach and colorectal cancer, but several subclasses of flavonoids, mostly in case-control studies, showed protective effects against stomach and colorectal cancer risk.

Flavonoids could affect cancer risk through their antiinflammatory and antitumor activities. Flavonoids exert their antiinflammatory activities by inhibiting cycloxygenase-2 (COX2) in colon cancer cells, and this is associated with a reduced risk of colorectal cancer[43]. Plant flavonoids induce apoptosis and suppress the growth of colon cancer cells by inhibiting the COX2- and Wnt/epidermal growth factor receptor/nuclear factor-κB-signaling pathways, which play crucial roles in colorectal cancer[44]. Quercetin inhibits tyrosine kinase activity, thus downregulating cell proliferation[45]. The antitumor effects of flavonoids have not been demonstrated conclusively, but it has been suggested that the free radical scavenging properties of flavonoids are closely related to beneficial effects on cancer risk, as flavonoids are more effective antioxidants than vitamin C, vitamin E and carotenoids[46]. Hydroxylation at the 3-position on the C ring, the increased number of hydroxyl groups in ring B, and a saturated 2-3 bond on the C ring showed enhanced scavenging activities[47,48].

Reduced risks of colorectal cancer were observed in the summary estimates for flavonols, flavan-3-ols, anthocyanidins, and proanthocyanidins in the present study, as well as in subgroup analyses. The intake of total dietary flavonols and dietary quercetin were associated with a significant reduction of stomach cancer risk. These results might be attributed to the antiinflammatory and antitumor effects of those nutrients. However, no reduced risk of stomach and colorectal cancer was observed in the summary estimates of total dietary flavonoids, including the subgroup analyses by sex and study design, in the present meta-analysis. The subclasses of flavonoids included in the calculations of total flavonoids were different across studies, as shown in Table 1. All studies included flavonols and flavones among the total dietary flavonoids, but flavan-3-ols, proanthocyanidins, which can contribute a considerable proportion of the total flavonoid intake, were not considered in most studies. These discrepancies can lead to highly heterogeneous results. However, the overall results were homogenous in terms of the effects of total flavonoids, except for the summary estimate of case-control studies, which showed moderate heterogeneity. Thus, dietary flavonoid intake might not be truly associated with stomach or colorectal cancer risk. Furthermore, dietary intake of quercetin, kaempferol, and myricetin showed no significant association with colorectal cancer risk, whereas total dietary flavonols showed statistically significant results for both cancer types. These results might be closely related to the design of the selected studies. The studies that investigated total flavonoids as well as the flavonols quercetin, kaempferol and myricetin were mostly cohort designs, while the studies that investigated total flavonols were mostly case-control designs. Case-control studies are more subject to recall bias, resulting in either an underestimate or overestimate of the risk estimates. Especially in stomach and colorectal cancer, patients might have intestinal discomfort prior to diagnosis, resulting in changes in dietary habits. Similar results were found in a meta-analysis of fruit and vegetable consumption[49]. A statistically significant association between the risk of stomach and colorectal cancer and fruit and vegetable consumption was observed only in the summary estimate of case-control studies. This summary risk estimate was highly heterogeneous, unlike that of the cohort studies, suggesting that bias might be introduced in case-control studies. Another explanation for the lack of association in cohort studies is the typically short follow-up times. In a meta-analysis of association between fruit and vegetable consumption and gastric cancer risk[50], a

Table 1 Selected studies on dietary flavonoids and risk of stomach and colorectal cancer

Ref.	Cancer site	Country	Study period	Case/control (*n*/*n*)	Dietary assessment method	Reported flavonoids	Included subclasses for total flavonoids	Intake comparison High *vs* low (mg/d)[1]	Controlled confounders
Garcia-Closas *et al*[20]	Stomach	Spain	1987-1989	354/354	Diet history	Q, K, M		Q4 *vs* Q1 mean (SD), Q: 7.1 (6.5), K: 1.2 (1.9), M: 0.65 (1.17)	Total energy intake, intake of nitrites, nitrosamines, vitamin C, total carotenoids (α-carotene, β-carotene, lutein, and lycopene) and other specific favonoids (quercetin, kaempferol, myricetin, and luteolin)
Lagiou *et al*[21]	Stomach	Greece	1981-1984	110/100	FFQ	F1, F2, F3, F4, An, I		F1: per 10.0, F2: per 0.3, F3: per 19.8, F4: per 135.1, An: per 40.4, I: per 2.0.	Age, gender, total energy intake, place of birth, BMI, height, years of education, smoking habits and duration of smoking, alcohol consumption, and fruit and vegetable consumption
Cotterchio *et al*[22]	Colorectum	Canada	1997-2000	1095/1890	FFQ	I		> 1.097 *vs* < 0.289.	Age, sex, and total energy intake
Rossi *et al*[23]	Colorectum	Italy	1992-1996	1953/4154	FFQ	T, F1, F2, F3, F4, An, I	F1, F2, F3, F4, An, I	T: > 191.1 *vs* < 75.3, F1: > 28.5 *vs* < 13.2, F2: > 0.7 *vs* < 0.3, F3: > 67.0 *vs* < 12.5, F4: > 88.5 *vs* < 20.8, An: > 31.7 *vs* < 5.3, I: > 33.9 *vs* < 14.4	Age, sex, energy intake, study center, family history of colorectal cancer, education, alcohol consumption, BMI, and occupational physical activity
Theodoratou *et al*[24]	Colorectum	Scotland	-	1456/1456	FFQ	F1, F2, F3, F4, Q, H, N, C, E		F1: > 36.75 *vs* < 16, F2: > 1.9 *vs* < 0.5, F3: > 45.2 *vs* < 16.7, F4: > 162.1 *vs* < 42.6, Q: > 22.9 *vs* < 11.7, H: > 21.1 *vs* < 3.95, N: > 19.7 *vs* < 3.8	Total energy intake, family history of colorectal cancer, total fiber intake, alcohol intake, NSAID intake, smoking, BMI, physical activity, and fruit and vegetable intake
Akhter *et al*[25]	Colorectum	Japan	2004-2005	721/697	FFQ	I		> 62.41 *vs* < 24.77	Age, sex, total energy intake, screening period, family history of colorectal cancer, cigarette smoking, alcohol consumption, BMI, physical activity, supplement use and non-steroidal anti-inflammatory drug use
Kyle *et al*[26]	Colorectum	United Kingdom	1998-2000	261/404	FFQ	F1, F3, F4		F1: > 40.4 *vs* < 19.3, F3: > 32.2 *vs* < 2.73, F4: > 188.8 *vs* < 67.1	Age, energy, family history, non-steroidal anti-inflammatory drugs, aspirin, Mn, riboflavin, vitamin C, folate
Rossi *et al*[27]	Stomach	Italy	1997-2007	230/547	FFQ	F1, F2, F3, F4, An, I, P		F1: > 32.3 *vs* < 13.2, F2: > 0.7 *vs* < 0.3, F3: > 56.8 *vs* < 12.9, F4: > 79.2 *vs* < 21.6, An: > 21.5 *vs* < 6.2, I: > 34.3 *vs* < 15.0, P: > 373.0 *vs* < 339.6	Age, sex, education, year of interview, BMI, tobacco smoking, and total energy intake
Rossi *et al*[28]	Colorectum	Italy	1992-1996	1953/4154	FFQ	P		> 486.6 *vs* < 202.5	Age, sex, study center, family history, education, alcohol consumption, BMI, physical activity and energy intake
Budhathoki *et al*[29]	Colorectum	Japan	2003-2003	816/815	FFQ	I		74.4 *vs* 15.5 (median)	Age, sex, total energy intake, resident area, parental colorectal cancer, smoking, alcohol use, BMI, type of job, and leisure time physical activity
Ekström *et al*[30]	Stomach (cardia and non cardia)	Sweden	1989-1995	C81, Non; 420 /1116	FFQ	Q		> 11.89 *vs* < 3.88	Age, sex, socioeconomic status, number of siblings, BMI, smoking and energy and salt intake

Zamora-Ros *et al*[31]	Colorectum	Spain	1996-1998	424/401	FFQ	T, F1, F2, F3, F4, An, I, P	F1, F2, F3, F4, An, I, P, Th	T: > 167.9 *vs* < 68.9, F1: > 11.5 *vs* < 5.1, F2: > 2.1 *vs* < 0.7, F3: > 17.7 *vs* < 3.7, F4: > 12.9 *vs* < 4.9, An: > 10.6 *vs* < 3.3, I: > 0.17 *vs* < 0.07, P: > 112.3 *vs* < 40.9	Age, sex, energy intake, BMI, alcohol and fiber intake, red and processed meat intake, tobacco consumption, physical activity, regular drugs, and family history of colorectal cancer
Djuric *et al*[32]	Colorectum	United States	2003-2005	1163/1501	FFQ	Q		> 10.29 *vs* < 4.33	Age, sex, physical activity at age 30-39, BMI, family history of colorectal cancer, highest education achieved, and nonsteroidal antiinflammatory drug use (NSAID) use, red meat, and total calcium intake
			Follow-up (yr)	Case (*n*)					
Hirvonen *et al*[33]	Colorectum, Stomach	Finland	6.1 (median)	C 133 S 111	Diet history	T	F1, F2	16.3 *vs* 4.2 (median)	Age and supplementation group
Knekt *et al*[34]	Colorectum, Stomach	Finland	30 (maximum)	C 90 S 74	Diet history	T, Q, K, M, H, N	F1, F2, F3	T: > 39.5 (F), 26.9 (M) *vs* < 8.5 (F), 4.3 (M), Q: > 4.7 (F), 3.9 (M) *vs* < 1.8 (F), 1.5 (M), K: > 0.9 (F), 0.8 (M) *vs* < 0.2 (F), 0.1 (M), M: > 0.2 (F), 0.11 (M) *vs* < 0.03 (F), 0.06 (M), H:> 26.8 (F), 15.4 (M) *vs* < 3.2 (F), 0 (M), N: > 7.7 (F), 4.7 (M) *vs* < 0.9 (F), 0 (M)	Age, sex, geographic area, occupation, smoking, and BMI
Lin *et al*[35]	Colorectum	United States	-	878	FFQ	T, Q, K, M	F1, F2	Q5 *vs* Q1 (NHS: > 31.1 *vs* < 9.6, HPFS: > 30.5 *vs* < 10.7)	Age, BMI, family history of colorectal cancer, history of colorectal polyps, prior sigmoidoscopy screening, physical activity, smoking status, red meat intake, alcohol consumption, total energy intake, total calcium intake, total folate intake, total fiber intake, aspirin use, and multivitamin use
Akhter *et al*[36]	Colorectum	Japan	7.6 (mean)	886	FFQ	I		Q4 *vs* Q1	Age, public health center area, history of diabetes mellitus, BMI, leisure time physical activity, cigarette smoking, alcohol drinking, and intake of vitamin D, dairy products, meat, fruit, vegetable, and fish, (F) + menopausal status and current use of female hormones
Mursu *et al*[37]	Colorectum	Finland	16.2 (mean)	55	Food recording	T, F1, F2, F3, F4, An	F1, F2, F3, F4, An	Q4 *vs* Q1 T: 416.3 *vs* 265.0 (mean)	Age and examination years, BMI, smoking status, pack-years of smoking, physical activity, intakes of alcohol, total fat and saturated fat, and energy adjusted intake of fiber, vitamin C and E
Simon *et al*[38]	Colorectum	Nether-lands	13.3	1271	FFQ	T, F4	F1, F2	T: > 36 *vs* < 16 (M), F4: > 84.3 *vs* < 44.4 (M), T: > 38.3 *vs* < 18.4 (F), F4: > 95.9 *vs* < 51.6 (F)	Age, family history of colorectal cancer, smoking status, alcohol intake, occupational physical activity at longest held job, BMI and processed meat intake
Wang *et al*[39]	Colorectum	United States	11.5 (mean)	305	FFQ	T	F1, F2	T: > 34.6 *vs* < 11.6	Age, race, total energy intake, and randomized treatment assignment, smoking, alcohol use, physical activity, postmenopausal status, hormone replacement therapy use, multivitamin use, BMI, family history of colorectal cancer, ovary cancer, and breast cancer, and intake of fruit and vegetables, fiber, folate, and saturated fat
Yang *et al*[40]	Colorectum	United States	6.4 (mean)	321	FFQ	I		T3 *vs* T1 34.8 *vs* 20.9 (mean)	Age, education, household income, BMI, physical activity, menopausal status, family history of colorectal cancer, total calorie intake, and average intakes of fruit, vegetables, red meat, nonsoy calcium, nonsoy fiber, and nonsoy folic acid

Ward *et al*[41]	Colorectum	Unitd States	9 (mean)	221	Food recording	I	Continuous	Age, height, weight, family history of colorectal cancer, smoking status, aspirin use, physical activity, and average daily intake of fat, energy, calcium, alcohol, and red and processed meats, (F) + oral contraceptive use, menopausal status, menopausal hormone therapy use, parity, breastfeeding, and surgical removal of ovaries
Hara *et al*[42]	Stomach	Japan		1249	FFQ	I	Q4 *vs* Q1 (median) 42.3 *vs* 9.2 (M) 41.8 *vs* 9.4 (F)	Age, public center area, BMI, smoking status, ethanol intake, family history of gastric cancer, vegetable, fruit, fish, salt, and total energy intake

[1]M and F represent male and female in this row. Q: Querectin; K: Kaempferol; M: Myricetin; H: Hesperidin; N: Naringenin; C: Catechin; E: Epicatechin; F1: Flavonols; F2: Flavones; F3: Flavanones; F4: Flavan-3-ols; An: Anthocyanidins; I: Isoflavones; P: Proanthocyanidins; Th: Theaflavins; BMI: Body mass index.

Table 2 Summary estimates of dietary flavonoids and stomach and colorectal cancer risk

	Combined					Colorectum					Stomach				
				Heterogeneity					Heterogeneity					Heterogeneity	
	n[a]	RR	95%CI	I^2	P^b	*n*	RR	95%CI	I^2	P^b	*n*	RR	95%CI	I^2	P^b
Total flavonoids	11	1.00	0.91-1.11	15.0%	0.301	9	1.00	0.90-1.11	28.2%	0.194	2	1.07	0.70-1.61	0%	0.464
Cohort	9	1.05	0.93-1.18	0%	0.517	7	1.04	0.92-1.18	9.6%	0.355	2	-	-	-	-
Case-control	-	-	-	-	-	2	0.81	0.50-1.29	67.9%	0.078[c]	0				
Female	-	-	-	-	-	3	0.93	0.84-1.04	0%	0.890	0				
Male	5	1.05	0.96-1.15	0%	0.514	4	1.05	0.96-1.15	18.6%	0.298	1				
Flavonols	7	0.71	0.63-0.80	10.7%	0.348	5	0.71	0.63-0.81	37.0%	0.175	2	0.68	0.46-0.99	0%	0.586
Case-control	6	0.70	0.62-0.79	0%	0.755	4	0.70	0.62-0.79	0%	0.510	2	-	-	-	-
Female	-	-	-	-	-	2	0.84	0.75-0.93	0%	0.361	0				
Male	-	-	-	-	-	3	0.87	0.79-0.96	45.4%	0.160	0				
Quercetin	8	0.81	0.68-0.97	44.1%	0.085[c]	4	0.90	0.80-1.03	42.2%	0.159	4	0.66	0.51-0.85	0%	0.472
Kaempferol	4	0.95	0.65-1.37	55.2%	0.082[c]	2	1.12	0.91-1.38	0%	0.979	2	0.73	0.31-1.71	71.8%	0.060[c]
Myricetin	4	1.15	0.87-1.51	0%	0.965	2	1.15	0.80-1.67	0%	0.607	2	1.13	0.75-1.71	0%	0.935
Flavones	6	0.84	0.74-0.96	44.8%	0.107	4	0.83	0.63-1.09	64.4%	0.038[c]	2	0.76	0.54-1.08	0%	0.635
Case-control	5	0.82	0.66-1.02	55.0%	0.064[c]	3	0.83	0.61-1.14	87.9%	0.016[c]	2	-	-	-	-
Female	-	-	-	-	-	2	0.94	0.84-1.04	0%	0.353	0				
Male	-	-	-	-	-	3	0.92	0.84-1.02	0%	0.477	0				
Flavanones	7	1.04	0.86-1.27	44.5%	0.094[c]	5	1.08	0.95-1.22	28.2%	0.234	2	0.72	0.49-1.07	38.3%	0.203
Case-control	6	1.05	0.85-1.30	53.3%	0.057[c]	4	1.08	0.95-1.23	46.4%	0.144	2	-	-	-	-
Female	-	-	-	-	-	2	1.01	0.89-1.14	0%	0.734	0				
Male	-	-	-	-	-	3	0.99	0.89-1.11	0%	0.583	0				
Hesperidin	3	1.12	0.90-1.40	0%	0.675	2	1.15	0.91-1.45	0%	0.587	1				
Naringenin	3	1.11	0.89-1.38	0%	0.732	2	1.13	0.90-1.42	0%	0.539	1				
Flavan-3-ols	9	0.88	0.80-0.97	16.3%	0.297	7	0.88	0.79-0.97	30.1%	0.198	2	0.91	0.66-1.26	0%	0.335
Cohort	-	-	-	-	-	3	0.91	0.76-1.08	26.9%	0.254	0				
Case-control	6	0.87	0.77-0.98	24.7%	0.249	4	0.86	0.76-0.98	46.6%	0.132	2	-	-	-	-
Female	-	-	-	-	-	3	0.94	0.87-1.02	54.7%	0.110	0				
Male	-	-	-	-	-	4	1.07	0.99-1.15	23.5%	0.270	0				
Anthocyanidines	5	0.75	0.64-0.89	21.9%	0.275	3	0.68	0.56-0.82	0%	0.868	2	1.03	0.73-1.43	0%	0.509
Case-control	4	0.76	0.64-0.90	37.9%	0.185	2	0.68	0.56-0.83	0%	0.668	2	-	-	-	-
Proanthocyanidines	3	0.55	0.35-0.87	74.4%	0.020[c]	2	0.72	0.61-0.85	0%	0.372	1				
Isoflavones	14	0.90	0.80-1.01	54.4%	0.008[c]	10	0.89	0.77-1.02	65.1%	0.003[c]	4	0.91	0.78-1.06	5.5%	0.206
Cohort	7	0.98	0.85-1.12	44.0%	0.098	5	1.02	0.90-1.15	43.1%	0.135	2	0.88	0.74-1.05	48.7%	0.163
Case-control	7	0.78	0.70-0.87	37.5%	0.143	5	0.76	0.68-0.85	36.2%	0.180	2	1.02	0.73-1.44	0%	0.430
Female	7	0.95	0.81-1.11	47.7%	0.075[c]	6	0.93	0.78-1.11	54.3%	0.052[c]	1				
Male	6	0.89	0.82-0.96	35.7%	0.170	5	0.90	0.83-0.99	42.0%	0.141	1				

[a]Selected study numbers; [b]*P* values for heterogeneity from *Q*-test; [c]Random effects model was used if $P < 0.1$. RR: Relative risk.

significant association was observed only in the meta-analyses of studies with more than 10 years of follow-up. Meta-regression also revealed that longer follow-up time was associated with lower risk of gastric cancer. The mean follow-up times were more than 10 years for all cohort studies reporting total dietary flavonoid intake in our study, except for Hirvonen *et al*[35]. A meta-analysis performed after excluding this study did not change the results. Thus, the hypothesis of an inverse association between dietary flavonoids and risk of stomach and colorectal cancer cannot be supported by our results.

Although this meta-analysis provided little evidence of an inverse association between stomach and colorectal cancer risk and flavonoid intake, several mechanisms supported by *in vitro* and animal studies remain biologically plausible. Antitumor effects were shown for quercetin, luteolin, and myricetin[51-54], and polyphenols extracted from apple and olive oil had a chemopreven-

tive effect in colon cancer cell lines or mice[55-57] and antioxidant activities in gastric mucosa[58,59]. Most subclasses of flavonoids were inversely associated in the present meta-analyses, although some bias might have been introduced. Thus, it is thought that dietary flavonoids are inversely associated with stomach or colorectal cancer risk, but this association is very small, making it difficult to detect. The United States Department of Agriculture (USDA) database for food flavonoids has been updated several times since 2003[60]. Most total dietary flavonoids are flavan-3-ols (82.5%), as estimated by USDA database and 24 h dietary records provided by United States adults[61]. The second greatest contributor was flavanones (7.6%), followed by flavonols (6.8%), anthocyanidins (1.6%), flavones (0.8%), and isoflavones (0.6%). However, the bioavailability of these compounds might be different. For example, tea flavonoids showed low absorption rates, whereas quercetin and isoflavones showed strong bioavailability. The main source of dietary flavonoid subclasses in Scotland in a database by Kyle *et al*[62] was tea[24], whereas the main sources of total flavonoids in Spain[31], calculated using the USDA database[60], were fruits (65.1%), followed by wine (14.4%), legumes (6.3%), and vegetables (4.2%). The methods used to estimate total flavonoids from dietary intake are poorly established[61]. Levels of exact individual flavonoid intake from food should be determined to confirm the true association between flavonoids and stomach and colorectal cancer risk.

The main limitation of this study is the small number of publications included. Especially in the case of stomach cancer, summary estimates could not be calculated in several subgroup analyses due to the limited number of studies. Because a significant association was found in the analyses of case-control studies which were subject to bias, the inverse association between subclasses of dietary flavonoid intake and cancer risk may be overestimated.

CONCLUSION

There was no clear evidence that dietary flavonoids are associated with reduced risk of stomach and colorectal cancer in the present meta-analysis. However, there is a possibility that there may be a weak association with stomach and colorectal cancer based on consistent associations for subclasses of flavonoids. The association of flavonoids with stomach and colorectal cancer could be relatively small and thus might only be detected with better methods of estimating true dietary flavonoid intake.

REFERENCES

1 **Ferlay J**, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; **127**: 2893-2917 [PMID: 21351269 DOI: 10.1002/ijc.25516]

2 **Sandhu MS**, White IR, McPherson K. Systematic review of the prospective cohort studies on meat consumption and colorectal cancer risk: a meta-analytical approach. *Cancer Epidemiol Biomarkers Prev* 2001; **10**: 439-446 [PMID: 11352852]

3 **Larsson SC**, Wolk A. Meat consumption and risk of colorectal cancer: a meta-analysis of prospective studies. *Int J Cancer* 2006; **119**: 2657-2664 [PMID: 16991129 DOI: 10.1002/ijc.22170]

4 **Larsson SC**, Orsini N, Wolk A. Processed meat consumption and stomach cancer risk: a meta-analysis. *J Natl Cancer Inst* 2006; **98**: 1078-1087 [PMID: 16882945 DOI: 10.1093/jnci/djj301]

5 **van Duijnhoven FJ**, Bueno-De-Mesquita HB, Ferrari P, Jenab M, Boshuizen HC, Ros MM, Casagrande C, Tjønneland A, Olsen A, Overvad K, Thorlacius-Ussing O, Clavel-Chapelon F, Boutron-Ruault MC, Morois S, Kaaks R, Linseisen J, Boeing H, Nöthlings U, Trichopoulou A, Trichopoulos D, Misirli G, Palli D, Sieri S, Panico S, Tumino R, Vineis P, Peeters PH, van Gils CH, Ocké MC, Lund E, Engeset D, Skeie G, Suárez LR, González CA, Sánchez MJ, Dorronsoro M, Navarro C, Barricarte A, Berglund G, Manjer J, Hallmans G, Palmqvist R, Bingham SA, Khaw KT, Key TJ, Allen NE, Boffetta P, Slimani N, Rinaldi S, Gallo V, Norat T, Riboli E. Fruit, vegetables, and colorectal cancer risk: the European Prospective Investigation into Cancer and Nutrition. *Am J Clin Nutr* 2009; **89**: 1441-1452 [PMID: 19339391 DOI: 10.3945/ajcn.2008.27120]

6 **Jenab M**, Riboli E, Ferrari P, Sabate J, Slimani N, Norat T, Friesen M, Tjønneland A, Olsen A, Overvad K, Boutron-Ruault MC, Clavel-Chapelon F, Touvier M, Boeing H, Schulz M, Linseisen J, Nagel G, Trichopoulou A, Naska A, Oikonomou E, Krogh V, Panico S, Masala G, Sacerdote C, Tumino R, Peeters PH, Numans ME, Bueno-de-Mesquita HB, Büchner FL, Lund E, Pera G, Sanchez CN, Sánchez MJ, Arriola L, Barricarte A, Quirós JR, Hallmans G, Stenling R, Berglund G, Bingham S, Khaw KT, Key T, Allen N, Carneiro F, Mahlke U, Del Giudice G, Palli D, Kaaks R, Gonzalez CA. Plasma and dietary vitamin C levels and risk of gastric cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST). *Carcinogenesis* 2006; **27**: 2250-2257 [PMID: 16774936 DOI: 10.1093/carcin/bgl096]

7 **Middleton E**. Effect of plant flavonoids on immune and inflammatory cell function. *Adv Exp Med Biol* 1998; **439**: 175-182 [PMID: 9781303 DOI: 10.1007/978-1-4615-5335-9_13]

8 **Lambert JD**, Hong J, Yang GY, Liao J, Yang CS. Inhibition of carcinogenesis by polyphenols: evidence from laboratory investigations. *Am J Clin Nutr* 2005; **81**: 284S-291S [PMID: 15640492]

9 **Beecher GR**. Overview of dietary flavonoids: nomenclature, occurrence and intake. *J Nutr* 2003; **133**: 3248S-3254S [PMID: 14519822]

10 **Neuhouser ML**. Dietary flavonoids and cancer risk: evidence from human population studies. *Nutr Cancer* 2004; **50**: 1-7 [PMID: 15572291 DOI: 10.1207/s15327914nc5001_1]

11 USDA Database for the Flavonoid Content of Selected Foods, Release 3 (2011). Available from: URL: http://www.ars.usda.gov/services/docs.htm?docid=6231

12 **Huxley RR**, Neil HA. The relation between dietary flavonol intake and coronary heart disease mortality: a meta-analysis of prospective cohort studies. *Eur J Clin Nutr* 2003; **57**: 904-908 [PMID: 12879084 DOI: 10.1038/sj.ejcn.1601624]

13 **Hooper L**, Kroon PA, Rimm EB, Cohn JS, Harvey I, Le Cornu KA, Ryder JJ, Hall WL, Cassidy A. Flavonoids, flavonoid-rich foods, and cardiovascular risk: a meta-analysis of randomized controlled trials. *Am J Clin Nutr* 2008; **88**: 38-50 [PMID: 18614722]

14 **Schroeter H**, Heiss C, Spencer JP, Keen CL, Lupton JR, Schmitz HH. Recommending flavanols and procyanidins for cardiovascular health: current knowledge and future needs. *Mol Aspects Med* 2010; **31**: 546-557 [PMID: 20854838 DOI: 10.1016/j.mam.2010.09.008]

15 **Sun CL**, Yuan JM, Koh WP, Yu MC. Green tea, black tea

and breast cancer risk: a meta-analysis of epidemiological studies. *Carcinogenesis* 2006; **27**: 1310-1315 [PMID: 16311246 DOI: 10.1093/carcin/bgi276]
16 **Tang N**, Wu Y, Zhou B, Wang B, Yu R. Green tea, black tea consumption and risk of lung cancer: a meta-analysis. *Lung Cancer* 2009; **65**: 274-283 [PMID: 19128856 DOI: 10.1016/j.lungcan.2008.12.002]
17 **Sterne JAC,** Bradburn MJ, Egger M. Meta-Analysis in Stata. In: Egger M, Smith GD. Altman DG, editors. Systematic Reviews in Health Care: Meta-analysis in Context. London, England: BMJ Publishing Group, 2001: 347-369
18 **Higgins JP**, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; **327**: 557-560 [PMID: 12958120 DOI: 10.1136/bmj.327.7414.]
19 **Ravasco P**, Monteiro-Grillo I, Marqués Vidal P, Camilo ME. Nutritional risks and colorectal cancer in a Portuguese population. *Nutr Hosp* ; **20**: 165-172 [PMID: 15989062]
20 **Garcia-Closas R**, Gonzalez CA, Agudo A, Riboli E. Intake of specific carotenoids and flavonoids and the risk of gastric cancer in Spain. *Cancer Causes Control* 1999; **10**: 71-75 [PMID: 10334645]
21 **Lagiou P**, Samoli E, Lagiou A, Peterson J, Tzonou A, Dwyer J, Trichopoulos D. Flavonoids, vitamin C and adenocarcinoma of the stomach. *Cancer Causes Control* 2004; **15**: 67-72 [PMID: 14970736]
22 **Cotterchio M**, Boucher BA, Manno M, Gallinger S, Okey A, Harper P. Dietary phytoestrogen intake is associated with reduced colorectal cancer risk. *J Nutr* 2006; **136**: 3046-3053 [PMID: 17116718]
23 **Rossi M**, Negri E, Talamini R, Bosetti C, Parpinel M, Gnagnarella P, Franceschi S, Dal Maso L, Montella M, Giacosa A, La Vecchia C. Flavonoids and colorectal cancer in Italy. *Cancer Epidemiol Biomarkers Prev* 2006; **15**: 1555-1558 [PMID: 16896049 DOI: 10.1158/1055-9965.EPI-06-0017]
24 **Theodoratou E**, Kyle J, Cetnarskyj R, Farrington SM, Tenesa A, Barnetson R, Porteous M, Dunlop M, Campbell H. Dietary flavonoids and the risk of colorectal cancer. *Cancer Epidemiol Biomarkers Prev* 2007; **16**: 684-693 [PMID: 17416758 DOI: 10.1158/1055-9965.EPI-06-0785]
25 **Akhter M**, Iwasaki M, Yamaji T, Sasazuki S, Tsugane S. Dietary isoflavone and the risk of colorectal adenoma: a case-control study in Japan. *Br J Cancer* 2009; **100**: 1812-1816 [PMID: 19417743 DOI: 10.1038/sj.bjc.6605088]
26 **Kyle JA**, Sharp L, Little J, Duthie GG, McNeill G. Dietary flavonoid intake and colorectal cancer: a case-control study. *Br J Nutr* 2010; **103**: 429-436 [PMID: 19732470 DOI: 10.1017/S0007114509991784]
27 **Rossi M**, Rosato V, Bosetti C, Lagiou P, Parpinel M, Bertuccio P, Negri E, La Vecchia C. Flavonoids, proanthocyanidins, and the risk of stomach cancer. *Cancer Causes Control* 2010; **21**: 1597-1604 [PMID: 20521092 DOI: 10.1007/s10552-010-9588-4]
28 **Rossi M**, Negri E, Parpinel M, Lagiou P, Bosetti C, Talamini R, Montella M, Giacosa A, Franceschi S, La Vecchia C. Proanthocyanidins and the risk of colorectal cancer in Italy. *Cancer Causes Control* 2010; **21**: 243-250 [PMID: 20012183 DOI: 10.1007/s10552-009-9455-3]
29 **Budhathoki S**, Joshi AM, Ohnaka K, Yin G, Toyomura K, Kono S, Mibu R, Tanaka M, Kakeji Y, Maehara Y, Okamura T, Ikejiri K, Futami K, Maekawa T, Yasunami Y, Takenaka K, Ichimiya H, Terasaka R. Soy food and isoflavone intake and colorectal cancer risk: the Fukuoka Colorectal Cancer Study. *Scand J Gastroenterol* 2011; **46**: 165-172 [PMID: 20969489 DOI: 10.3109/00365521.2010.522720]
30 **Ekström AM**, Serafini M, Nyrén O, Wolk A, Bosetti C, Bellocco R. Dietary quercetin intake and risk of gastric cancer: results from a population-based study in Sweden. *Ann Oncol* 2011; **22**: 438-443 [PMID: 20688844]
31 **Zamora-Ros R**, Not C, Guinó E, Luján-Barroso L, García RM, Biondo S, Salazar R, Moreno V. Association between habitual dietary flavonoid and lignan intake and colorectal cancer in a Spanish case-control study (the Bellvitge Colorectal Cancer Study). *Cancer Causes Control* 2013; **24**: 549-557 [PMID: 22588680 DOI: 10.1007/s10552-012-9992-z]
32 **Djuric Z**, Severson RK, Kato I. Association of dietary quercetin with reduced risk of proximal colon cancer. *Nutr Cancer* 2012; **64**: 351-360 [PMID: 22429001 DOI: 10.1080/01635581.2012.658950]
33 **Hirvonen T,** Virtamo J, Korhonen P, Albanes D, Pietinen P. Flavonol and flavone intake and the risk of cancer in male smokers (Finland). *Cancer Causes Control* 2001; **12:** 789-796 [PMID: 11714106]
34 **Knekt P**, Kumpulainen J, Järvinen R, Rissanen H, Heliövaara M, Reunanen A, Hakulinen T, Aromaa A. Flavonoid intake and risk of chronic diseases. *Am J Clin Nutr* 2002; **76**: 560-568 [PMID: 12198000]
35 **Lin J**, Zhang SM, Wu K, Willett WC, Fuchs CS, Giovannucci E. Flavonoid intake and colorectal cancer risk in men and women. *Am J Epidemiol* 2006; **164**: 644-651 [PMID: 16923774 DOI: 10.1093/aje/kwj296]
36 **Akhter M**, Inoue M, Kurahashi N, Iwasaki M, Sasazuki S, Tsugane S. Dietary soy and isoflavone intake and risk of colorectal cancer in the Japan public health center-based prospective study. *Cancer Epidemiol Biomarkers Prev* 2008; **17**: 2128-2135 [PMID: 18708407 DOI: 10.1158/1055-9965.EPI-08-0182]
37 **Mursu J**, Nurmi T, Tuomainen TP, Salonen JT, Pukkala E, Voutilainen S. Intake of flavonoids and risk of cancer in Finnish men: The Kuopio Ischaemic Heart Disease Risk Factor Study. *Int J Cancer* 2008; **123**: 660-663 [PMID: 18338754 DOI: 10.1002/ijc.23421]
38 **Simons CC**, Hughes LA, Arts IC, Goldbohm RA, van den Brandt PA, Weijenberg MP. Dietary flavonol, flavone and catechin intake and risk of colorectal cancer in the Netherlands Cohort Study. *Int J Cancer* 2009; **125**: 2945-2952 [PMID: 19530252 DOI: 10.1002/ijc.24645]
39 **Wang L**, Lee IM, Zhang SM, Blumberg JB, Buring JE, Sesso HD. Dietary intake of selected flavonols, flavones, and flavonoid-rich foods and risk of cancer in middle-aged and older women. *Am J Clin Nutr* 2009; **89**: 905-912 [PMID: 19158208 DOI: 10.3945/ajcn.2008.26913]
40 **Yang G**, Shu XO, Li H, Chow WH, Cai H, Zhang X, Gao YT, Zheng W. Prospective cohort study of soy food intake and colorectal cancer risk in women. *Am J Clin Nutr* 2009; **89**: 577-583 [PMID: 19073792 DOI: 10.3945/ajcn.2008.26742]
41 **Ward HA**, Kuhnle GG, Mulligan AA, Lentjes MA, Luben RN, Khaw KT. Breast, colorectal, and prostate cancer risk in the European Prospective Investigation into Cancer and Nutrition-Norfolk in relation to phytoestrogen intake derived from an improved database. *Am J Clin Nutr* 2010; **91**: 440-448 [PMID: 20007303 DOI: 10.3945/ajcn.2009.28282]
42 **Hara A**, Sasazuki S, Inoue M, Iwasaki M, Shimazu T, Sawada N, Yamaji T, Tsugane S. Isoflavone intake and risk of gastric cancer: a population-based prospective cohort study in Japan. *Am J Clin Nutr* 2012; **95**: 147-154 [PMID: 22170362 DOI: 10.3945/ajcn.111.020479]
43 **Mutoh M**, Takahashi M, Fukuda K, Komatsu H, Enya T, Matsushima-Hibiya Y, Mutoh H, Sugimura T, Wakabayashi K. Suppression by flavonoids of cyclooxygenase-2 promoter-dependent transcriptional activity in colon cancer cells: structure-activity relationship. *Jpn J Cancer Res* 2000; **91**: 686-691 [PMID: 10920275 DOI: 10.1111/j.1349-7006.2000.tb01000.x]
44 **Suh Y**, Afaq F, Johnson JJ, Mukhtar H. A plant flavonoid fisetin induces apoptosis in colon cancer cells by inhibition of COX2 and Wnt/EGFR/NF-kappaB-signaling pathways. *Carcinogenesis* 2009; **30**: 300-307 [PMID: 19037088 DOI: 10.1093/carcin/bgn269]
45 **So FV**, Guthrie N, Chambers AF, Moussa M, Carroll KK. Inhibition of human breast cancer cell proliferation and delay of mammary tumorigenesis by flavonoids and citrus

juices. *Nutr Cancer* 1996; **26**: 167-181 [PMID: 8875554 DOI: 10.1080/01635589609514473]
46 **Dai J**, Mumper RJ. Plant phenolics: extraction, analysis and their antioxidant and anticancer properties. *Molecules* 2010; **15**: 7313-7352 [PMID: 20966876 DOI: 10.3390/molecules15107313]
47 **Magnani L,** Gaydou EM, Hubaud JC. Spectrophotometric measurement of antioxidant properties of flavones and flavonols against superoxide anion. *Anal Chim Acta* 2000; **411:** 209-216 [DOI: 10.1016/S0003-2670(00)00717-0]
48 **Heim KE**, Tagliaferro AR, Bobilya DJ. Flavonoid antioxidants: chemistry, metabolism and structure-activity relationships. *J Nutr Biochem* 2002; **13**: 572-584 [PMID: 12550068]
49 **Riboli E**, Norat T. Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk. *Am J Clin Nutr* 2003; **78**: 559S-569S [PMID: 12936950]
50 **Lunet N**, Lacerda-Vieira A, Barros H. Fruit and vegetables consumption and gastric cancer: a systematic review and meta-analysis of cohort studies. *Nutr Cancer* 2005; **53**: 1-10 [PMID: 16351501 DOI: 10.1207/s15327914nc5301_1]
51 **Watkin B**. A question of attitudes. *Nurs Mirror Midwives J* 1976; **142**: 39 [PMID: 1045344 DOI: 10.1207/S15327914NC340113]
52 **González-Segovia R**, Quintanar JL, Salinas E, Ceballos-Salazar R, Aviles-Jiménez F, Torres-López J. Effect of the flavonoid quercetin on inflammation and lipid peroxidation induced by Helicobacter pylori in gastric mucosa of guinea pig. *J Gastroenterol* 2008; **43**: 441-447 [PMID: 18600388 DOI: 10.1007/s00535-008-2184-7]
53 **Seelinger G**, Merfort I, Wölfle U, Schempp CM. Anti-carcinogenic effects of the flavonoid luteolin. *Molecules* 2008; **13**: 2628-2651 [PMID: 18946424 DOI: 10.3390/molecules13102628]
54 **Ko CH**, Shen SC, Lee TJ, Chen YC. Myricetin inhibits matrix metalloproteinase 2 protein expression and enzyme activity in colorectal carcinoma cells. *Mol Cancer Ther* 2005; **4**: 281-290 [PMID: 15713899]
55 **Fini L**, Hotchkiss E, Fogliano V, Graziani G, Romano M, De Vol EB, Qin H, Selgrad M, Boland CR, Ricciardiello L. Chemopreventive properties of pinoresinol-rich olive oil involve a selective activation of the ATM-p53 cascade in colon cancer cell lines. *Carcinogenesis* 2008; **29**: 139-146 [PMID: 17999988 DOI: 10.1093/carcin/bgm255]
56 **Fini L**, Selgrad M, Fogliano V, Graziani G, Romano M, Hotchkiss E, Daoud YA, De Vol EB, Boland CR, Ricciardiello L. Annurca apple polyphenols have potent demethylating activity and can reactivate silenced tumor suppressor genes in colorectal cancer cells. *J Nutr* 2007; **137**: 2622-2628 [PMID: 18029474]
57 **Fini L**, Piazzi G, Daoud Y, Selgrad M, Maegawa S, Garcia M, Fogliano V, Romano M, Graziani G, Vitaglione P, Carmack SW, Gasbarrini A, Genta RM, Issa JP, Boland CR, Ricciardiello L. Chemoprevention of intestinal polyps in ApcMin/+ mice fed with western or balanced diets by drinking annurca apple polyphenol extract. *Cancer Prev Res* (Phila) 2011; **4**: 907-915 [PMID: 21383028 DOI: 10.1158/1940-6207.CAPR-10-0359]
58 **D'Argenio G**, Mazzone G, Tuccillo C, Grandone I, Gravina AG, Graziani G, Fogliano V, Romano M. Apple polyphenol extracts prevent aspirin-induced damage to the rat gastric mucosa. *Br J Nutr* 2008; **100**: 1228-1236 [PMID: 18482463 DOI: 10.1017/S0007114508988747]
59 **Romano M**, Vitaglione P, Sellitto S, D'Argenio G. Nutraceuticals for protection and healing of gastrointestinal mucosa. *Curr Med Chem* 2012; **19**: 109-117 [PMID: 22300083]
60 USDA database for the flavonoid contents of selected foods, Release 3 (2003)
61 **Chun OK**, Chung SJ, Song WO. Estimated dietary flavonoid intake and major food sources of U.S. adults. *J Nutr* 2007; **137**: 1244-1252 [PMID: 17449588]
62 **Kyle J,** Duthie G. Flavonoids in foods. In: Andersen ØM, Markham KR, editors. Flavonoids: Chemistry, biochemistry and applications. Boca Raton: CRC Press, 2006

P- Reviewers Lin J, Passamonti S, Romano M
S- Editor Song XX **L- Editor** Cant MR **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i7.1020

World J Gastroenterol 2013 February 21; 19(7): 1020-1029
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

TOPIC HIGHLIGHT

Jung Eun Lee, ScD, Assistant Professor, *Series Editor*

Consumption of red and processed meat and esophageal cancer risk: Meta-analysis

Yuni Choi, Sujin Song, Yoonju Song, Jung Eun Lee

Yuni Choi, Jung Eun Lee, Department of Food and Nutrition, Sookmyung Women's University, Seoul 140-742, South Korea
Sujin Song, Department of Food and Nutrition, Seoul National University, Seoul 151-742, South Korea
Yoonju Song, School of Human Ecology, Catholic University of Korea, Bucheon, Gyeonggi Do 420-743, South Korea
Author contributions: Choi Y and Lee JE designed the study; Choi Y drafted the manuscript and conducted data analysis; Choi Y, Song S and Lee JE contributed to the selection of studies and data extraction, and the interpretation of the results; all authors critically reviewed the manuscript and gave final approval of the version to be published.
Supported by The Sookmyung Women's University Research Grant (2012)
Correspondence to: Dr. Jung Eun Lee, Department of Food and Nutrition, Sookmyung Women's University, 52 Hyochang-won-gil, Youngsan-gu, Seoul 140-742, South Korea. junglee@sm.ac.kr
Telephone: +82-2-20777560 Fax: +82-2-7109479
Received: September 19, 2012 Revised: November 19, 2012
Accepted: December 5, 2012
Published online: February 21, 2013

Abstract

To summarize the evidence about the association between red and processed meat intake and the risk of esophageal cancer, we systematically searched the PubMed and EMBASE databases up to May 2012, with a restriction to English publications, and the references of the retrieved articles. We combined the study-specific relative risks (RRs) and 95%CI, comparing the highest with the lowest categories of consumption by using a random-effects model. A total of 4 cohort studies and 23 case-control studies were included in the meta-analysis. The combined RRs (95%CI) of the cohort studies comparing the highest and lowest categories were 1.26 (1.00-1.59) for red meat and 1.25 (0.83-1.86) for processed meat. For the case-control studies, the combined RRs (95%CI) comparing the highest and lowest categories were 1.44 (1.16-1.80) for red meat and 1.36 (1.07-1.74) for processed meat. Findings from this meta-analysis suggest that a higher consumption of red meat was associated with a greater risk of esophageal cancer.

© 2013 Baishideng. All rights reserved.

Key words: Cohort study; Case-control study; Meta-analysis; Red meat; Processed meat; Esophageal cancer

Choi Y, Song S, Song Y, Lee JE. Consumption of red and processed meat and esophageal cancer risk: Meta-analysis. *World J Gastroenterol* 2013; 19(7): 1020-1029 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i7/1020.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i7.1020

INTRODUCTION

The incidence rate of esophageal cancer ranked eighth worldwide, accounting for 3.8% of all new cancers, and its mortality rate ranked sixth, accounting for 5.4% of all cancer deaths in 2008[1]. The most predominant histological types of esophageal cancer are esophageal squamous cell carcinoma (ESCC) and esophageal adenocarcinoma (EAC), representing distinct characteristics in patterns of cancer development and risk factors[2].

Given that mutagenic compounds such as heterocyclic amines (HCAs), polycyclic aromatic hydrocarbons (PAHs), and N-nitroso compounds (NOCs) generated from red and processed meats were associated with cancer development[3], concerns about a high incidence of esophageal cancer related to a high consumption of red and processed meats have been increasing. In 2007, a consensus report of experts assembled by the World Cancer Research Fund (WCRF) and the American Institute for Cancer Research (AICR)[4] concluded through review of studies published up to 2004 that there were suggestive but inconclusive associations between red and processed meat consumption

and esophageal cancer risk. The WCRF/AICR expert report also indicated that the lack of consistent results may be because of insufficient data, especially from prospective cohort studies. Another review of studies published up to 2005[5] suggested a possible increased risk of esophageal cancer with processed meat (9 case-control studies) and combined white and red meat (2 cohort and 18 case-control studies); however, this study concluded that more prospective data involving a larger number of cases would be needed to determine the association between meat consumption and the risk of esophageal cancer.

Since the completion of the two reviews, the results of the large prospective studies as well as new or updated case-control studies that examined association between red and processed meats and esophageal cancer risk have been published, but no meta-analysis of the prospective cohort studies has been reported. We, therefore, performed a meta-analysis of large prospective cohort and case-control studies to summarize the association between red and processed meat intake and the risk of esophageal cancer. We also quantified the dose-response relationships in the analysis of the cohort studies.

SEARCH STRATEGY

Two authors (Choi Y, Song S) independently performed a systematic search of published articles using the PubMed and EMBASE databases up to May 2012[6]. We used the following search terms: "oesophageal or esophageal or esophagus or oesophagus" and "cancer or neoplasm or carcinoma" and "cohort or prospective or case-control" and "food or diet or meat". We also reviewed the reference lists from the retrieved articles and those from previous review studies to identify additional relevant studies that may not have been identified by our database searches.

INCLUSION CRITERIA

Studies were included in our meta-analysis if they met the following criteria: (1) either a cohort or case-control design was used; (2) relative risk (RR) estimates and the 95%CI were provided for the association between red and/or processed meat intake and esophageal cancer; (3) the outcomes of interest were either the overall incidence of esophageal cancer or the two main histological subtypes, ESCC or EAC; and (4) the study was published in English. We included studies that reported the associations of esophageal cancer with exposures identified as "red meat" or "processed meat" and individual food items within the two groups. Studies generally included beef, pork, minced meat, lamb, veal, and offal (*e.g.*, liver, kidney) for unprocessed red meat and sausage, ham, bacon, salami, luncheon meat, or frankfurters, and any types of meat that were processed by smoking, curing, salting, or the addition of preservatives for processed meat. We excluded studies providing no apparent classification of meat or studies reporting a combination of red and white meat (*e.g.*, poultry). If data were duplicated in more than 1 study, the latest studies were included.

DATA EXTRACTION

We independently extracted the following data from each study, according to the meta-analysis of observational studies in epidemiology guidelines[6], and any discrepancies were resolved by discussion: the first author's last name, the publication year, the country where the study was conducted, the study period, the age range of the subjects, the number of cases and controls or the cohort size, the measures and comparison levels of the exposures, the multivariate adjusted RRs with corresponding 95%CI for the highest *vs* lowest categories of red or processed meat intake, and the variables that were adjusted for in the analysis. For each study, we used the most fully adjusted RRs in the multivariate model. Any disagreements were resolved through consensus. The same two authors assessed the quality of the studies based on the Newcastle-Ottawa Scale, which ranged from 1 to 9 stars[7]. The average score for each study was used in the analysis.

STATISTICAL ANALYSIS

We conducted separate meta-analyses for case-control and cohort studies, using results that compared red and processed meat intake as well as those that assessed each type individually. We also performed a meta-analysis combining both case-control and cohort studies. Using a random-effects model that considered both within and between study variation[8], we combined the study-specific multivariate RRs and 95%CI, comparing the highest and the lowest categories of red and processed meat intake.

We assessed the statistical heterogeneity among the studies by using Q and I^2 statistics[9], where significance was reached at $P < 0.1$. Publication bias was evaluated by using the Egger asymmetry test[10], with significant level at $P < 0.05$. We investigated the potential sources of heterogeneity among the studies by conducting subgroup and meta-regression analyses for histological subtype (ESCC and EAC), sex (males, females, and both sexes), study location (Asia, Europe, North America, and South America), study quality, and confounders adjusted for in the analysis [alcohol, smoking, body mass index (BMI), and fruit and/or vegetable]. We also conducted the sensitivity analysis for case-control and cohort studies separately, omitting each study individually to evaluate whether the results could have been affected substantially by any one study.

In a sensitivity analysis, we estimated a dose-response for combined RRs for 100 g/d increments of red or processed meats for 3 cohort studies[11-13], which are less prone to selection or recall bias than case-control studies. We did not include one study (Yu *et al*[14]), that presented binary categories of exposure for a dose-response analysis. For two studies[11,13], the estimates were rescaled into 100 g/d increments. All statistical analyses were performed with Stata software, version 11 (Stata Corp., College Station, TX, United States). $P < 0.05$ considered statistically significant.

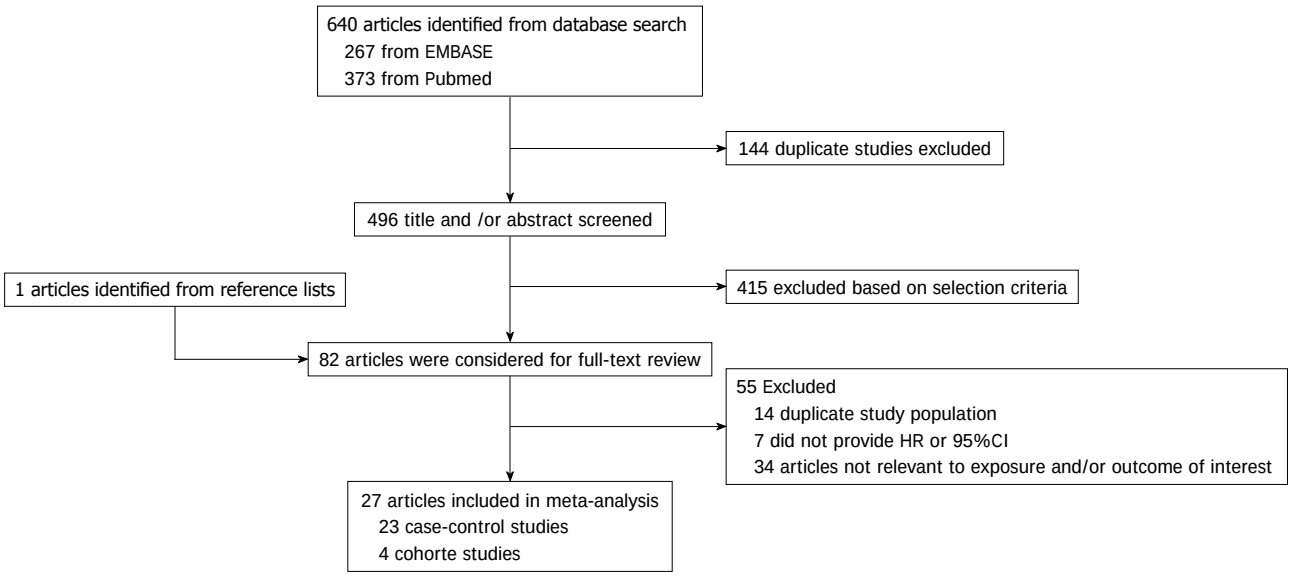


Figure 1 Selection of studies included in meta-analysis.

LITERATURE SEARCH

The preliminary literature search yielded 640 articles. Of these, 81 articles and 1 additional article identified from the reference lists were considered for further review (Figure 1). After the full-text review, 7 articles that did not provide RRs or 95%CI, 14 articles that used duplicated study populations, and 34 articles that were unrelated to exposure or outcomes of interest were excluded. A total of 27 articles were included in the meta-analysis; 22 articles (4 cohort and 18 case-control studies) that reported findings on red meat and 18 articles (3 cohorts and 15 case-controls) that reported findings on processed meat were included in the meta-analysis.

RED MEAT INTAKE

We identified 4 cohorts studies[11-14] involving 2324 cases and 1 149 981 participants and 18 case-control studies[15-32] involving 5165 cases and 26 350 control subjects (Table 1). Two of the 22 studies reported results for both ESCC and EAC, 16 studies reported the results for either EAC or ESCC, and 6 reported results for overall esophageal cancer without the histological subtypes. Six studies were conducted in Asia, 6 in Europe, 7 in United States, and 3 in South America. The studies used either a food frequency questionnaire (FFQ) or a structured questionnaire form to measure red meat intake. Fifteen studies provided RR estimates that were adjusted for alcohol intake, 16 for smoking habit, 12 for BMI, and 7 for fruit and/or vegetable intake. Eight studies were given a score of 7 stars or above, representing a high quality of studies[7]. The combined RRs (95%CI) comparing the highest and lowest categories of red meat intake were 1.26 (1.00-1.59) for the 4 cohort studies and 1.44 (1.16-1.80) for the 18 case-control studies (Figure 2A). There was no evidence of heterogeneity among the cohort studies ($P = 0.15$, $I^2 = 35.3\%$), but there was a heterogeneity among the case-control studies ($P < 0.01$, $I^2 = 72.8\%$). Combining the two types of study design resulted in an overall combined RR of 1.38 (95%CI: 1.17-1.64; P for heterogeneity: $P < 0.01$, $I^2 = 67.1\%$). Excluding a single study did not substantially influence the combined estimates of the cohort or case-control studies. There was no statistical evidence of publication bias according to the Egger asymmetry test ($P = 0.79$ for cohort studies and $P = 0.34$ for case-control studies). Dose-response associations were examined in 3[11-13] of 4 cohort studies, showing the combined RRs of 1.05 (95%CI: 0.91-1.21; P for heterogeneity = 0.42, $I^2 = 0.2\%$) for every 100 g/d increment of red meat intake. The associations did not vary significantly by histological subtypes, study location, sex, and study quality (Table 2). In addition, the associations did not differ by adjusted confounding factors including alcohol, smoking, BMI, and fruit and vegetable intakes (data not shown).

PROCESSED MEAT INTAKE

We conducted a meta-analysis of 3 cohort studies[11-13], which included 1162 cases and 1 137 288 participants and 15 case-control studies[15,16,19-21,24,25,27,30,32-37], which included 3851 cases and 10 064 controls (Table 1). Two of the 18 studies examined both ESCC and EAC as the primary endpoints, 13 studies reported the results for either EAC or ESCC and 5 did not differentiate between histological subtypes. Five studies were conducted in Asia, 7 in Europe, 5 in United States, and 1 in South America. The studies used either a FFQ or a structured questionnaire form to measure processed meat intake. Fourteen studies provided RR estimates that were adjusted for alcohol intake, 15 for smoking habit, 10 for BMI, and 8 for fruit and/or vegetable intake. Six studies were given a score of 7 or greater, indicating a high methodological quality[7].

Table 1 Characteristics of the studies included in the meta analysis

Ref.	Study period	Sex	No. of cases	No. cohorts or controls	Dietary assessment	Exposure and comparison level	Adjusted RR (95%CI)	Study quality[1]	Adjustment for confounders
Cohort studies									
Keszei *et al*[11]	1986-2002	M	ESCC: 107	120 852	FFQ 150 items	Red meat		9	Age, smoking (including years and numbers per day), total energy, BMI, alcohol drinking, vegetable, fruit, education, non-occupational PA
		F	EAC: 145			ESCC			
		M				Q5 *vs* Q1	2.66 (0.94-7.48)		
		F				T3 *vs* T1	0.87 (0.42-1.79)		
						EAC			
		M				Q5 *vs* Q1	0.57 (0.28-1.19)		
		F				T3 *vs* T1	1.09 (0.44-2.75)		
						Processed meat			
						ESCC			
		M				Q5 *vs* Q1	3.47 (1.21-9.94)		
		F				T3 *vs* T1	0.63 (0.28-1.44)		
						EAC			
		M				Q5 *vs* Q1	0.94 (0.46-1.89)		
		F				T3 *vs* T1	0.58 (0.22-1.50)		
Cross *et al*[12]	1995-2006	C	ESCC: 215	494 979	FFQ 124 items	Red meat (Q5 *vs* Q1)		9	Age, sex, BMI, education, ethnicity, smoking, alcohol drinking, PA at work, vigorous PA, daily intakes of fruit, vegetable, saturated fat, energy
			EAC: 630			ESCC	1.79 (1.07-3.01)		
						EAC	1.15 (0.84-1.57)		
						Processed meat (Q5 *vs* Q1)			
						ESCC	1.32 (0.83-2.10)		
						EAC	1.08 (0.81-1.43)		
González *et al*[13]	1992-1998	C	EAC: 65	521 457	FFQ 88-266 items	Red meat (T3 *vs* T1)	1.67 (0.75-3.72)	8	Sex, height, weight, education, smoking, smoking intensity, work and leisure PA, intakes of alcohol, energy, vegetable, citrus fruit, non-citrus fruit, types of meat intake were mutually adjusted
						Processed meat (T3 *vs* T1)	3.54 (1.57-7.99)		
Yu *et al*[14]	1974-1989	C	All: 1162	12 693	Questionnaire 15 items	Pork (never *vs* regular/occasional)	1.37 (1.11-1.68)	7	Age, sex
Case-control studies									
Ward *et al*[15]	1988-1993	C	EAC: 124	449	Questionnaire 100 items	Red meat (> 157.2 g/d *vs* ≤ 73.8 g/d)	2.85 (1.00-8.16)	5	Age, sex, race, vital status, year of birth, sex, No. of cigarettes per day, BMI, intakes of retinoic acid, folate, riboflavin, zinc, carbohydrate, protein, total energy.
						Processed meat (> 52.3 g/d *vs* ≤ 16.1 g/d)	1.40 (0.62-3.15)		
De Stefani *et al*[16]	1996-2004	C	ESCC: 234	2020	FFQ 64 items	Red meat (T3 *vs* T1)	4.97 (2.98-8.29)	7	Age, sex, residence, education, BMI, smoking, drinking, mate temperature, total energy, total intakes of vegetable and fruit, scored pattern
						Processed meat (T3 *vs* T1)	0.76 (0.51-1.13)		
Gao *et al*[17]	1997-2005	C	ESCC: 600	1514	Questionnaire 35 items	Red meat (> weekly *vs* monthly/seldom/never)	1.37 (1.03-1.82)	5	Age, sex, geographic region
Wu *et al*[18]	2003-2007	C	All: 1495	3819	FFQ	Red meat (Q4 *vs* Q1)	1.13 (0.94-1.36)	7	Age, sex, education, previous income, BMI, pack-years smoking, weekly ethanol intake, study area
Hajizadeh *et al*[19]	N/A	C	ESCC: 47	96	FFQ 168 items	Red meat (T3 *vs* T1)	2.47 (0.76-7.96)	6	Age, sex, education, tobacco smoking, symptomatic gastroesophageal reflux, BMI, total energy
						Processed meat (T3 *vs* T1)	1.10 (0.36-2.47)		
O'Doherty *et al*[20]	2002-2005	C	EAC: 221	256	FFQ 101 items	Red meat (Q4 *vs* Q1)	3.15 (1.38-7.20)	7	Age, sex, smoking, BMI 5 yr before interview date, education, job type, Intakes of energy, fruit, vegetable, alcohol (g/d), *Helicobacter pylori* infection, nonsteroidal anti-inflammatory drug use 5 yr before, interview date, gastroesophageal reflux symptoms, location, types of meat intake were mutually adjusted
						Processed meat (Q4 *vs* Q1)	1.41 (0.67-2.95)		

Sapkota *et al*[21]	1999-2003	C	ESCC: 187	1110	Questionnaire 23 items	Red meat (≥ 1/wk *vs* < 1/mo) Processed meat (≥ 1 time/wk *vs* < 1 time/mo)	0.62 (0.19-2.09) 1.12 (0.52-2.41)	6	Age, sex, country, tobacco pack-year, education, BMI, frequency of alcohol consumption, vegetable, fruit consumption
Navarro Silvera *et al*[22]	1993-1995	C	EAC: 282	687	FFQ 104 items	Red meat (high *vs* low)	3.02 (1.65-5.52)	7	Age, sex, study site, race, proxy status, income, education, BMI, No. of smoking cigarettes per day, intakes of beer, wine, liquor, and energy
Wang *et al*[23]	2004-2006	M F	ESCC: 355	408	Questionnaire	Pork (often *vs* none/seldom)	2.06 (1.42-2.99) 1.91 (1.16-3.16)	5	Age, sex, marital status, education
Wu *et al*[24]	1992-1997	C	EAC: 206	1308	Questionnaire 124 items	Red meat (Q4 *vs* Q1) Processed meat (Q4 *vs* Q1)	1.29 (0.8-2.2) 1.23 (0.7-2.1)	5	Age, sex, race, birthplace, education, smoking, BMI, reflux, use of vitamins, total energy
Chen *et al*[25]	1988-1993	C	EAC: 124	449	Questionnaire 54 items	Red meat (Q4 *vs* Q1) Processed meat (Q4 *vs* Q1)	1.4 (0.61-3.2) 1.7 (0.71-3.9)	7	Age, sex, energy intake, respondent type, BMI, alcohol drinking, smoking, education, family history, vitamin supplement use, age squared for EAC
Takezaki *et al*[26]	1988-1997	M	All: 284	11 888	Questionnaire	Beef (≥ 3/wk *vs* ≤ 3/mo)	0.9 (0.6-1.5)	5	Age, year and season of visit, smoking, drinking
Bosetti *et al*[27]	1992-1997	C	ESCC:304	743	FFQ 78 items	Red meat (Q5 *vs* Q1) Processed meat (Q5 *vs* Q1)	1.93 (1.09-3.41) 1.39 (0.85-2.26)	5	Age, sex, area of residence, education, tobacco smoking, alcohol drinking, non-alcohol energy
Rolón *et al*[28]	1988-1991	C	All: 131	379	FFQ	Red meat (highest *vs* lowest)	3.8 (1.3-11.0)	5	Age, sex, alcohol, smoking, design variable of the study, hospital group, intakes of red meats, fats, fish, milk
Castelletto *et al*[29]	1986-1989	C	ESCC: 131	261	FFQ 10 food groups	Beef (≥ daily *vs* < daily)	0.6 (0.3-0.9)	6	Age, sex, design variable, hospital, education, No. of cigarettes smoking per day, intakes of alcohol, barbecued meat, potatoes, raw vegetables, cooked vegetables
Tavani *et al*[30]	1984-1992	C	All: 46	230	FFQ 14 items	Ham (Q3 *vs* Q1) Liver (Q2 *vs* Q1)	1.4 (0.6-3.3) 1.1 (0.5-2.3)	5.5	Age, sex, education, total alcohol intake
Rogers *et al*[31]	1983-1987	C	All: 127	466	FFQ 125 items	Beef (≥ 1/wk *vs* < 1/wk) As a main dish As a sandwich Pork (≥ 1/wk *vs* < 1/wk)	 0.8 (0.4-1.4) 1.0 (0.6-1.7) 1.2 (0.8-2.5)	5	Age, sex, pack-years of cigarette, drink-years of alcohol, energy intake, beta-carotene intake, ascorbic acid intake
Yu *et al*[32]	1975-1981	C	Beef: 267 Fried bacon or ham: 265 Barbecued or smoked meat: 268	Beef: 267 Fried bacon or ham: 265 Barbecued or smoked meat: 268	Questionnaire 10 food groups	Beef (≥ 5/wk *vs* ≤ 1/wk) Fried bacon or ham (≤ 1/wk *vs* ≥ 5/wk) Barbecued or smoked meat (≥ 2/wk *vs* ≤ 1/wk)	1.3 (0.6-2.7) 2.0 (1.1-3.5) 1.7 (0.9-3.0)	5	Age, sex, race
Chen *et al*[33]	1996-2005	M	ESCC: 320	709	Questionnaire 6 items	Cured meat (≥ 1/wk *vs* < 1/wk)	0.8 (0.4-1.4)	5	Age, educational level, ethnicity, source of hospital, smoking, alcohol drinking, areca nut chewing
Yang *et al*[34]	2003-2004	C	All: 185	185	Questionnaire 9 Items	Processed meat (> 3 meals/wk *vs* < 1 meal/wk)	0.66 (0.31-1.41)	5.5	Family history of esophageal cancer, occupation, smoking, drinking, eating hot food, eating speed, intakes of vegetables, fruit, pickled vegetables, fresh meat, egg, tea, water supply
Levi *et al*[35]	1992-2002	C	All:138	660	FFQ 79 items	Processed meat (> 3.2 freq/wk *vs* < 0.8 freq/wk)	4.48 (2.05-9.79)	6	Age, sex, education, smoking, intakes of alcohol, energy, fruit and vegetable intake
Li *et al*[36]	1997-2000	C	All:1248	1248	Questionnaire 12 items	Sowbelly (daily *vs* < 1/wk)	2.28 (1.6-3.3)	5	Age, sex, income, residence, occupation, alcohol, tobacco

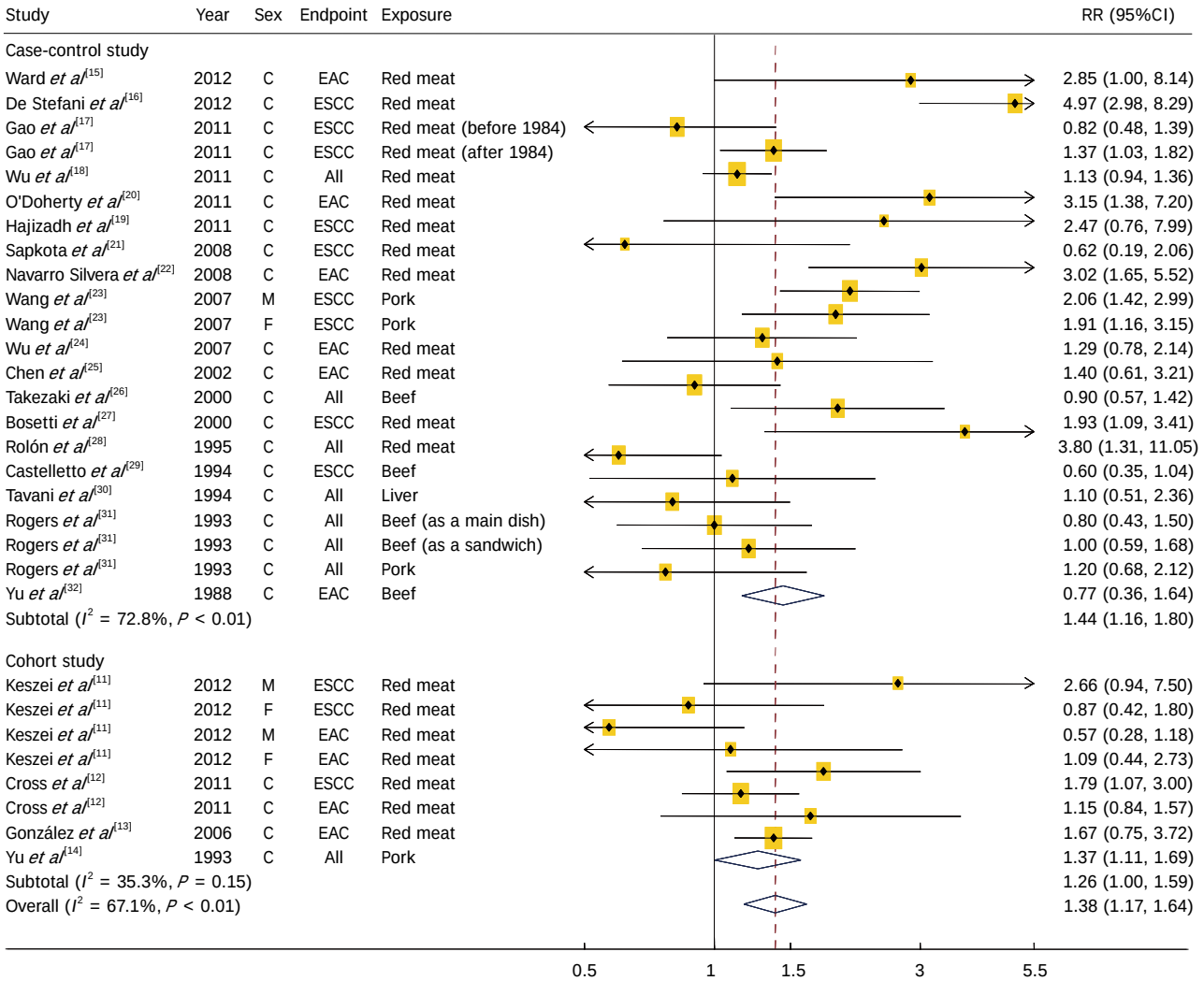
[1]Study quality was assessed using the Newcastle-Ottawa Scale (range: 1-9 stars). RR: Relative risk; M: Male; F: Female; C: Combined males and females; ESCC: Esophageal squamous cell carcinoma; EAC: Esophageal adenocarcinoma; FFQ: Food frequency questionnaire; BMI: Body mass index; PA: Physical activity; N/A: Not available.

Table 2 Combined relative risks and 95%CI for esophageal cancer associated with red meat or processed meat by other factors in both cohort and case-control studies

Factors	Red meat				Processed meat			
	Studies (*n*)	Ref.	RR (95%CI)	*P* for heterogeneity	Studies (*n*)	Ref.	RR (95%CI)	*P* for heterogeneity
Histological subtypes								
EAC	9	[11-13,15,20,22,24,25,32]	1.42 (1.02-1.98)	0.19	8	[11-13,15,20,24,25,32]	1.38 (1.07-1.78)	0.3
ESCC	9	[11,12,16,17,19,21,23,27,29]	1.55 (1.10-2.17)		7	[11,12,16,19,21,27,33]	1.08 (0.80-1.44)	
Study location								
Asia	6	[14,17,18,19,23,26]	1.33 (1.09-1.62)	0.67	5	[19,33,34,36,37]	1.09 (0.61-1.95)	0.65
Europe	6	[11,13,20,21,27,30]	1.33 (0.86-2.07)		7	[11,13,20,21,27,30,35]	1.49 (0.99-2.23)	
United States	7	[12,15,22,24,25,31,32]	1.32 (1.03-1.70)		5	[12,15,21,25,32]	1.30 (1.08-1.57)	
South America	3	[16,28,29]	2.20 (0.48-10.04)		1	[16]	0.76 (0.51-1.13)	
Sex								
Male	3	[11,23,26]	1.26 (0.66-2.41)	0.88	2	[11,33]	1.24 (0.58-2.65)	0.14
Female	2	[11,23]	1.31 (0.78-2.21)		1	[11]	0.61 (0.33-1.13)	
Both	19	[12-22,24,25,27-32]	1.42 (1.17-1.71)		16	[12,13,15,16,19-21,24,25,27,30,32,34-37]	1.43 (1.15-1.77)	
Study quality[1]								
≥ 7	8	[11-14,16,18,20,22]	1.60 (1.20-2.13)	0.23	6	[11-13,16,20,25]	1.20 (0.88-1.62)	0.42
< 7	14	[15,17,19,21,23-32]	1.25 (1.02-1.54)		12	[15,19,21,24,27,30,32-37]	1.43 (1.11-1.86)	

[1]Study quality was assessed using the Newcastle-Ottawa Scale (range, 1-9 stars); RR: Relative risk; ESCC: Esophageal squamous cell carcinoma; EAC: Esophageal adenocarcinoma.

A



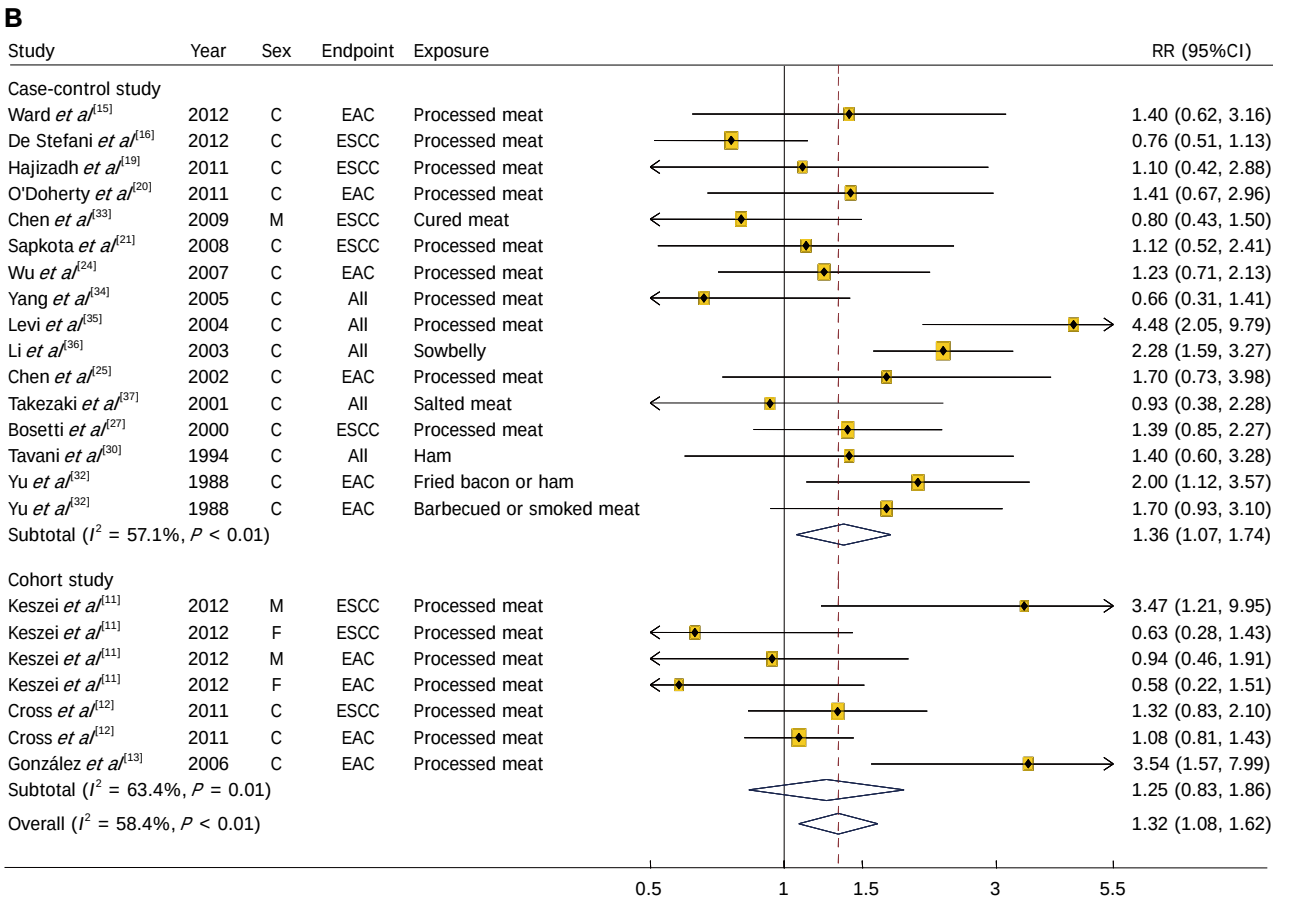


Figure 2 The combined relative risks and 95%CI of esophageal cancer risk for the highest *vs* lowest categories of red meat (A) and processed meat (B). M: Male; F: Female; C: Combined males and females; ESCC: Esophageal squamous cell carcinoma; EAC: Esophageal adenocarcinoma.

In a meta-analysis of the 15 case-control studies, we found that the highest categories of processed meat intake were associated with a 36% increase in esophageal cancer risk when compared with the lowest categories (95%CI: 1.07-1.74; Figure 2B); however, we found a non-significant, positive association when we examined only the cohort studies (RR: 1.25; 95%CI: 0.83-1.86). When we examined whether an individual study was the source of heterogeneity among either the cohort or case-control studies, there were heterogeneities between the case-control studies (P < 0.01, I^2 = 57.1%) and the cohort studies (P = 0.01, I^2 = 63.4%). When the results from the cohort and case-control studies were combined, the overall combined RR comparing the highest and the lowest category of processed meat was 1.32 (95%CI: 1.08-1.62; P for heterogeneity: P < 0.01, I^2 = 58.4%). The heterogeneity observed between the prospective studies of processed meat intake and esophageal cancer risk was no longer significant (P = 0.12) after excluding a study by González *et al*[13]. However, excluding any one case-control study from the analysis did not influence the heterogeneity findings observed among case-control studies.

No publication bias was found for either the cohort or case-control studies (P = 0.65 for the cohort studies and P = 0.80 for the case-control studies). In a dose-response meta-analysis of 3 cohort studies, we found that each 100 g/d increase in processed meat intake was positively, but not significantly, associated with esophageal cancer risk (RR: 1.37; 95%CI: 0.88-2.13). There was no evidence of heterogeneity (P = 0.17, I^2 = 33.5%).

When stratifying the analyses by histological subtypes, study location, sex, and study quality, we found no significant differences in the associations, although the magnitude of the associations differed slightly in these subgroups (Table 2). The associations also did not vary by adjusted confounding factors including alcohol, smoking, BMI, and fruit and vegetable intakes (data not shown).

DISCUSSION

To our knowledge, this is the first systematic meta-analysis of cohort and case-control studies to summarize the evidence regarding the association between red or processed meat intake and the risk of esophageal cancer. High red meat consumption was associated with a 38% higher risk of esophageal cancer compared to low consumption in a meta-analysis of both case-control and cohort studies. A 26% higher risk of esophageal cancer was observed among those who had high red meat intake compared to those with low intake in a meta-analysis of 4 cohort studies. With regard to processed meat, we found a higher risk of esophageal cancer with high processed

meat intake compared to low intake in a meta-analysis of case-control studies, but the combined estimate of cohort studies did not reach statistical significance. Prospective cohort studies are less prone to selection or recall bias compared to case-control studies, which is critical in research of diet and cancer etiology. Therefore, a significant association in only the case-control studies and not in the meta-analysis of the 3 cohort studies could not provide adequate supportive evidence of an increased risk associated with processed meat consumption. However, the results for more prospective cohort studies need to be reported to obtain a clearer conclusion.

There are possible underlying mechanisms linking the consumption of red and processed meats and the incidence of cancer. HCAs and PAHs are chemical compounds with mutagenic potential that are formed when meat is boiled, fried, or grilled at high temperatures[3]. Animal studies have suggested that these two mutagenic compounds may induce changes in DNA, possibly promoting carcinogenesis[3,38]. Another class of meat-related mutagen is NOCs, the majority of which are potent carcinogens[39] formed either endogenously or exogenously. Processed meat is typically preserved by adding nitrate or nitrite, which increases the formation of NOCs[3]. Heme iron, largely derived from red meat sources, has been suggested to promote the endogenous formation of NOCs[40]. There is only limited epidemiological evidence, however, to suggest that the dietary intake of nitrite or nitrosamine is positively associated with the risk of esophageal cancer[5]. The esophagus is frequently exposed to these dietary mutagenic and/or carcinogenic compounds as stomach and colon, permitting food to pass from the esophagus into the stomach. While the specific mechanism by which meat causes esophageal cancer has not been fully elucidated, one likely reason may involve the potential for increase the susceptibility to carcinogenesis by repeated exposure of esophagus to the mutagenic and/or carcinogenic compounds, given their effects on carcinogenesis in animal models[3,38,39].

The results from the subgroup and meta-regression analysis could not completely explain the potential sources of between-study heterogeneity because we did not observe statistically significant differences by histological subtype, study location, sex, or study quality. For red meat intake, it appeared that a single study did not substantially influence the overall combined RR, whereas, the observed heterogeneity among the prospective studies of processed meat intake and esophageal cancer risk disappeared when the study by González *et al*[13] was excluded. However, the observed heterogeneity among the case-control studies of processed meat intake and esophageal cancer risk was not martially altered in sensitivity analyses excluding one study at a time.

Our meta-analysis had some limitations. Although the majority of the studies adjusted for known potential confounding factors, there may be a possibility that unidentified or residual confounding factors remained that were not adjusted for in the multivariate analysis or by covariates inadequately measured. Most studies, however, adjusted for alcohol and smoking, both of which are established risk factors for esophageal cancer. Additionally, we found an increased risk of esophageal cancer with high red meat intake in a meta-analysis of well-scored studies, which were relatively recent and adjusted for various potential confounding factors. The random measurement error of meat consumption that occurred during dietary assessment or the systematic error resulting from recall or selection bias in the case-control studies may have influenced our findings; however, we found a statistically significant association between red meat intake and esophageal cancer risk in a meta-analysis of prospective studies, which supports the hypothesis that red meat intake increases the risk of esophageal cancer.

Our meta-analysis also included several strengths. Our meta-analysis updated the recent large prospective and case-control studies with a larger number of cases that were not included in previous reviews. In particular, the inclusion of new data from large cohort studies, which were unavailable when earlier conclusions of these associations were made by the WCRF/AICR expert panel[4] or by a review study[5], enabled us to provide more unbiased evidence compared to the review that included only case-control studies. The findings from this meta-analysis were not subject to publication bias, indicating that the probability of publishing a study did not rely on the strength and direction of the associations.

CONCLUSION

The findings from our meta-analysis of either prospective cohort or case-control studies suggest that a high consumption of red meat may increase the risk of esophageal cancer. Although we found an increased risk in a meta-analysis of the case-control studies for processed meat intake in relation to esophageal cancer risk, the prospective cohort studies did not strongly support this evidence. There is a need for further large scale prospective studies to determine whether processed meat intake increases the risk of esophageal cancer. Moreover, further studies evaluating the effect of red or processed meat intake on individual histological subtypes of esophageal cancer are warranted.

ACKNOWLEDGMENTS

We thank Dr. Cross for data provision for our dose-response meta-analysis part.

REFERENCES

1 **Ferlay J**, Shin HR, Bray F, Forman D, Mathers C, Parkin DM. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. *Int J Cancer* 2010; **127**: 2893-2917 [PMID: 21351269 DOI: 10.1002/ijc.25516]

2 **Kamangar F**, Chow WH, Abnet CC, Dawsey SM. Environmental causes of esophageal cancer. *Gastroenterol Clin North Am* 2009; **38**: 27-57, vii [PMID: 19327566 DOI: 10.1016/j.gtc.2009.01.004]

3 **Cross AJ**, Sinha R. Meat-related mutagens/carcinogens in the etiology of colorectal cancer. *Environ Mol Mutagen* 2004;

44: 44-55 [PMID: 15199546 DOI: 10.1002/em.20030]
4 **World Cancer Research Fund / American Institute for Cancer Research.** Food, Nutrition, Physical Activity, and the Prevention of Cancer: A Global Perspective. Washington DC: AICR, 2007
5 **Jakszyn P**, Gonzalez CA. Nitrosamine and related food intake and gastric and oesophageal cancer risk: a systematic review of the epidemiological evidence. *World J Gastroenterol* 2006; **12**: 4296-4303 [PMID: 16865769]
6 **Stroup DF**, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis Of Observational Studies in Epidemiology (MOOSE) group. *JAMA* 2000; **283**: 2008-2012 [PMID: 10789670 DOI: 10.1001/jama.283.15.2008]
7 **Wells G,** Shea B, O'Connell D, Peterson J, Welch V, Losos M, Tugwell P. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analysis. UK: University of Liverpool, 2012
8 **DerSimonian R**, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; **7**: 177-188 [PMID: 3802833 DOI: 10.1016/0197-2456(86)90046-2]
9 **Higgins JP**, Thompson SG. Quantifying heterogeneity in a meta-analysis. *Stat Med* 2002; **21**: 1539-1558 [PMID: 12111919 DOI: 10.1002/sim.1186]
10 **Egger M**, Davey Smith G, Schneider M, Minder C. Bias in meta-analysis detected by a simple, graphical test. *BMJ* 1997; **315**: 629-634 [PMID: 9310563 DOI: 10.1136/bmj.315.7109.629]
11 **Keszei AP**, Schouten LJ, Goldbohm RA, van den Brandt PA. Red and processed meat consumption and the risk of esophageal and gastric cancer subtypes in The Netherlands Cohort Study. *Ann Oncol* 2012; **23**: 2319-2326 [PMID: 22351741 DOI: 10.1093/annonc/mdr615]
12 **Cross AJ**, Freedman ND, Ren J, Ward MH, Hollenbeck AR, Schatzkin A, Sinha R, Abnet CC. Meat consumption and risk of esophageal and gastric cancer in a large prospective study. *Am J Gastroenterol* 2011; **106**: 432-442 [PMID: 20978481 DOI: 10.1038/ajg.2010.415]
13 **González CA**, Jakszyn P, Pera G, Agudo A, Bingham S, Palli D, Ferrari P, Boeing H, del Giudice G, Plebani M, Carneiro F, Nesi G, Berrino F, Sacerdote C, Tumino R, Panico S, Berglund G, Simán H, Nyrén O, Hallmans G, Martinez C, Dorronsoro M, Barricarte A, Navarro C, Quirós JR, Allen N, Key TJ, Day NE, Linseisen J, Nagel G, Bergmann MM, Overvad K, Jensen MK, Tjonneland A, Olsen A, Bueno-de-Mesquita HB, Ocke M, Peeters PH, Numans ME, Clavel-Chapelon F, Boutron-Ruault MC, Trichopoulou A, Psaltopoulou T, Roukos D, Lund E, Hemon B, Kaaks R, Norat T, Riboli E. Meat intake and risk of stomach and esophageal adenocarcinoma within the European Prospective Investigation Into Cancer and Nutrition (EPIC). *J Natl Cancer Inst* 2006; **98**: 345-354 [PMID: 16507831 DOI: 10.1093/jnci/djj071]
14 **Yu Y**, Taylor PR, Li JY, Dawsey SM, Wang GQ, Guo WD, Wang W, Liu BQ, Blot WJ, Shen Q. Retrospective cohort study of risk-factors for esophageal cancer in Linxian, People's Republic of China. *Cancer Causes Control* 1993; **4**: 195-202 [PMID: 8318635]
15 **Ward MH**, Cross AJ, Abnet CC, Sinha R, Markin RS, Weisenburger DD. Heme iron from meat and risk of adenocarcinoma of the esophagus and stomach. *Eur J Cancer Prev* 2012; **21**: 134-138 [PMID: 22044848 DOI: 10.1097/CEJ.0b013e32834c9b6c]
16 **De Stefani E**, Deneo-Pellegrini H, Ronco AL, Boffetta P, Correa P, Aune D, Mendilaharsu M, Acosta G, Silva C, Landó G, Luaces ME. Meat consumption, cooking methods, mutagens, and risk of squamous cell carcinoma of the esophagus: a case-control study in Uruguay. *Nutr Cancer* 2012; **64**: 294-299 [PMID: 22242927 DOI: 10.1080/01635581.2012.648299]
17 **Gao Y**, Hu N, Han XY, Ding T, Giffen C, Goldstein AM, Taylor PR. Risk factors for esophageal and gastric cancers in Shanxi Province, China: a case-control study. *Cancer Epidemiol* 2011; **35**: e91-e99 [PMID: 21846596 DOI: 10.1016/j.canep.2011.06.006]
18 **Wu M**, Zhang ZF, Kampman E, Zhou JY, Han RQ, Yang J, Zhang XF, Gu XP, Liu AM, van't Veer P, Kok FJ, Zhao JK. Does family history of cancer modify the effects of lifestyle risk factors on esophageal cancer? A population-based case-control study in China. *Int J Cancer* 2011; **128**: 2147-2157 [PMID: 20602339 DOI: 10.1002/ijc.25532]
19 **Hajizadeh B**, Jessri M, Moasheri SM, Rad AH, Rashidkhani B. Fruits and vegetables consumption and esophageal squamous cell carcinoma: a case-control study. *Nutr Cancer* 2011; **63**: 707-713 [PMID: 21614725 DOI: 10.1080/01635581.2011.563028]
20 **O'Doherty MG**, Cantwell MM, Murray LJ, Anderson LA, Abnet CC. Dietary fat and meat intakes and risk of reflux esophagitis, Barrett's esophagus and esophageal adenocarcinoma. *Int J Cancer* 2011; **129**: 1493-1502 [PMID: 21455992 DOI: 10.1002/ijc.26108]
21 **Sapkota A**, Hsu CC, Zaridze D, Shangina O, Szeszenia-Dabrowska N, Mates D, Fabiánová E, Rudnai P, Janout V, Holcatova I, Brennan P, Boffetta P, Hashibe M. Dietary risk factors for squamous cell carcinoma of the upper aerodigestive tract in central and eastern Europe. *Cancer Causes Control* 2008; **19**: 1161-1170 [PMID: 18512121 DOI: 10.1007/s10552-008-9183-0]
22 **Navarro Silvera SA**, Mayne ST, Risch H, Gammon MD, Vaghan TL, Chow WH, Dubrow R, Schoenberg JB, Stanford JL, West AB, Rotterdam H, Blot WJ, Fraumeni JF. Food group intake and risk of subtypes of esophageal and gastric cancer. *Int J Cancer* 2008; **123**: 852-860 [PMID: 18537156 DOI: 10.1002/ijc.23544]
23 **Wang JM**, Xu B, Rao JY, Shen HB, Xue HC, Jiang QW. Diet habits, alcohol drinking, tobacco smoking, green tea drinking, and the risk of esophageal squamous cell carcinoma in the Chinese population. *Eur J Gastroenterol Hepatol* 2007; **19**: 171-176 [PMID: 17273005 DOI: 10.1097/MEG.0b013e32800ff77a]
24 **Wu AH**, Tseng CC, Hankin J, Bernstein L. Fiber intake and risk of adenocarcinomas of the esophagus and stomach. *Cancer Causes Control* 2007; **18**: 713-722 [PMID: 17562192 DOI: 10.1007/s10552-007-9014-8]
25 **Chen H**, Ward MH, Graubard BI, Heineman EF, Markin RM, Potischman NA, Russell RM, Weisenburger DD, Tucker KL. Dietary patterns and adenocarcinoma of the esophagus and distal stomach. *Am J Clin Nutr* 2002; **75**: 137-144 [PMID: 11756071]
26 **Takezaki T**, Shinoda M, Hatooka S, Hasegawa Y, Nakamura S, Hirose K, Inoue M, Hamajima N, Kuroishi T, Matsuura H, Tajima K. Subsite-specific risk factors for hypopharyngeal and esophageal cancer (Japan). *Cancer Causes Control* 2000; **11**: 597-608 [PMID: 10977104 DOI: 10.1023/A:1008909129756]
27 **Bosetti C**, La Vecchia C, Talamini R, Simonato L, Zambon P, Negri E, Trichopoulos D, Lagiou P, Bardini R, Franceschi S. Food groups and risk of squamous cell esophageal cancer in northern Italy. *Int J Cancer* 2000; **87**: 289-294 [PMID: 10861489]
28 **Rolón PA**, Castellsagué X, Benz M, Muñoz N. Hot and cold mate drinking and esophageal cancer in Paraguay. *Cancer Epidemiol Biomarkers Prev* 1995; **4**: 595-605 [PMID: 8547825]
29 **Castelletto R**, Castellsague X, Muñoz N, Iscovich J, Chopita N, Jmelnitsky A. Alcohol, tobacco, diet, mate drinking, and esophageal cancer in Argentina. *Cancer Epidemiol Biomarkers Prev* 1994; **3**: 557-564 [PMID: 7827585]
30 **Tavani A**, Negri E, Franceschi S, La Vecchia C. Risk factors for esophageal cancer in lifelong nonsmokers. *Cancer Epidemiol Biomarkers Prev* 1994; **3**: 387-392 [PMID: 7920205]
31 **Rogers MA**, Thomas DB, Davis S, Vaughan TL, Nevissi AE. A case-control study of element levels and cancer of the upper aerodigestive tract. *Cancer Epidemiol Biomarkers Prev* 1993; **2**: 305-312 [PMID: 8348053]
32 **Yu MC**, Garabrant DH, Peters JM, Mack TM. Tobacco, al-

cohol, diet, occupation, and carcinoma of the esophagus. *Cancer Res* 1988; **48**: 3843-3848 [PMID: 3378219]
33 **Chen YK**, Lee CH, Wu IC, Liu JS, Wu DC, Lee JM, Goan YG, Chou SH, Huang CT, Lee CY, Hung HC, Yang JF, Wu MT. Food intake and the occurrence of squamous cell carcinoma in different sections of the esophagus in Taiwanese men. *Nutrition* 2009; **25**: 753-761 [PMID: 19394796 DOI: 10.1016/j.nut.2009.02.002]
34 **Yang CX**, Wang HY, Wang ZM, Du HZ, Tao DM, Mu XY, Chen HG, Lei Y, Matsuo K, Tajima K. Risk factors for esophageal cancer: a case-control study in South-western China. *Asian Pac J Cancer Prev* 2005; **6**: 48-53 [PMID: 15780032]
35 **Levi F**, Pasche C, Lucchini F, Bosetti C, La Vecchia C. Processed meat and the risk of selected digestive tract and laryngeal neoplasms in Switzerland. *Ann Oncol* 2004; **15**: 346-349 [PMID: 14760132 DOI: 10.1093/annonc/mdh060]
36 **Li K**, Yu P. Food groups and risk of esophageal cancer in Chaoshan region of China: a high-risk area of esophageal cancer. *Cancer Invest* 2003; **21**: 237-240 [PMID: 12743989 DOI: 10.1081/CNV-120016420]
37 **Takezaki T**, Gao CM, Wu JZ, Ding JH, Liu YT, Zhang Y, Li SP, Su P, Liu TK, Tajima K. Dietary protective and risk factors for esophageal and stomach cancers in a low-epidemic area for stomach cancer in Jiangsu Province, China: comparison with those in a high-epidemic area. *Jpn J Cancer Res* 2001; **92**: 1157-1165 [PMID: 11714439 DOI: 10.1111/j.1349-7006.2001.tb02135.x]
38 **Sugimura T**, Wakabayashi K, Nakagama H, Nagao M. Heterocyclic amines: Mutagens/carcinogens produced during cooking of meat and fish. *Cancer Sci* 2004; **95**: 290-299 [PMID: 15072585 DOI: 10.1111/j.1349-7006.2004.tb03205.x]
39 **Mirvish SS**. Role of N-nitroso compounds (NOC) and N-nitrosation in etiology of gastric, esophageal, nasopharyngeal and bladder cancer and contribution to cancer of known exposures to NOC. *Cancer Lett* 1995; **93**: 17-48 [PMID: 7600541 DOI: 10.1016/0304-3835(95)03786-V]
40 **Cross AJ**, Pollock JR, Bingham SA. Haem, not protein or inorganic iron, is responsible for endogenous intestinal N-nitrosation arising from red meat. *Cancer Res* 2003; **63**: 2358-2360 [PMID: 12750250]

P- Reviewer Marjanovic G **S- Editor** Song XX
L- Editor A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i7.1030

World J Gastroenterol 2013 February 21; 19(7): 1030-1039
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

TOPIC HIGHLIGHT

Jung Eun Lee, ScD, Assistant Professor, *Series Editor*

Current trends in the development and application of molecular technologies for cancer epigenetics

Hyeran Jang, Hyunjin Shin

Hyeran Jang, Section of Endocrinology, Boston University School of Medicine, Boston, MA 02118, United States
Hyunjin Shin, Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Harvard School of Public Health, Boston, MA 02215, United States
Author contributions: Jang H and Shin H contributed equally to this work.
Correspondence to: Hyunjin Shin, PhD, Department of Biostatistics and Computational Biology, Dana-Farber Cancer Institute, Harvard School of Public Health, 665 Huntington Avenue, Boston, MA 02215, United States. ulexis.dfci@gmail.com
Telephone: +1-617-5518730 Fax: +1-617-5517871
Received: September 19, 2012 Revised: January 7, 2013
Accepted: January 29, 2013
Published online: February 21, 2013

Abstract

Current progress in epigenetic research supports the view that diet and dietary components are important in cancer etiology by enhancing or inhibiting carcinogenesis. Since diet and dietary factors may significantly contribute to the causation and progression of many cancers, it is important to find the molecular mechanisms of action of such dietary factors for cancer prevention and treatment. Recently, the role of epigenetic mechanisms in the cancer development and progression has attracted more attention as additional evidence along with traditional DNA sequence based mechanisms such as mutations and structural re-arrangements. Such an increasing interest in cancer epigenetics has also accelerated the development and application of molecular assays and tools for DNA methylation detection and histone modification enrichment analysis. In this paper, key assays and methods for epigenetic research are reviewed and discussed in terms of their utility and usability. In addition, more advanced methods for genome-wide analysis are introduced as part of upcoming research trends and directions.

© 2013 Baishideng. All rights reserved.

Key words: Cancer; Epigenetic; Technology; Butyrate; Histone modifications; Diet; Methyl sources

Jang H, Shin H. Current trends in the development and application of molecular technologies for cancer epigenetics. *World J Gastroenterol* 2013; 19(7): 1030-1039 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i7/1030.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i7.1030

INTRODUCTION

Diet and dietary factors play an important role in many biological processes and are also involved in the regulation of pathological progressions including cancers. Several epidemiological and preclinical studies suggested that increased intake of bioactive dietary components may modulate cancer risk. Many studies provide compelling evidence that part of the anti-cancer properties contributed to several bioactive dietary components may relate to modulation of epigenetic process including DNA methylation and histone protein modifications. Here, we provide a brief overview of dietary modulation of DNA methylation and histone modifications and its potential role in cancer prevention. Also, we will discuss several new epigenetic methods to help understand the effect of dietary factors on epigenetic modifications.

EPIGENETICS AND CANCER

The growing interest in the role of epigenetics in cancer came from the demonstration that epigenetic modifications are involved in tumor development and progression. Epigenetics can be defined as phenomena that alter the expression of the information in the genome at the transcriptional, translational, or posttranslational

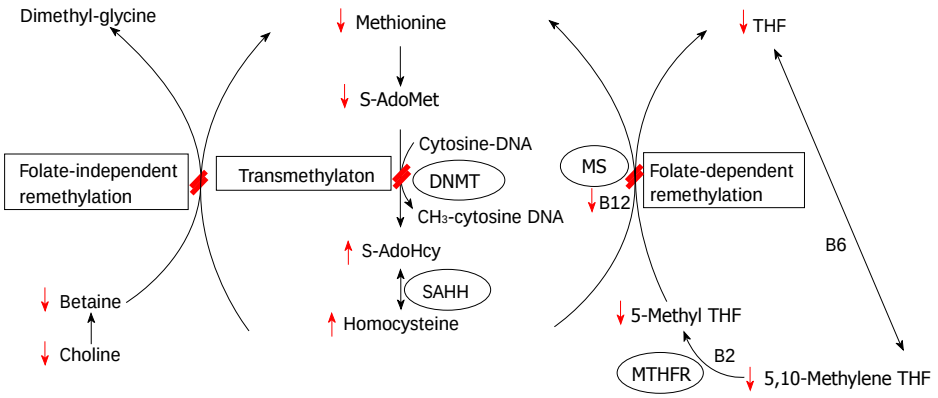


Figure 1 Effect of methyl-deficiency on biological methylation pathway. S-AdoMet: S-adenosylmethionine; S-AdoHcy: S-adensylhomocysteine; DNMT: DNA methyltransferase; SAHH: S-adenosylhomocysteine hydrolase; MS: Methionine synthase; THF: Tetrahydrofolate; MTHFR: Methylenetetrahydrofolate reductase.

level without change in DNA sequence[1,2]. Epigenetic information is maintained to preserve cellular identity in normal cells, while cancer cells are characterized by profound alteration of epigenetic regulation[3-7]. The overall disruption of epigenetic phenomena is a common feature of all human tumors and includes alteration of DNA methylation and histone modification patterns[8]. DNA methylation patterns of neoplastic cells have been recognized as being substantially altered compared with normal cells[3,4]. Two types of changes in the DNA methylation pattern can occur in cancer: global DNA hypomethylation and hypermethylation of CpG islands which are associated with gene silencing[3,4,7]. DNA in eukaryotic cells is intimately associated with a family of small, basic histone proteins forming a highly ordered and condensed DNA-protein complex termed chromatin. Because of this chromatin structure, changes in DNA methylation in cancer cells are not isolated events; they occur in the context of more complex epigenetic deregulation[9]. Chromatin is the physiological template of the genetic information and is composed of DNA, histones, and other chromosomal proteins. The fundamental repeating unit of chromatin is the nucleosome octamer, which consists of 147 base pairs of DNA wrapped around 2 copies each of histones H2A, H2B, H3 and H4[10]. The amino-terminal tails of histones are subject to posttranslational modifications, including acetylation, methylation, phosphorylation, ubiquitination, SUMOylation, and ADP-ribosylation[11], and multiple histone modifications may occur on a given histone tail[12]. Histone modifications patterns distinguish the structure of chromatin status, in particular, acetylation of histone H3 and H4 is associated with active gene expression with open chromatin structure. Histone acetylation is regulated by several enzymes such as histone acetyltransferase and histone deacetylases activity. Aberrations in post-translational modifications of histones have been shown to occur in cancer cells. Although alterations in global histone modification patterns in cancer cells have remained unknown, recent studies on global histone modifications at specific amino acids have been suggested as predictive clinical outcomes for various cancers[13-15]. Additionally, a number of studies have been focused only on changes of a particular histone modification at individual gene promoters in cancer cells.

DNA METHYLATION

Methyl-deficient diet induced hepatocarcinogenesis

In last 4 decades, researchers have developed various tools for exploring DNA methylation, and started to apply those new technologies to the field of nutrition science. The methyl-deficient model of endogenous hepatocarcinogenesis is one of which in DNA methylation has been extensively studied. This animal model is unique in that dietary omission rather than chemical carcinogens addition can lead to tumor formation[16]. Specifically, deficiency of the major dietary sources of methyl groups - methionine, choline, folic acid and vitamin B12 - leads to the development of liver cancers in rats and certain mouse strains[17-19]. From early 1990, these animal models have shown that the methyl-deficiency is associated with several defects, including genome-wide DNA hypomethylation and gene-specific hypermethylation[20-22]. Importantly, the aberrant epigenetic alterations imposed by this diet have been hypothesized to be the primary mechanism responsible for malignant transformation of rat liver cells[15,20,22,23]. Figure 1 displays a simplified version of biological methylation pathway from one-carbon metabolism, emphasizing that various dietary methyl sources (methionine, choline, various coenzymatic forms of folate and vitamin B2, B6 and B12) play an important roles in DNA methylation. Methyl source deficiency has marked effects on the flow of one-carbon units through this web of reactions as the effect of methyl source deficiency are highlighted in red. The major effect observed in methyl deficiency models is a rapid decrease in hepatic S-adenosylmethionine (SAdoMet) levels and genomic DNA hypomethylation. In other recent studies examining the early stages of hepatocarcinogenesis induced by methyl deficiency in rats, substantial alterations in other aspects of the epigenetic machinery have been observed, including aberrant expression of DNA methyltransferases and methyl-CpG

Table 1 DNA methylation analyses used in methyl-deficient model of hepatocarcinogenesis in rodents

Dietary component	Model	Observations	Methylation assay	Ref.
Amino acid-defined diet lacking choline, methionine, folic acid and vitamin B12	Rat	Depletion of SAdoMet and DNA hypomethylation	Liver DNA methyltransferase activity assay with labeled SAdoMet	[20]
Amino acid-defined diet lacking choline, methionine, folic acid and vitamin B12	Rat	Hypomethylation of CCGG site of c-myc, c-fos and c-Ha-ras	Enzyme digestion by *Hpa* Ⅱ/*Msp* Ⅰ	[21]
Diet low in methionine lacking in choline and folic acid	Rat	Hypermethylation of $p16^{INK4A}$	MS-PCR	[94]
Diet low in methionine lacking in choline and folic acid	Rat	Decrease in the total percent of methylated CCGG sites in DNA	*Hpa* Ⅱ/*Msp* Ⅰ-based cytosine extension assay	[22]
Diet low in methionine lacking in choline and folic acid	Rat	Depletion of S-AdoMet, decrease in S-AdoMet/S-AdoHcy and global DNA hypomethylation	*Hpa* Ⅱ/*Msp* Ⅰ-based cytosine extension assay	[23]
Diet low in methionine lacking in choline and folic acid	Rat	Hypomethylation of ID element and LINE-1 in preneoplastic livers and liver tumors; Decrease in histone H4-Lys20 trimethylation and increase in histone H3-Lys9 trimethylation; Decrease in histone H4-Lys20 trimethylation at the LINE-1 regulatory region	ID methylation by methylation-sensitive McrBC-PCR array; LINE-1 methylation by COBRA-assay; global histone methylation by Western blotting; LINE-1-associated histone methylation by ChIP	[15]
Diet deficient in methionine lacking in choline and folic acid	Rat	Changes in the DNA methylation machinery	Indirect methods by DNA methyltransferases and Methyl CpG binding proteins	[24]
Amino acid-defined diet lacking choline	Rat	Hypermethylation of upstream of E-cadherin and Cx26	Bisulfite sequencing	[95]
Diet deficient in methionine lacking in choline and folic acid	Rat	global loss of DNA methylation; hypermethylation of CpG islands	Global DNA methylation by cytosine extension assay and [^{3}H-methyl] incorporation; CpG island methylation by [^{32}P]dGTP incorporation	[30]
Diet deficient in methionine lacking in choline and folic acid	Mouse	Global DNA hypomethylation; substantial loss of repetitive sequences (LINE-1, SINES, IAP elements) cytosine methylation. Increase in histone H3-Lys9 trimethylation and decrease in histone H4-Lys20 trimethylation	Global DNA methylation by cytosine extension assay; methylation-sensitive McrBC-qPCR assay; global histone modifications by Western blot	[96]
Diet deficient in methionine lacking in choline and folic acid	Mouse	Detection of CpG island methylation profiles	MeDIP	[97]

S-AdoMet: S-adenosylmethionine; ID: Identifier; LINE-1: Long interspersed nucleotide elements; SINES: Short interspersed nuclear elements; IAP: Intracisternal A-particle; MS-PCR: Methylation-specific polymerase chain reaction; ChIP: Chromatin immunoprecipitation; MeDIP: Methylated DNA immunoprecipitation.

binding proteins[24], defects in histone methyltransferase protein expression and histone posttranslational modifications[15]. In Table 1, various DNA methylation assays were summarized in methyl-deficient model of hepatocarcinogenesis in rodents.

Genomic DNA methylation assays

One of widely used methods for global DNA methylation assay is a radioassay that utilizes the enzyme *Sss* I DNA methyltransferase to catalyze the *de novo* methylation of the CpG sites with radiolabeled [^{3}H]-SAdoMet, a universal methyl donor *in vitro*[20,25-28]. And another method was developed thanks to the discovery of methylation-sensitive restriction endonucleases. In 1999, Pogribny *et al*[29] developed a new method based on methylation-sensitive endonucleases followed by single nucleotide extension with radiolabeled [^{3}H]-dCTP. This cytosine extension assay was used in various studies of methyl-deficient model of hepatocarcinogenesis for genomic DNA methylation[22,23,29,30]. These enzyme based methods have wide variations in precision as a result of inconsistency in the activity of methyl-sensitive endonucleases and the instability of methyltransferase activity[31]. In 2002, Friso *et al*[32] developed a method for quantitative determination of 5-methyl-2'deoxycytidine using liquid chromatography/electrospray ionization/mass spectrometry (LC/ESI/MS). This method allows accurate measurement of the absolute amount of 5-methyl-2'deoxycytidine relative to the total amount of cytosine residues, furthermore, it requires relatively lower amount of DNA and has a shorter run time for each sample than other high-performance liquid chromatography-based methods[33-35]. DNA methylation assay by LC/ESI/MS has been widely used for quantitative DNA methylation in animal studies and population-based studies in the light of its greater reproducibility and precision in large number of samples[32,36-40].

Gene-specific DNA methylation measurements

DNA methylation has long been recognized as an important factor on the silencing of genes, therefore it has become important to know the methylation status of individual CpG site. The first generation of DNA methylation detection assay is Southern blot or polymerase chain reaction (PCR) amplification that follows the enzyme digestion with methylation-sensitive restriction endonucleases[41-46]. Currently, the most commonly used methods for gene-specific DNA methylation can be categorized into three major methods.

Bisulfite DNA sequencing and methylation-specific

PCR: Treatment of DNA with bisulfite converts cytosine residue to uracil, but leaves 5-methylcytosine residue unaffected. Bisulfite sequencing involves chemical conversion of cytosine to uracil, followed by PCR, and DNA sequencing[47]. While providing single-base resolution, the high cost and labor-intensive steps limit the use of this method for high-throughput analyses[48]. Methylation-specific-PCR also employs bisulfite conversion, but avoids the need to sequence the area of interest. Instead, methyl-specific and unmethyl-specific primer sets are designed, to distinguish methylated from unmethylated DNA in bisulfate-converted DNA[49]. This method is powerful to explore CpG islands with high methylation density, as increased numbers of CpG in the primer increase the specificity of the assay. However, these two methods using bisulfite conversion are not currently suitable for whole-genome analysis on multiple samples but commonly used for data validation from array-based methods.

Methods that focus specific single-CpG: These include Combined Bisulfite Restriction Analysis (COBRA)[50], MethyLight[51], and bisulfite pyrosequencing[52]. In COBRA, the combination of bisulfite conversion and PCR amplification is used, therefore it results in sequence conversion (unmethylated cytosine residue to thymidine and methylated cytosine to cytosine) which can lead to new methylation-dependent restriction enzyme sites. The following digestion of the PCR product with at least one CpG site in the recognition sequence only proceeds if the CpG site is protected from bisulfite conversion by methylation. For this reason, the signal ratio of restriction products indicating methylation to undigested PCR product representing unmethylated sequences can be used as a measure for the methylation level of this specific CpG. MethyLight is a bisulfite-dependent, fluorescence-based, quantitative real-time PCR method for DNA methylation. MethyLight relies on methylation-specific priming combined with methylation-specific fluorescent probing. This combination of methylation-specific detection principles results in a highly methylation-specific detection technology, with an accompanying ability to sensitively detect very low frequencies of hypermethylated alleles. Bisulfite pyrosequencing has been used to analyze bisulfite-converted DNA without using methylation-specific PCR. Following PCR amplification of the region of interest, pyrosequencing is used to determine the bisulfite-converted sequence of specific CpG sites in the region. The ratio of cytosine to thymidine at individual sites can be determined quantitatively based on the amount of cytosine and thymidine incorporation during the sequence extension. While the methods mentioned above are sensitive, specific, and relatively inexpensive, none of these methods is suitable for analysis of the whole genome, which includes about 28 million CpGs.

Microarray-based methods: These enable to interrogate larger numbers of CpG, there are three major types of microarray-based methylation analysis. Direct hybridization to CpG island arrays is the first high-throughput approach capable of detecting DNA methylaion in genes across several CpG sites. Based on the bisulfite modification of DNA, this method utilizes methylation-specific oligonucleotides arrayed on glass slides for detection of all possible methylation in target DNA[53]. Methylated DNA immunoprecipitation (MeDIP) is also a large-scale, genome-wide purification method that is used to enrich for methylated DNA sequence using antibody raised against 5-methylcytosine[54]. DNA from MeDIP can be used for either array-based hybridization (MeDIP-chip) or high-throughput sequencing (MeDIP-seq). Although MeDIP helps generate comprehensive DNA methylation profiles, both applications have their typical limitation of array-based technology, restricted resolution. The HELP assay (*Hpa*Ⅱ tiny fragment enrichment by ligation-mediated PCR) is comparative isoschizomer profiling of DNA methylation[55]. DNA is digested by *Hpa*Ⅱ in parallel with *Msp* Ⅰ (resistant to DNA methylation), and then the *Hpa*Ⅱ and *Msp* Ⅰ products are either amplified by ligation-mediated PCR and hybridized using separate fluorochromes to a customized array, or directly sequenced[56]. These high-throughput array-based approaches for DNA methylation are relatively inexpensive tool suitable for genome-wide analysis, therefore, help to target aberrant methylation patterns in various cancer models. Furthermore, methylation profiling achieved from high throughput methods will offer differentially methylated regions to understand the effect of dietary factors on epigenetic modifications in cancer, subsequently, provide insight in prevention strategies to reduce the burden of cancer.

HISTONE MODIFICATION

Histone deacetylase inhibition by butyrate

In addition to the effects on DNA methylation, dietary components can affect posttranslational modifications of histones. The dietary agent best studied in histone modifications is the short chain fatty acid butyrate which is generated in the colon as a result of bacterial fermentation of dietary fiber. Higher intake of dietary fiber is associated with reduced risk of colorectal cancer[57,58]. The molecular mechanisms underlying this anti-cancer effect of dietary fiber are poorly understood, however, the strongest evidence is based on the anti-carcinogenic actions of butyrate. Butyrate can be found at millimolar concentrations in the lumen of the colon[59], and has inhibitory effects on types Ⅰ and Ⅱ histone deacetylase enzymes. Butyrate-induced alterations in histone marks, especially acetylation at histone H3 and/or H4, have been associated with several processes, including cellular differentiation[60,61], cell cycle arrest[62-64], apoptosis[65-67], and inhibition of invasion[68] in a number of cancer cell studies. Table 2 summarized some of evidence of the effects of butyrate on histone acetylation. Although butyrate has strong marks

Table 2 A summary of selected evidence for effects of butyrate in histone modification and histone modification assays in cancer cell culture models

Dietary component	Cell culture model	Observations	Histone modification assay	Ref.
Sodium butyrate	SW620 human colon carcinoma cells	Increased global histone H4 acetylation	Western blot	[70]
Sodium butyrate	A375 human melanoma and S91 mouse melanoma	Increased global histone H4 acetylation	Western blot	[98]
Sodium butyrate	Colo-320 human colon cancer cells	Increased acetylation of histone H3 and H4 within CDKN1A promoter site	ChIP	[64]
Sodium butyrate	EBC-1 human lung epithelial cells	Increased histone H3 and H4 acetylation associated with promoter of cathelicidin	ChIP	[99]
Sodium butyrate	HepG2 human hepatocarcinoma	Increased global histone H3 and H4 acetylation; Genome-wide changes in acetylation of DNA-bound histones	Western blot; ChIP-chip (ChIP and microarray hybridization)	[100]

CDKN1A: Cyclin-dependent kinase inhibitor 1A; ChIP: Chromatin immunoprecipitation.

on histone acetylation, a small fraction of cellular genes is regulated in response to butyrate[69-71]. Therefore, it should be noted that site-specific approach by chromatin immunoprecipitation (ChIP) based experimental tools will provide a better understanding on the chemopreventive effects of butyrate, showing gene-specific histone acetylation and its associated gene expression.

Global histone acetylation assays

The first estimates for the rate of acetylation turnover were measured by pulse, pulse-chase, and steady-state acetylation labeling in hepatoma tissue culture cells in 1975[72]. Boffa *et al*[73] showed that sodium butyrate suppressed histone deacetylation *in vivo* and *in vitro* by measuring the kinetics of [^{3}H] acetate release from histone proteins. Since specific antibodies to modified histones were developed, Western blot has been used to detect histone modifications. As shown in Table 2, butyrate-induced histone acetylation was confirmed by Western blot in many studies.

Gene-specific histone acetylation measurements

ChIP: The antibodies to acetylated histone H3 and H4 have been used for ChIP to determine histone acetylation in specific regions of gene promoter and other regulatory regions. ChIP is a specialized immunoprecipitation used to detect the covalent interaction between the DNA sequence and DNA-binding proteins such as transcription factors or histone proteins. ChIP using histone antibodies is able to determine the specific location in the genome that various histone modifications are associated with, indicating the target of the histone modifiers[74]. For example, ChIP experiment unveiled that butyrate induced an increase in histone H3 and H4 acetylation within the CDKN1A promoter, which regulates the p21 protein, in Colo-320 human colon cancer cells[64]. Due to its ability to precisely detect the DNA binding of modified histones, transcription factors, and non-histone chromosomal proteins, ChIP has been widely used to generate and test numerous hypotheses regarding transcriptional and epigenetic regulations. However, it remains to be still challenging to conduct ChIP on an "epigenome" level, since ensuring an antibody of high specificity is often laborious and time-consuming. Another important concern with ChIP scalability is the maximum range of target regions that can be investigated by a single assay. For instance, a typical experiment of ChIP coupled with qPCR is designed to measure the enrichment levels of a DNA binding protein at a handful of sites (*e.g.*, gene promoters). However, in general, even a single epigenetic event in the cell pervasively occurs over a wide range of genomic regions, often involving thousands of genes and their associated regulatory elements. Thus, it becomes more important to have an ability to run the assay on a genome-wide scale for having a more balanced and unbiased perspective on the underlying mechanisms. Coupled with genomic profiling technologies such as tiling arrays or next generation sequencing (NGS), ChIP can be extended over the whole genome. In the following sections, we will introduce two major methods coupled with ChIP that enable epigenome-scale research of histone marks and transcription factors.

ChIP-chip: ChIP-chip is based on the combination of ChIP and a genomic tiling array technology (*i.e.*, chip), in which DNA sequences extracted after ChIP hybridize with probes that are designed to cover the whole genome or specific regions of interest such as promoter[75,76]. Due to bias in microarray hybridization, a control experiment using chromatin input or DNA from non-specific immunoprecipitation (IP) (*e.g.*, IP against immunoglobulin G) is often recommended. Most algorithms for ChIP-chip are designed to compute the normalized ratio between the hybridizations of ChIP and control after removing random and/or systemic noise. Then, they call binding sites as those significantly enriched in ChIP over control[77-79]. Since its emergence in the early and mid 2000s, ChIP-chip has been widely adopted in many transcriptional and epigenetic regulation studies, assisting scientists to more understand the role of each histone mark in physiological and pathological processes[80-83]. However, the utility of ChIP-chip is heavily restricted by a tiling array probe design, which determines the resolution of

the measurement (*i.e.*, intervals between adjacent probes) and the regions that can be explored (*e.g.*, omission of repetitive sequence areas). These weaknesses of ChIP-chip have accelerated the major platform shift to NGS.

ChIP-Seq: In ChIP-Seq, the extracted DNA sequences are directly sequenced using a NGS technology instead of being hybridized onto tiling arrays. NGS refers to sequencing technologies that newly emerged since the mid 2000s as an alternative to the traditional automated Sanger sequencing. NGS is characterized as massive parallel sequencing of template DNA or RNA (cDNA) molecules by a relatively short length ranging over 50-400 bp[84]. One advantage of ChIP-Seq over ChIP-chip is that ChIP-Seq does not require any predefined array design, which allows a more unbiased assay at a much higher resolution (100-1000 bp in ChIP-chip *vs* 10-100 bp in ChIP-Seq). Since NGS generally produces a notoriously large amount of data than array-based methods, more powerful bioinformatics support is essential for data processing and analysis[85,86]. Bioinformatics analysis for ChIP-Seq in epigenetic research includes the pre-processing for sequence data such as quality control and read mapping, the identification of candidate sites enriched by the target histone mark, and further down-stream analysis for revealing biological implications of the observations from the precedent steps[85,86].

In cancer studies, the down-stream analysis is focused on finding the most associated genes or regulatory elements (*e.g.*, promoters or enhancers) with the histone mark of interest and investigating how these genes and regulatory elements can be understood in the context of biological pathways. Since Barski *et al*[87] and Wang *et al*[88] studies on 19 histone methylations and 18 histone acetylations using the human CD4+ T cell, many studies have been done to understand the biological implications of histone marks in normal conditions[80-82]. However, due to the plasticity of epigenome and heterogeneity of cancer, cancer epigenetics of examining histone modifications on a genome scale still remains in its beginning stage. For this reason, most of currently on-going efforts in cancer epigenetics still largely target DNA methylation (*e.g.*, The Cancer Genome Atlas, http://cancergenome.nih.gov/)[89]. Therefore, it will be a long-term goal to accumulate the knowledge on cancer epigenetics from histone modifications and use it for cancer studies, which will require a great amount of public and private investments. Another interesting research direction is an attempt to comprehend how genetic variations lead to epigenetic changes in cancer. In 2011, several studies have been published about the possibility of multiple chromatin remodelers and histone enzymes as potential oncogenes or tumor suppressor genes[90-93]. These studies suggest that the disruption of chromatin remodelers and histone enzymes due to driving somatic mutations in their coding regions may cause aberrant epigenetic changes, which eventually lead to cancer development or evolution in at least several cancer indications. Such approach is particularly interesting because it may be able to provide a genuine perspective on the target histone mark by observing somatic mutations in several key chromatin remodelers and histone enzymes.

CONCLUSION

In conclusion, a number of aberrant epigenetic modifications have been found in cancer cells, and diet and dietary factors play an important role to prevent cancer as well as to stimulate carcinogenesis. The use of epigenetic technology offers significant advantages to study the epigenetic mechanisms of cancer development and progression. Also, the newly developed technologies for epigenetic study expand the scope of nutrition study in the field of cancer research by helping monitor and pin down specific epigenetic pathways in diet-related cancers.

REFERENCES

1 **Egger G**, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. *Nature* 2004; **429**: 457-463 [PMID: 15164071 DOI: 10.1038/nature02625]

2 **Trosko JE**, Upham BL. The emperor wears no clothes in the field of carcinogen risk assessment: ignored concepts in cancer risk assessment. *Mutagenesis* 2005; **20**: 81-92 [PMID: 15784692]

3 **Jones PA**, Baylin SB. The fundamental role of epigenetic events in cancer. *Nat Rev Genet* 2002; **3**: 415-428 [PMID: 12042769 DOI: 10.1038/nrg816]

4 **Feinberg AP**, Tycko B. The history of cancer epigenetics. *Nat Rev Cancer* 2004; **4**: 143-153 [PMID: 14732866 DOI: 10.1038/nrc1279]

5 **Lund AH**, van Lohuizen M. Epigenetics and cancer. *Genes Dev* 2004; **18**: 2315-2335 [PMID: 15466484]

6 **Hake SB**, Xiao A, Allis CD. Linking the epigenetic 'language' of covalent histone modifications to cancer. *Br J Cancer* 2004; **90**: 761-769 [PMID: 14970850 DOI: 10.1038/sj.bjc.6601575]

7 **Esteller M**. Aberrant DNA methylation as a cancer-inducing mechanism. *Annu Rev Pharmacol Toxicol* 2005; **45**: 629-656 [PMID: 15822191 DOI: 10.1146/annurev.pharmtox.45.120403.095832]

8 **Esteller M**. Epigenetics provides a new generation of oncogenes and tumour-suppressor genes. *Br J Cancer* 2006; **94**: 179-183 [PMID: 16404435]

9 **Fraga MF**, Esteller M. Towards the human cancer epigenome: a first draft of histone modifications. *Cell Cycle* 2005; **4**: 1377-1381 [PMID: 16205112]

10 **Luger K**, Mäder AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* 1997; **389**: 251-260 [PMID: 9305837 DOI: 10.1038/38444]

11 **Strahl BD**, Allis CD. The language of covalent histone modifications. *Nature* 2000; **403**: 41-45 [PMID: 10638745 DOI: 10.1038/47412]

12 **Fischle W**, Wang Y, Allis CD. Binary switches and modification cassettes in histone biology and beyond. *Nature* 2003; **425**: 475-479 [PMID: 14523437 DOI: 10.1038/nature02017]

13 **Seligson DB**, Horvath S, Shi T, Yu H, Tze S, Grunstein M, Kurdistani SK. Global histone modification patterns predict risk of prostate cancer recurrence. *Nature* 2005; **435**: 1262-1266 [PMID: 15988529 DOI: 10.1038/nature03672]

14 **Tzao C**, Tung HJ, Jin JS, Sun GH, Hsu HS, Chen BH, Yu CP, Lee SC. Prognostic significance of global histone modifications in resected squamous cell carcinoma of the esophagus. *Mod Pathol* 2009; **22**: 252-260 [PMID: 18953329 DOI: 10.1038/

modpathol.2008.172]
15 **Pogribny IP**, Ross SA, Tryndyak VP, Pogribna M, Poirier LA, Karpinets TV. Histone H3 lysine 9 and H4 lysine 20 trimethylation and the expression of Suv4-20h2 and Suv-39h1 histone methyltransferases in hepatocarcinogenesis induced by methyl deficiency in rats. *Carcinogenesis* 2006; **27**: 1180-1186 [PMID: 16497704 DOI: 10.1093/carcin/bgi364]
16 **Ghoshal AK**, Farber E. The induction of liver cancer by dietary deficiency of choline and methionine without added carcinogens. *Carcinogenesis* 1984; **5**: 1367-1370 [PMID: 6488458 DOI: 10.1093/carcin/5.10.1367]
17 **Newberne PM**. Lipotropic factors and oncogenesis. *Adv Exp Med Biol* 1986; **206**: 223-251 [PMID: 3035898]
18 **Poirier LA**. Methyl group deficiency in hepatocarcinogenesis. *Drug Metab Rev* 1994; **26**: 185-199 [PMID: 8082564 DOI: 10.3109/03602539409029790]
19 **Denda A**, Kitayama W, Kishida H, Murata N, Tsutsumi M, Tsujiuchi T, Nakae D, Konishi Y. Development of hepatocellular adenomas and carcinomas associated with fibrosis in C57BL/6J male mice given a choline-deficient, L-amino acid-defined diet. *Jpn J Cancer Res* 2002; **93**: 125-132 [PMID: 11856475 DOI: 10.1111/j.1349-7006.2002.tb01250.x]
20 **Wainfan E**, Poirier LA. Methyl groups in carcinogenesis: effects on DNA methylation and gene expression. *Cancer Res* 1992; **52**: 2071s-2077s [PMID: 1544143]
21 **Christman JK**, Sheikhnejad G, Dizik M, Abileah S, Wainfan E. Reversibility of changes in nucleic acid methylation and gene expression induced in rat liver by severe dietary methyl deficiency. *Carcinogenesis* 1993; **14**: 551-557 [PMID: 8472313 DOI: 10.1093/carcin/14.4.551]
22 **Pogribny IP**, James SJ, Jernigan S, Pogribna M. Genomic hypomethylation is specific for preneoplastic liver in folate/methyl deficient rats and does not occur in non-target tissues. *Mutat Res* 2004; **548**: 53-59 [PMID: 15063136 DOI: 10.1016/j.mrfmmm.2003.12.014]
23 **Pogribny IP**, Ross SA, Wise C, Pogribna M, Jones EA, Tryndyak VP, James SJ, Dragan YP, Poirier LA. Irreversible global DNA hypomethylation as a key step in hepatocarcinogenesis induced by dietary methyl deficiency. *Mutat Res* 2006; **593**: 80-87 [PMID: 16144704 DOI: 10.1016/j.mrfmmm.2005.06.028]
24 **Ghoshal K**, Li X, Datta J, Bai S, Pogribny I, Pogribny M, Huang Y, Young D, Jacob ST. A folate- and methyl-deficient diet alters the expression of DNA methyltransferases and methyl CpG binding proteins involved in epigenetic gene silencing in livers of F344 rats. *J Nutr* 2006; **136**: 1522-1527 [PMID: 16702315]
25 **Christman JK**, Weich N, Schoenbrun B, Schneiderman N, Acs G. Hypomethylation of DNA during differentiation of Friend erythroleukemia cells. *J Cell Biol* 1980; **86**: 366-370 [PMID: 6931107 DOI: 10.1083/jcb.86.2.366]
26 **Balaghi M**, Wagner C. DNA methylation in folate deficiency: use of CpG methylase. *Biochem Biophys Res Commun* 1993; **193**: 1184-1190 [PMID: 8323540 DOI: 10.1006/bbrc.1993.1750]
27 **Kim YI**, Giuliano A, Hatch KD, Schneider A, Nour MA, Dallal GE, Selhub J, Mason JB. Global DNA hypomethylation increases progressively in cervical dysplasia and carcinoma. *Cancer* 1994; **74**: 893-899 [PMID: 8039116]
28 **Kim YI**, Pogribny IP, Basnakian AG, Miller JW, Selhub J, James SJ, Mason JB. Folate deficiency in rats induces DNA strand breaks and hypomethylation within the p53 tumor suppressor gene. *Am J Clin Nutr* 1997; **65**: 46-52 [PMID: 8988912]
29 **Pogribny I**, Yi P, James SJ. A sensitive new method for rapid detection of abnormal methylation patterns in global DNA and within CpG islands. *Biochem Biophys Res Commun* 1999; **262**: 624-628 [PMID: 10471374 DOI: 10.1006/bbrc.1999.1187]
30 **Pogribny IP**, Shpyleva SI, Muskhelishvili L, Bagnyukova TV, James SJ, Beland FA. Role of DNA damage and alterations in cytosine DNA methylation in rat liver carcinogenesis induced by a methyl-deficient diet. *Mutat Res* 2009; **669**: 56-62 [PMID: 19442675 DOI: 10.1016/j.mrfmmm.2009.05.003]
31 **Oakeley EJ**. DNA methylation analysis: a review of current methodologies. *Pharmacol Ther* 1999; **84**: 389-400 [PMID: 10665836 DOI: 10.1016/S0163-7258(99)00043-1]
32 **Friso S**, Choi SW, Dolnikowski GG, Selhub J. A method to assess genomic DNA methylation using high-performance liquid chromatography/electrospray ionization mass spectrometry. *Anal Chem* 2002; **74**: 4526-4531 [PMID: 12236365 DOI: 10.1021/ac020050h]
33 **Kuo KC**, McCune RA, Gehrke CW, Midgett R, Ehrlich M. Quantitative reversed-phase high performance liquid chromatographic determination of major and modified deoxyribonucleosides in DNA. *Nucleic Acids Res* 1980; **8**: 4763-4776 [PMID: 7003544 DOI: 10.1093/nar/8.20.4763]
34 **Christman JK**. Separation of major and minor deoxyribonucleoside monophosphates by reverse-phase high-performance liquid chromatography: a simple method applicable to quantitation of methylated nucleotides in DNA. *Anal Biochem* 1982; **119**: 38-48 [PMID: 7072940 DOI: 10.1016/0003-2697(82)90662-5]
35 **Kaur H**, Halliwell B. Measurement of oxidized and methylated DNA bases by HPLC with electrochemical detection. *Biochem J* 1996; **318** (Pt 1): 21-23 [PMID: 8761446]
36 **Friso S**, Choi SW, Girelli D, Mason JB, Dolnikowski GG, Bagley PJ, Olivieri O, Jacques PF, Rosenberg IH, Corrocher R, Selhub J. A common mutation in the 5,10-methylenetetrahydrofolate reductase gene affects genomic DNA methylation through an interaction with folate status. *Proc Natl Acad Sci USA* 2002; **99**: 5606-5611 [PMID: 11929966 DOI: 10.1073/pnas.062066299]
37 **Choi SW**, Friso S, Ghandour H, Bagley PJ, Selhub J, Mason JB. Vitamin B-12 deficiency induces anomalies of base substitution and methylation in the DNA of rat colonic epithelium. *J Nutr* 2004; **134**: 750-755 [PMID: 15051821]
38 **Friso S**, Girelli D, Trabetti E, Olivieri O, Guarini P, Pignatti PF, Corrocher R, Choi SW. The MTHFR 1298A& gt; C polymorphism and genomic DNA methylation in human lymphocytes. *Cancer Epidemiol Biomarkers Prev* 2005; **14**: 938-943 [PMID: 15824167 DOI: 10.1158/1055-9965.EPI-04-0601]
39 **Keyes MK**, Jang H, Mason JB, Liu Z, Crott JW, Smith DE, Friso S, Choi SW. Older age and dietary folate are determinants of genomic and p16-specific DNA methylation in mouse colon. *J Nutr* 2007; **137**: 1713-1717 [PMID: 17585020]
40 **Lim U**, Flood A, Choi SW, Albanes D, Cross AJ, Schatzkin A, Sinha R, Katki HA, Cash B, Schoenfeld P, Stolzenberg-Solomon R. Genomic methylation of leukocyte DNA in relation to colorectal adenoma among asymptomatic women. *Gastroenterology* 2008; **134**: 47-55 [PMID: 18166347 DOI: 10.1053/j.gastro.2007.10.013]
41 **Bird AP**. Use of restriction enzymes to study eukaryotic DNA methylation: II. The symmetry of methylated sites supports semi-conservative copying of the methylation pattern. *J Mol Biol* 1978; **118**: 49-60 [PMID: 625057 DOI: 10.1016/0022-2836(78)90243-7]
42 **Waalwijk C**, Flavell RA. MspI, an isoschizomer of hpaII which cleaves both unmethylated and methylated hpaII sites. *Nucleic Acids Res* 1978; **5**: 3231-3236 [PMID: 704354 DOI: 10.1093/nar/5.9.3231]
43 **Razin A**, Riggs AD. DNA methylation and gene function. *Science* 1980; **210**: 604-610 [PMID: 6254144 DOI: 10.1126/science.6254144]
44 **Feinberg AP**, Vogelstein B. Hypomethylation distinguishes genes of some human cancers from their normal counterparts. *Nature* 1983; **301**: 89-92 [PMID: 6185846 DOI: 10.1038/301089a0]
45 **Goelz SE**, Vogelstein B, Hamilton SR, Feinberg AP. Hypomethylation of DNA from benign and malignant human colon neoplasms. *Science* 1985; **228**: 187-190 [PMID: 2579435

DOI: 10.1126/science.2579435]
46 **Singer-Sam J**, LeBon JM, Tanguay RL, Riggs AD. A quantitative HpaII-PCR assay to measure methylation of DNA from a small number of cells. *Nucleic Acids Res* 1990; **18**: 687 [PMID: 1689825 DOI: 10.1093/nar/18.3.687]
47 **Frommer M**, McDonald LE, Millar DS, Collis CM, Watt F, Grigg GW, Molloy PL, Paul CL. A genomic sequencing protocol that yields a positive display of 5-methylcytosine residues in individual DNA strands. *Proc Natl Acad Sci USA* 1992; **89**: 1827-1831 [PMID: 1542678 DOI: 10.1073/pnas.89.5.1827]
48 **Eckhardt F**, Lewin J, Cortese R, Rakyan VK, Attwood J, Burger M, Burton J, Cox TV, Davies R, Down TA, Haefliger C, Horton R, Howe K, Jackson DK, Kunde J, Koenig C, Liddle J, Niblett D, Otto T, Pettett R, Seemann S, Thompson C, West T, Rogers J, Olek A, Berlin K, Beck S. DNA methylation profiling of human chromosomes 6, 20 and 22. *Nat Genet* 2006; **38**: 1378-1385 [PMID: 17072317 DOI: 10.1038/ng1909]
49 **Herman JG**, Graff JR, Myöhänen S, Nelkin BD, Baylin SB. Methylation-specific PCR: a novel PCR assay for methylation status of CpG islands. *Proc Natl Acad Sci USA* 1996; **93**: 9821-9826 [PMID: 8790415 DOI: 10.1073/pnas.93.18.9821]
50 **Xiong Z**, Laird PW. COBRA: a sensitive and quantitative DNA methylation assay. *Nucleic Acids Res* 1997; **25**: 2532-2534 [PMID: 9171110 DOI: 10.1093/nar/25.12.2532]
51 **Eads CA**, Danenberg KD, Kawakami K, Saltz LB, Blake C, Shibata D, Danenberg PV, Laird PW. MethyLight: a high-throughput assay to measure DNA methylation. *Nucleic Acids Res* 2000; **28**: E32 [PMID: 10734209 DOI: 10.1093/nar/28.8.e32]
52 **Dupont JM**, Tost J, Jammes H, Gut IG. De novo quantitative bisulfite sequencing using the pyrosequencing technology. *Anal Biochem* 2004; **333**: 119-127 [PMID: 15351288 DOI: 10.1016/j.ab.2004.05.007]
53 **Gitan RS**, Shi H, Chen CM, Yan PS, Huang TH. Methylation-specific oligonucleotide microarray: a new potential for high-throughput methylation analysis. *Genome Res* 2002; **12**: 158-164 [PMID: 11779841 DOI: 10.1101/gr.202801]
54 **Weber M**, Davies JJ, Wittig D, Oakeley EJ, Haase M, Lam WL, Schübeler D. Chromosome-wide and promoter-specific analyses identify sites of differential DNA methylation in normal and transformed human cells. *Nat Genet* 2005; **37**: 853-862 [PMID: 16007088 DOI: 10.1038/ng1598]
55 **Khulan B**, Thompson RF, Ye K, Fazzari MJ, Suzuki M, Stasiek E, Figueroa ME, Glass JL, Chen Q, Montagna C, Hatchwell E, Selzer RR, Richmond TA, Green RD, Melnick A, Greally JM. Comparative isoschizomer profiling of cytosine methylation: the HELP assay. *Genome Res* 2006; **16**: 1046-1055 [PMID: 16809668 DOI: 10.1101/gr.5273806]
56 **Allinen M**, Beroukhim R, Cai L, Brennan C, Lahti-Domenici J, Huang H, Porter D, Hu M, Chin L, Richardson A, Schnitt S, Sellers WR, Polyak K. Molecular characterization of the tumor microenvironment in breast cancer. *Cancer Cell* 2004; **6**: 17-32 [PMID: 15261139 DOI: 10.1016/j.ccr.2004.06.010]
57 **Bingham SA**. Mechanisms and experimental and epidemiological evidence relating dietary fibre (non-starch polysaccharides) and starch to protection against large bowel cancer. *Proc Nutr Soc* 1990; **49**: 153-171 [PMID: 2172992 DOI: 10.1079/PNS19900021]
58 **Murphy N**, Norat T, Ferrari P, Jenab M, Bueno-de-Mesquita B, Skeie G, Dahm CC, Overvad K, Olsen A, Tjønneland A, Clavel-Chapelon F, Boutron-Ruault MC, Racine A, Kaaks R, Teucher B, Boeing H, Bergmann MM, Trichopoulou A, Trichopoulos D, Lagiou P, Palli D, Pala V, Panico S, Tumino R, Vineis P, Siersema P, van Duijnhoven F, Peeters PH, Hjartaker A, Engeset D, González CA, Sánchez MJ, Dorronsoro M, Navarro C, Ardanaz E, Quirós JR, Sonestedt E, Ericson U, Nilsson L, Palmqvist R, Khaw KT, Wareham N, Key TJ, Crowe FL, Fedirko V, Wark PA, Chuang SC, Riboli E. Dietary fibre intake and risks of cancers of the colon and rectum in the European prospective investigation into cancer and nutrition (EPIC). *PLoS One* 2012; **7**: e39361 [PMID: 22761771 DOI: 10.1371/journal.pone.0039361]
59 **Topping DL**, Clifton PM. Short-chain fatty acids and human colonic function: roles of resistant starch and nonstarch polysaccharides. *Physiol Rev* 2001; **81**: 1031-1064 [PMID: 11427691]
60 **McIntyre A**, Gibson PR, Young GP. Butyrate production from dietary fibre and protection against large bowel cancer in a rat model. *Gut* 1993; **34**: 386-391 [PMID: 8386131 DOI: 10.1136/gut.34.3.386]
61 **Gobbi G**, Di Marcantonio D, Micheloni C, Carubbi C, Galli D, Vaccarezza M, Bucci G, Vitale M, Mirandola P. TRAIL up-regulation must be accompanied by a reciprocal PKCε down-regulation during differentiation of colonic epithelial cell: implications for colorectal cancer cell differentiation. *J Cell Physiol* 2012; **227**: 630-638 [PMID: 21465464 DOI: 10.1002/jcp.22765]
62 **Gope R**, Gope ML. Effect of sodium butyrate on the expression of retinoblastoma (RB1) and P53 gene and phosphorylation of retinoblastoma protein in human colon tumor cell line HT29. *Cell Mol Biol* (Noisy-le-grand) 1993; **39**: 589-597 [PMID: 8220069]
63 **Nakano K**, Mizuno T, Sowa Y, Orita T, Yoshino T, Okuyama Y, Fujita T, Ohtani-Fujita N, Matsukawa Y, Tokino T, Yamagishi H, Oka T, Nomura H, Sakai T. Butyrate activates the WAF1/Cip1 gene promoter through Sp1 sites in a p53-negative human colon cancer cell line. *J Biol Chem* 1997; **272**: 22199-22206 [PMID: 9268365 DOI: 10.1074/jbc.272.35.22199]
64 **Fang JY**, Chen YX, Lu J, Lu R, Yang L, Zhu HY, Gu WQ, Lu LG. Epigenetic modification regulates both expression of tumor-associated genes and cell cycle progressing in human colon cancer cell lines: Colo-320 and SW1116. *Cell Res* 2004; **14**: 217-226 [PMID: 15225415 DOI: 10.1038/sj.cr.7290222]
65 **Janson W**, Brandner G, Siegel J. Butyrate modulates DNA-damage-induced p53 response by induction of p53-independent differentiation and apoptosis. *Oncogene* 1997; **15**: 1395-1406 [PMID: 9333015 DOI: 10.1038/sj.onc.1201304]
66 **Terui T**, Murakami K, Takimoto R, Takahashi M, Takada K, Murakami T, Minami S, Matsunaga T, Takayama T, Kato J, Niitsu Y. Induction of PIG3 and NOXA through acetylation of p53 at 320 and 373 lysine residues as a mechanism for apoptotic cell death by histone deacetylase inhibitors. *Cancer Res* 2003; **63**: 8948-8954 [PMID: 14695212]
67 **Bernhard D**, Ausserlechner MJ, Tonko M, Löffler M, Hartmann BL, Csordas A, Kofler R. Apoptosis induced by the histone deacetylase inhibitor sodium butyrate in human leukemic lymphoblasts. *FASEB J* 1999; **13**: 1991-2001 [PMID: 10544182]
68 **Kuwajima A**, Iwashita J, Murata J, Abe T. The histone deacetylase inhibitor butyrate inhibits melanoma cell invasion of Matrigel. *Anticancer Res* 2007; **27**: 4163-4169 [PMID: 18225587]
69 **Van Lint C**, Emiliani S, Verdin E. The expression of a small fraction of cellular genes is changed in response to histone hyperacetylation. *Gene Expr* 1996; **5**: 245-253 [PMID: 8723390]
70 **Mariadason JM**, Corner GA, Augenlicht LH. Genetic reprogramming in pathways of colonic cell maturation induced by short chain fatty acids: comparison with trichostatin A, sulindac, and curcumin and implications for chemoprevention of colon cancer. *Cancer Res* 2000; **60**: 4561-4572 [PMID: 10969808]
71 **Davie JR**. Inhibition of histone deacetylase activity by butyrate. *J Nutr* 2003; **133**: 2485S-2493S [PMID: 12840228]
72 **Jackson V**, Shires A, Chalkley R, Granner DK. Studies on highly metabolically active acetylation and phosphorylation of histones. *J Biol Chem* 1975; **250**: 4856-4863 [PMID: 168194]
73 **Boffa LC**, Vidali G, Mann RS, Allfrey VG. Suppression of histone deacetylation in vivo and in vitro by sodium butyr-

ate. *J Biol Chem* 1978; **253**: 3364-3366 [PMID: 649576]
74 **Collas P**. The current state of chromatin immunoprecipitation. *Mol Biotechnol* 2010; **45**: 87-100 [PMID: 20077036 DOI: 10.1007/s12033-009-9239-8]
75 **de Magalhães JP**, Finch CE, Janssens G. Next-generation sequencing in aging research: emerging applications, problems, pitfalls and possible solutions. *Ageing Res Rev* 2010; **9**: 315-323 [PMID: 19900591 DOI: 10.1016/j.arr.2009.10.006]
76 **Ho JW**, Bishop E, Karchenko PV, Nègre N, White KP, Park PJ. ChIP-chip versus ChIP-seq: lessons for experimental design and data analysis. *BMC Genomics* 2011; **12**: 134 [PMID: 21356108 DOI: 10.1186/1471-2164-12-134]
77 **Johnson WE**, Li W, Meyer CA, Gottardo R, Carroll JS, Brown M, Liu XS. Model-based analysis of tiling-arrays for ChIP-chip. *Proc Natl Acad Sci USA* 2006; **103**: 12457-12462 [PMID: 16895995 DOI: 10.1073/pnas.0601180103]
78 **Song JS**, Johnson WE, Zhu X, Zhang X, Li W, Manrai AK, Liu JS, Chen R, Liu XS. Model-based analysis of two-color arrays (MA2C). *Genome Biol* 2007; **8**: R178 [PMID: 17727723 DOI: 10.1186/gb-2007-8-8-r178]
79 **Ji H**, Jiang H, Ma W, Johnson DS, Myers RM, Wong WH. An integrated software system for analyzing ChIP-chip and ChIP-seq data. *Nat Biotechnol* 2008; **26**: 1293-1300 [PMID: 18978777 DOI: 10.1038/nbt.1505]
80 **Consortium EP.** The ENCODE (ENCyclopedia Of DNA Elements) Project. *Science* 2004; **306**: 636-640 [PMID: 15499007 DOI: 10.1126/science.1105136]
81 **Liu T**, Rechtsteiner A, Egelhofer TA, Vielle A, Latorre I, Cheung MS, Ercan S, Ikegami K, Jensen M, Kolasinska-Zwierz P, Rosenbaum H, Shin H, Taing S, Takasaki T, Iniguez AL, Desai A, Dernburg AF, Kimura H, Lieb JD, Ahringer J, Strome S, Liu XS. Broad chromosomal domains of histone modification patterns in C. elegans. *Genome Res* 2011; **21**: 227-236 [PMID: 21177964 DOI: 10.1101/gr.115519.110]
82 **Roy S**, Ernst J, Kharchenko PV, Kheradpour P, Negre N, Eaton ML, Landolin JM, Bristow CA, Ma L, Lin MF, Washietl S, Arshinoff BI, Ay F, Meyer PE, Robine N, Washington NL, Di Stefano L, Berezikov E, Brown CD, Candeias R, Carlson JW, Carr A, Jungreis I, Marbach D, Sealfon R, Tolstorukov MY, Will S, Alekseyenko AA, Artieri C, Booth BW, Brooks AN, Dai Q, Davis CA, Duff MO, Feng X, Gorchakov AA, Gu T, Henikoff JG, Kapranov P, Li R, MacAlpine HK, Malone J, Minoda A, Nordman J, Okamura K, Perry M, Powell SK, Riddle NC, Sakai A, Samsonova A, Sandler JE, Schwartz YB, Sher N, Spokony R, Sturgill D, van Baren M, Wan KH, Yang L, Yu C, Feingold E, Good P, Guyer M, Lowdon R, Ahmad K, Andrews J, Berger B, Brenner SE, Brent MR, Cherbas L, Elgin SC, Gingeras TR, Grossman R, Hoskins RA, Kaufman TC, Kent W, Kuroda MI, Orr-Weaver T, Perrimon N, Pirrotta V, Posakony JW, Ren B, Russell S, Cherbas P, Graveley BR, Lewis S, Micklem G, Oliver B, Park PJ, Celniker SE, Henikoff S, Karpen GH, Lai EC, MacAlpine DM, Stein LD, White KP, Kellis M. Identification of functional elements and regulatory circuits by Drosophila modENCODE. *Science* 2010; **330**: 1787-1797 [PMID: 21177974 DOI: 10.1126/science.1198374]
83 **Bernstein BE**, Stamatoyannopoulos JA, Costello JF, Ren B, Milosavljevic A, Meissner A, Kellis M, Marra MA, Beaudet AL, Ecker JR, Farnham PJ, Hirst M, Lander ES, Mikkelsen TS, Thomson JA. The NIH Roadmap Epigenomics Mapping Consortium. *Nat Biotechnol* 2010; **28**: 1045-1048 [PMID: 20944595 DOI: 10.1038/nbt1010-1045]
84 **Metzker ML**. Sequencing technologies - the next generation. *Nat Rev Genet* 2010; **11**: 31-46 [PMID: 19997069 DOI: 10.1038/nrg2626]
85 **Kharchenko PV**, Tolstorukov MY, Park PJ. Design and analysis of ChIP-seq experiments for DNA-binding proteins. *Nat Biotechnol* 2008; **26**: 1351-1359 [PMID: 19029915 DOI: 10.1038/nbt.1508]
86 **Park PJ**. ChIP-seq: advantages and challenges of a maturing technology. *Nat Rev Genet* 2009; **10**: 669-680 [PMID: 19736561 DOI: 10.1038/nrg2641]
87 **Barski A**, Cuddapah S, Cui K, Roh TY, Schones DE, Wang Z, Wei G, Chepelev I, Zhao K. High-resolution profiling of histone methylations in the human genome. *Cell* 2007; **129**: 823-837 [PMID: 17512414 DOI: 10.1016/j.cell.2007.05.009]
88 **Wang Z**, Zang C, Rosenfeld JA, Schones DE, Barski A, Cuddapah S, Cui K, Roh TY, Peng W, Zhang MQ, Zhao K. Combinatorial patterns of histone acetylations and methylations in the human genome. *Nat Genet* 2008; **40**: 897-903 [PMID: 18552846 DOI: 10.1038/ng.154]
89 **Cancer Genome Atlas Research Network.** Comprehensive genomic characterization defines human glioblastoma genes and core pathways. *Nature* 2008; **455**: 1061-1068 [PMID: 18772890 DOI: 10.1038/nature07385]
90 **Morin RD**, Mendez-Lago M, Mungall AJ, Goya R, Mungall KL, Corbett RD, Johnson NA, Severson TM, Chiu R, Field M, Jackman S, Krzywinski M, Scott DW, Trinh DL, Tamura-Wells J, Li S, Firme MR, Rogic S, Griffith M, Chan S, Yakovenko O, Meyer IM, Zhao EY, Smailus D, Moksa M, Chittaranjan S, Rimsza L, Brooks-Wilson A, Spinelli JJ, Ben-Neriah S, Meissner B, Woolcock B, Boyle M, McDonald H, Tam A, Zhao Y, Delaney A, Zeng T, Tse K, Butterfield Y, Birol I, Holt R, Schein J, Horsman DE, Moore R, Jones SJ, Connors JM, Hirst M, Gascoyne RD, Marra MA. Frequent mutation of histone-modifying genes in non-Hodgkin lymphoma. *Nature* 2011; **476**: 298-303 [PMID: 21796119 DOI: 10.1038/nature10351]
91 **Pasqualucci L**, Trifonov V, Fabbri G, Ma J, Rossi D, Chiarenza A, Wells VA, Grunn A, Messina M, Elliot O, Chan J, Bhagat G, Chadburn A, Gaidano G, Mullighan CG, Rabadan R, Dalla-Favera R. Analysis of the coding genome of diffuse large B-cell lymphoma. *Nat Genet* 2011; **43**: 830-837 [PMID: 21804550 DOI: 10.1038/ng.892]
92 **Gui Y**, Guo G, Huang Y, Hu X, Tang A, Gao S, Wu R, Chen C, Li X, Zhou L, He M, Li Z, Sun X, Jia W, Chen J, Yang S, Zhou F, Zhao X, Wan S, Ye R, Liang C, Liu Z, Huang P, Liu C, Jiang H, Wang Y, Zheng H, Sun L, Liu X, Jiang Z, Feng D, Chen J, Wu S, Zou J, Zhang Z, Yang R, Zhao J, Xu C, Yin W, Guan Z, Ye J, Zhang H, Li J, Kristiansen K, Nickerson ML, Theodorescu D, Li Y, Zhang X, Li S, Wang J, Yang H, Wang J, Cai Z. Frequent mutations of chromatin remodeling genes in transitional cell carcinoma of the bladder. *Nat Genet* 2011; **43**: 875-878 [PMID: 21822268 DOI: 10.1038/ng.907]
93 **Li M**, Zhao H, Zhang X, Wood LD, Anders RA, Choti MA, Pawlik TM, Daniel HD, Kannangai R, Offerhaus GJ, Velculescu VE, Wang L, Zhou S, Vogelstein B, Hruban RH, Papadopoulos N, Cai J, Torbenson MS, Kinzler KW. Inactivating mutations of the chromatin remodeling gene ARID2 in hepatocellular carcinoma. *Nat Genet* 2011; **43**: 828-829 [PMID: 21822264 DOI: 10.1038/ng.903]
94 **Pogribny IP**, James SJ. De novo methylation of the p16INK4A gene in early preneoplastic liver and tumors induced by folate/methyl deficiency in rats. *Cancer Lett* 2002; **187**: 69-75 [PMID: 12359353]
95 **Tsujiuchi T**, Shimizu K, Itsuzaki Y, Onishi M, Sugata E, Fujii H, Honoki K. CpG site hypermethylation of E-cadherin and Connexin26 genes in hepatocellular carcinomas induced by a choline-deficient L-Amino Acid-defined diet in rats. *Mol Carcinog* 2007; **46**: 269-274 [PMID: 17295234 DOI: 10.1002/mc.20268]
96 **Pogribny IP**, Tryndyak VP, Bagnyukova TV, Melnyk S, Montgomery B, Ross SA, Latendresse JR, Rusyn I, Beland FA. Hepatic epigenetic phenotype predetermines individual susceptibility to hepatic steatosis in mice fed a lipogenic methyl-deficient diet. *J Hepatol* 2009; **51**: 176-186 [PMID: 19450891 DOI: 10.1016/j.jhep.2009.03.021]
97 **Tryndyak VP**, Han T, Muskhelishvili L, Fuscoe JC, Ross SA, Beland FA, Pogribny IP. Coupling global methylation

and gene expression profiles reveal key pathophysiological events in liver injury induced by a methyl-deficient diet. *Mol Nutr Food Res* 2011; **55**: 411-418 [PMID: 20938992 DOI: 10.1002/mnfr.201000300]

98 **Demary K**, Wong L, Spanjaard RA. Effects of retinoic acid and sodium butyrate on gene expression, histone acetylation and inhibition of proliferation of melanoma cells. *Cancer Lett* 2001; **163**: 103-107 [PMID: 11163113]

99 **Kida Y**, Shimizu T, Kuwano K. Sodium butyrate up-regulates cathelicidin gene expression via activator protein-1 and histone acetylation at the promoter region in a human lung epithelial cell line, EBC-1. *Mol Immunol* 2006; **43**: 1972-1981 [PMID: 16423398 DOI: 10.1016/j.molimm.2005.11.014]

100 **Rada-Iglesias A**, Enroth S, Ameur A, Koch CM, Clelland GK, Respuela-Alonso P, Wilcox S, Dovey OM, Ellis PD, Langford CF, Dunham I, Komorowski J, Wadelius C. Butyrate mediates decrease of histone acetylation centered on transcription start sites and down-regulation of associated genes. *Genome Res* 2007; **17**: 708-719 [PMID: 17567991 DOI: 10.1101/gr.5540007]

P- Reviewer Prasad KK, Lakatos PL
S- Editor Huang XZ **L- Editor** A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i7.1040

World J Gastroenterol 2013 February 21; 19(7): 1040-1048
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

REVIEW

Use of thiopurines in inflammatory bowel disease

Pascal Frei, Luc Biedermann, Ole Haagen Nielsen, Gerhard Rogler

Pascal Frei, Luc Biedermann, Gerhard Rogler, Division of Gastroenterology and Hepatology, Department of Internal Medicine, University Hospital of Zürich, CH-8091 Zürich, Switzerland
Ole Haagen Nielsen, Department of Gastroenterology, Medical Section, Herlev Hospital, University of Copenhagen, DK-2730 Herlev, Denmark
Author contributions: Frei P wrote the initial draft of the manuscript; Nielsen OH and Rogler G contributed to the conception and design of the review and supervised the review; Biedermann L contributed to the supportive work and revision of the manuscript.
Correspondence to: Gerhard Rogler, MD, PhD, Division of Gastroenterology and Hepatology, Department of Internal Medicine, University Hospital of Zurich, Raemistrasse 100, CH-8091 Zürich, Switzerland. gerhard.rogler@usz.ch
Telephone: +41-44-2559519 Fax: +41-44-2559497
Received: June 5, 2012 Revised: July 16, 2012
Accepted: August 14, 2012
Published online: February 21, 2013

Abstract

The use of thiopurines as immunosuppression for the treatment of refractory or chronic active inflammatory bowel disease is established for both Crohn's disease and ulcerative colitis. Nevertheless, many questions remain concerning the optimal treatment regimens of azathioprine, 6-mercaptopurine and thioguanine. We will briefly summarize dose recommendations, indications for thiopurine therapy and side effects which are relevant in clinical practice. We discuss some currently debated topics, including the combination of azathioprine and allopurinol, switching of thiopurine therapy in case of side effects, the use of azathioprine in pregnancy, the infection risk using thiopurines and the evidence when to stop thiopurines. Excellent reviews have been published on the thiopurine metabolic pathway which will not be discussed here in detail.

© 2013 Baishideng. All rights reserved.

Key words: Thiopurines; Inflammatory bowel disease; Crohn's disease; Ulcerative colitis; Immunosuppression; Infection

Frei P, Biedermann L, Nielsen OH, Rogler G. Use of thiopurines in inflammatory bowel disease. *World J Gastroenterol* 2013; 19(7): 1040-1048 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i7/1040.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i7.1040

DOSING AND MONITORING OF THIOPURINES

Thiopurines used for inflammatory bowel disease (IBD) treatment are azathioprine (AZA), 6-mercaptopurine (6-MP) and occasionally also 6-thioguanine (6-TG). The most commonly used thiopurine is AZA. The hepatic enzyme glutathione-S-transferase rapidly cleaves the prodrug AZA to 6-MP[1], which is then metabolized in both liver and gut by several enzymes: (1) thiopurine-s-methyltransferase (TPMT) catalysing 6-MP to 6-methyl-MP (6-MMP); (2) xanthine oxidase catalyzing 6-MP to thiourea; and (3) hypoxanthine-guanine-phosphoribosyltransferase converting 6-MP to 6-thioguanine nucleotides (6-TGN). 6-TG is the final effector-metabolite[2] which slowly accumulates in cells, and this metabolite is probably responsible for the delayed onset of action after 10-12 wk[3].

Dose recommendations for AZA and 6-MP vary slightly between Western guidelines, with a daily dose of 2-3 mg/kg AZA and 1-1.5 mg/kg 6-MP recommended by the AGA[4], and a daily dose of 1.5-2.5 mg/kg AZA and 0.75-1.5 mg/kg 6-MP recommended by the European Crohn's and Colitis Organisation (ECCO)[5]. However, these recommendations do not necessarily hold true for other ethnicities. Several Japanese studies showed that Japanese IBD patients might reach sufficient 6-TGN values with substantially lower AZA and 6-MP dosages in adults[6,7], children and adolescents[8]. If 6-TG is exceptionally used, it should be started at a much lower dosage of approximately 20 mg per day and should not exceed

25 mg daily[9]. However, it has to be strengthened that there are limited indications for use of 6-TG in IBD. 6-TG can be used in IBD patients who need a thiopurine for maintenance therapy but who are intolerant (or resistant) to 5-aminosalicylic acid (5-ASA) [in ulcerative colitis (UC)], AZA, 6-MP and methotrexate [in Crohn's disease (CD)], and who do not have an option for surgery[9]. Biological therapy could be considered as an alternative to 6-TG in this setting. The ECCO guidelines[5] concluded in 2006 that thioguanine cannot currently be recommended for maintenance of CD due to a high frequency of liver abnormalities (mainly nodular regenerative hyperplasia). According to the current literature, this might, however, be explained by the use of too high dosages of 6-TG resulting in 6-TGN levels exceeding 1000 pmol/8 × 10^8 erythrocytes which is much more than the target levels of 250-500 pmol/8 × 10^8 erythrocytes usually recommended when using AZA or 6-MP[10-12]. Thus, if 6-TG is used as therapy for selected IBD patients, the 6-TGN levels should be controlled regularly combined with close monitoring of liver values[13], hematology and especially liver biopsy after one, three and then every three years[9]. However, the histopathological diagnosis of nodular regenerative hyperplasia is difficult with a very poor inter-observer agreement, even among experts[14]. Alternatively, non-invasive monitoring such as magnetic resonance imaging may be considered[15].

The dose recommendations as mentioned above are based on "Western" or "Caucasian" guidelines/evidence and are linked to the assumption that the TPMT pathway is "normal". A diminished TPMT activity due to mutations of the *TPMT* gene leads to toxic levels of 6-TGN, which is a risk factor for drug-induced myelosuppression[16]. TPMT mutations are not uncommon in Caucasian populations (mutation prevalence approximately 10%), but rarer in *e.g.*, Chinese patients (5%) or even very rare in Japanese patients (1%)[17], which questions the usefulness of routine TPMT monitoring in these patients. The role of pharmacogenetics has recently been addressed in an excellent review by Chouchana *et al*[18] and is shortly summarized below.

To achieve an optimal therapy, it seems crucial to find the correct concentration of intracellular 6-TGN (serum levels are of no value). Concentrations that are too high lead to myelosuppression, whereas concentrations that are too low result in a lack of efficacy in IBD[16,19]. TGN-levels can be measured irrespective of the time of intake of thiopurines. The commonly recommended 6-TGN concentration is 235-500 pmol/8 × 10^8 erythrocytes. In a meta-analysis[20], patients with 6-TGN levels above the threshold value of 235 were more likely to be in remission than those below the threshold value (62% *vs* 36%, $P < 0.001$). The usefulness of therapeutic drug monitoring has recently been supported in a Dutch study[21]. However, the minimum effective dose is unknown. No dose-response study has ever been carried out, but at least one study showed that an increase in AZA dosage (2.02-2.72 mg/kg daily) can induce response in some patients[22]. Since there is only a very weak correlation between thiopurine dosage and 6-TGN levels[20] and some ethnic variability[6,7], the authors believe that 6-TGN levels should be regularly measured. Measuring the 6-TGN levels alleviates uncertainties when AZA therapy is combined with 5-ASA in UC[23]. In a recent and well-performed prospective study, the addition of 2 g of 5-ASA increased the total amount of 6-TGN by 50%, and methylmercaptopurine ribonucleotide levels were reduced after adding 4 g of 5-ASA[24]. Further studies are needed to show whether 6-TGN measurements might be useful to improve thiopurine therapy.

TPMT MONITORING

Beside the above mentioned measurement of thiopurine metabolites, TPMT genotyping (analysis of single nucleotide polymorphisms which influence the TPMT activity) and phenotyping (measuring the TMPT activity) can be applied before therapy with AZA or 6-MP is initiated. A recent study concluded that genotyping should be favoured for pre-treatment TPMT function since it is the more reliable test[25]. This evaluation may help to identify slow metabolizers which are at risk of toxicity[4]. Nevertheless, a recently meta-analysis stated that there is not yet enough evidence to advocate for routine TPMT testing[26]. The United States Food and Drug Administration (FDA), but not the ECCO, recommends TPMT monitoring before the initiation of thiopurine therapy. In 2011, the first guideline for a thiopurine starting dose according to TPMT phenotype/genotype was developed[27]. A reduction to 30%-70% of the full dose is recommended in patients with an intermediate activity (heterozygote). In patients with low/absent activity (homozygous mutant or compound heterozygote), an alternative drug should be considered or AZA starting dose must be reduced by a 10-fold, administered thrice weekly, and under close monitoring. For patients with intermediate and low/absent TPMT activity, a therapeutic drug monitoring is recommended four weeks after treatment initiation. Patients with a very high activity might benefit from an increase in AZA dosage up to 3.0 mg/kg per day[18]. Nevertheless, it is important to note that TPMT testing does not predict the long-term risk of myelosuppression or idiosyncratic adverse events such as fever, arthralgias, hepatitis or pancreatitis[28]. Regular hematologic monitoring remains necessary[26], initially at least every second week until the patient has been on a stable dose for a month; in the later course at least every third month[29,30], including a complete blood count including a differential count, especially to check the lymphocyte level but also platelets as well as amylase, and liver enzyme levels[4,30,31].

INDICATIONS FOR AZATHIOPRINE OR 6-MERCAPTOPURINE

No evidence for induction of remission in active IBD

There is not enough evidence to recommend thiopurines

for inducing remission in active IBD[32] based on five randomized controlled trials (RCTs) in active CD[33-37] and on two RCTs in active UC[38,39].

Prevention of relapse in quiescent IBD

In quiescent CD, AZA and 6-MP are effective at preventing a relapse based on two RCTs comparing AZA with placebo[33,37], and three RCTs comparing continued AZA *vs* withdrawal in patients who had been successfully maintained on AZA[40-42]. These studies showed a significantly higher relapse rate in the placebo groups as compared with the active treatment[32]. Additionally, a RCT evaluating glucocorticoid-dependent CD patients suggested that AZA was better than placebo at reducing the need for glucocorticoids[43].

In quiescent UC, AZA and 6-MP are effective at preventing a relapse based on three RCTs comparing AZA with placebo[38,39,44]. Overall, 60% of patients can be kept in remission if AZA is continued for 9-12 mo[32]. Similarly, another RCT which evaluated AZA withdrawal in 79 patients, who had been maintained on this drug for a minimum of 6 mo, showed a significant reduction on the relapse rate on AZA *vs* placebo after one year (36% *vs* 59%)[45] .

Post-operative treatment of CD with thiopurines

Two RCTs in postoperative CD suggest that AZA or 6-MP is superior to placebo at preventing recurrence after surgery[46,47]. Furthermore, thiopurines administered postoperatively significantly reduced the clinical recurrence rates in population-based cohorts[48,49]. Thus, thiopurines might be a useful strategy, although anti-tumour necrosis factors (anti-TNFs) might be more efficient in preventing CD relapses postoperatively[50,51], especially among high risk patients (smokers, perforating disease or $\geqslant$ second operation)[50].

Treatment of fistulising CD with thiopurines

A meta-analysis in fistulizing CD demonstrates a significantly higher response rate to AZA and 6-MP as compared to placebo (54% *vs* 21%)[52]. The analyzed studies included patients with perianal, enterocutaneous, enteroenteric and rectovaginal fistulas. However, only in a minority of patients, AZA and 6-MP will lead to a complete fistula closure, but symptoms such as inflammation, discharge and discomfort are often substantially reduced. Since response of fistulas to these drugs will need several months, immunosuppression will be reasonable only as second-line treatment if fistulas don't necessitate immediate surgery. Antibiotics are commonly used as first-line treatment and need to be started in parallel[53].

An overview of indications for thiopurines in IBD is given in Table 1.

ADVERSE EVENTS

AZA and 6-MP have similar side effects leading to discontinuation of therapy in 39% of patients in a large Dutch cohort[54], however, rates of intolerance are usually far lower. Most adverse events occur within the first 3 mo[55]. More than 50% of AZA intolerant patients tolerate 6-MP long-term[56]. Common side effects can be divided into dose-dependent and idiosyncratic side effects.

The major dose-dependent side effect of thiopurines is drug-induced myelosuppression which is observed in 2%-5% of Caucasian patients[57,58]. It can occur at any time with 25% of cases appearing beyond the first year[55]. Asian (or at least Japanese patients[6,7]) have a higher risk of myelotoxicity. It has been speculated that viral infections might contribute to myelosuppression, however, clear evidence is missing. Determining TPMT activity before starting thiopurines might avoid early myelosuppression, but regular haematological monitoring remains necessary in every patient.

Infectious complications are mostly dose-dependent, but sometimes idiosyncratic side effects. Infectious complications during thiopurine therapy can occur even in the absence of a dose-dependent leukopenia[4,59,60], especially when using a combination of thiopurines with corticosteroids which may induce a dose-dependent lymphocyte depletion. We recommend avoiding a lymphopenia of $< 600/\mu L$ based on data from a rheumatological study[31]. Doing this, severe immunodeficiency is likely to be avoided[31] Hepatotoxicity can manifest as early drug-induced hepatitis, nodular regenerative hyperplasia after years of therapy, sinusoidal dilatation or fibrosis[61]. It is important to note that IBD *per se* is a risk factor for nodular regenerative hyperplasia[62]. Hepatotoxicity induced by thiopurines seems more often dose dependent than idiosyncratic. In many patients, elevated transaminases respond to dose reduction. In a prospective monocentric cohort, 21 of 123 patients showed a transient or constant elevation of alanine aminotransferase levels[63]. If liver enzymes are repeatedly elevated, thiopurines need to be discontinued.

The most frequent idiosyncratic side effects are nausea, vomiting, and malaise in up to 15% of all patients[64]. Some advocate to slowly increase the dosage when thiopurine therapy is started, or to take it before nighttime. However, the best way to start thiopurine therapy still has to be determined[4]. Other common side effects are headache, fatigue, anorexia, weight loss, stomatitis, alopecia, arthralgia, muscular weakness and rash, which may occur in more than 10% of patients. If these side effects are reported, it should be determined whether they disappear after dose reduction[4,59,60,65,66]. In case of arthralgias/myalgias upon AZA, a switch to 6-MP can be explored[55].

Pancreatitis is an important idiosyncratic side effect and occurs in up to 4% of the patients[4,60], especially during the first weeks of treatment[67]. A minor and asymptomatic increase in serum amylase ("pancreatic hyperenzymemia") is frequently observed, but only poorly understood and not discussed in current guidelines. Some authors prefer to reduce dosing, or stop treatment

Table 1 Indications for thiopurines in inflammatory bowel disease

	Indication	No indication
Crohn's disease	Maintaining remission in moderate (to severe) CD (any site of disease especially for extensive disease) Maintaining remission in CD with early relapse (< 3 mo after the last flare) or frequent flares (more than two per year) Fistulizing CD (in combination with antibiotics, if no early start of anti-TNF or surgery necessary) Postoperative prevention of CD recurrence (unless high-risk situation such as repeated surgery or current smoker) In combination with anti-TNFs in case of severe CD (rapid step-up or top-down)	Induction of remission (as a sole therapy in active disease)
Ulcerative colitis (treated with 5-ASA at optimal dose unless intolerant)	Maintaining remission in steroid-dependent UC Maintaining remission in UC with early relapse requiring steroids Maintaining remission in UC with frequent flares requiring steroids Maintaining remission in UC after induction of remission by ciclosporin, tacrolimus, or *i.v.* steroids Acute or chronic refractory pouchitis	Induction of remission (as a sole therapy in active disease)

CD: Crohn's disease; UC: Ulcerative colitis; TNF: Tumour necrosis factor; 5-ASA: 5-aminosalicylic acid.

in case of lack of biochemical response[68]. Thiopurines must be discontinued if amylase increase is associated with typical pain symptoms (*i.e.*, toxic pancreatitis). After AZA-induced pancreatitis, a switch to 6-MP is not recommended since these patients are less likely to tolerate 6-MP[64]; However, evidence against the use of 6-MP in AZA-induced pancreatitis is weak. As earlier outlined, 6-TG is a debated alternative to AZA and 6-MP in case of intolerance[9], which is, however, not recommended by some authors[69,70].

WHEN AND HOW TO CHANGE THIOPURINE THERAPY IN CASE OF SIDE EFFECTS?

In the case of idiosyncratic side effects, it might be reasonable to change from AZA to 6-MP, as discussed above. Furthermore, in so-called preferential 6-MMP metabolizers which achieve only low 6-TGN levels due to high 6-MMP levels with associated hepatotoxicity, adding low-dose allopurinol as a XO inhibitor to dose-decreased AZA (25%-33% of intended dose[71], might switch the AZA metabolism to 6-TGN instead of 6MMP. Close monitoring of 6-TGN metabolites and hematology is in such cases necessary (weekly during the first month, then every other week for the next month)[69]. This strategy often allows to reach therapeutic levels of 6-TGN and clinical remission, but may also to reduce or even alleviate nausea as one of the major early side effects in more than 80% of patients[72]. Interestingly it seems possible to achieve higher 6-TGN and lower 6-MMP levels in preferential 6-MMP metabolizers by simply splitting the daily thiopurine dose[73] or by switching to 6-MP.

MALIGNANT COMPLICATIONS

Treatment with AZA/6-MP is associated with a potential risk of developing lymphoma[74], including hepatosplenic T-cell lymphoma (HSTCL)[75]. Although the relative risk of lymphoma is increased four to five-fold[74,76], the absolute risk still remains rather small, and currently available data show that the benefits of thiopurines used in IBD greatly outweigh its risks[77]. The same holds true for non-melanoma skin cancer[78-80], which in a large cohort of 108.518 IBD patients collected from 1997 to 2009 was shown to occur significantly more often in IBD patients on thiopurines especially among those with CD than controls[79], correlating with the length of receiving thiopurines. Of 32 cases of nonmelanoma skin cancer in a large cohort[80], only 5 cancers occurred in immunomodulator-naïve patients, but 9 and 18 cancers occurred in patients who had previously taken or were currently on thiopurine therapy. Based on these studies, a dermatologic exam should be considered before and regularly during immunomodulator therapy; especially in elderly patients. It should in this context be mentioned that skin protection is rather crucial.

WHEN SHOULD THIOPURINE MONOTHERAPY BE STOPPED?

When to stop a successful immunosuppression is one of the most difficult decisions in IBD therapy. Five studies focussing on this question were recently reviewed by Clarke and Regueiro[81]. According to a randomized, controlled study from 2005, even CD patients who were in remission on AZA for at least 3.5 year profited from prolonged AZA therapy (relapse risk 8% *vs* 21% on placebo)[40]. Similar results have been gained from other studies[82]. Five years after stopping AZA, 3/4 of patients suffer from relapse. Nevertheless, a treatment stop seems justified since almost all patients re-treated with thiopurines were able to regain remission (23 of 24 patients; many with a combined short course of glucocorticoids)[83]. Independent predictors for a flare are a C-reactive protein-level of > 20 mg/L, a haemoglobin-level < 12 mg/dL, and an absolute neutrophil count of > 4×10^9/L at baseline. Since more than half of the patients with risk factors experienced a clinical flare-up within 24 mo (compared to only 15% of patients without negative predictors), a continuous course of thiopurine therapy is recommended for these patients.

In UC, several studies have shown that a prolonged AZA therapy helps to reduce the risk of relapse. Six months is certainly the minimum length of immunosuppression (with at least 3 mo disease-free interval off glu-

cocorticoids)[84,85], but other studies showed that 18 mo is significantly better than 6 mo[81].

In clinical practice, decision on the length of immunosuppressive therapy means weighing benefits of immunosuppression on IBD and risk of malignancies and other complications. The risk factors should be analysed before immunosuppression is stopped. Best candidates for stopping immunosuppression will probably be IBD patients in deep, prolonged remission with a short duration of time between diagnosis and immunomodulator treatment[81]. Nonetheless, prospective RCTs are needed for the development of evidence-based tapering schemes regarding AZA and 6-MP treatment, both in CD as well as in UC.

IMMUNOMODULATORS AND BIOLOGIC THERAPY

Many experts in the field currently advocate a "top down" approach with early combination therapy for moderate to severe CD based on evidence from three infliximab and azathioprine studies in CD[86-88], including the famous SONIC trial[86]. Nevertheless caution is advised as the number needed to treat for the combination therapy is 8, meaning that only one out of 8 patients will definitely benefit from the combination. For the combination of AZA and adalimumab, there are only retrospective and hardly convincing data[89]. Future trials need to determine whether it is crucial to start with anti-TNFs and thiopurines at the same time, or whether a sequential addition of thiopurines to anti-TNFs will have the same effect in the patients experiencing a benefit from this combination.

Furthermore, there are not enough available data on how long the concomitant use of immunomodulators should be maintained in patients receiving infliximab. There are no studies which documented a sustained efficacy of concomitant use of immunomodulators beyond 6-12 mo[90,91]. If therapy should be reduced, then AZA and not infliximab may be stopped. In the recently published prospective STORI (Stop Infliximab in Patients With Crohn's Disease) study on infliximab stop in patients in remission under combined immunosuppression, nearly half of all patients suffered from relapse within 1 year when infliximab was stopped despite continuing azathioprine[92]. In contrary, the risk of relapse after stopping AZA seems lower than after stopping infliximab. Thus, in a single referral center observational study on CD patients who stopped AZA after being in remission under combined AZA and infliximab for at least 6 mo, 15% of patients relapsed after 1 year[93].

THIOPURINES IN PREGNANCY AND LACTATION

Even though thiopurines belong to FDA pregnancy category D, the risk seems minimal. Two studies[94,95] did not find any evidence for an increased risk of pregnancy-related complications. Probably, the only major side effect of thiopurines is the risk of preterm birth[95-97], which, however, might be simply a disease effect. Furthermore, thiopurine levels in breast milk of mothers treated with AZA seem harmless[98] and without any long-term effects in these babies. Thus, it can be concluded that thiopurines can be administered safely to women with IBD, prior to and at the time at conception, as well as during pregnancy and lactation. Indeed, in a recent survey among gastroenterologists showed that more than 90% would recommend continuous thiopurine treatment[99].

CONCLUSION

Indications for use of thiopurines in IBD and ways to monitor therapy have been well established. In clinical practice, the possibility of 6-TGN measurement to monitor therapy seems underused. It is likely that broader use of TGN monitoring, and a combination of thiopurines with low dose allopurinol in case of inefficiency or side effects allows to (achieve or) maintain clinical remission in more patients. For the thiopurine/allopurinol combination, but also for 6-TG therapy, prospective trials to document the benefit (and risk) are urgently needed. Furthermore, more trials are needed to elucidate whether an early "top down" approach for combination of thiopurines with anti-TNF will bring a long-term benefit for patients as compared to a step-up approach in case of a non-response to thiopurines. Criteria when a successful thiopurine therapy for maintenance of remission can be stopped also need to be elucidated in future prospective trials. Risk communication with patients, but also referring physicians, about benefit, side effects and the above mentioned uncertainties remain challenging. Benefits are the quality of life gained by medically maintained remission; the avoidance of surgery (in CD and UC) and avoidance of colorectal cancer through efficient anti-inflammatory therapy. Risks are, however, (opportunistic) infections, lymphomas such as HSTCL and side-effects such as pancreatitis.

Accordingly, thiopurine therapy remains a hot topic in IBD.

REFERENCES

1 **Watanabe A**, Hobara N, Nagashima H. Demonstration of enzymatic activity converting azathioprine to 6-mercaptopurine. *Acta Med Okayama* 1978; **32**: 173-179 [PMID: 29442 DOI: 10.1097/00007691-199810000-00002]

2 **Yatscoff RW**, Aspeslet LJ. The monitoring of immunosuppressive drugs: a pharmacodynamic approach. *Ther Drug Monit* 1998; **20**: 459-463 [PMID: 9780118]

3 **Su C**, Lichtenstein GR. Treatment of inflammatory bowel disease with azathioprine and 6-mercaptopurine. *Gastroenterol Clin North Am* 2004; **33**: 209-234, viii [PMID: 15177535 DOI: 10.1016/j.gtc.2004.02.004 DOI: 10.1053/j.gastro.2006.01.047]

4 **Lichtenstein GR**, Abreu MT, Cohen R, Tremaine W. American Gastroenterological Association Institute medical position statement on corticosteroids, immunomodulators, and infliximab in inflammatory bowel disease. *Gastroenterology* 2006; **130**: 935-939 [PMID: 16530531]

5 **Dignass A**, Van Assche G, Lindsay JO, Lémann M, Söderholm J, Colombel JF, Danese S, D'Hoore A, Gassull M, Gomollón F, Hommes DW, Michetti P, O'Morain C, Oresland T, Windsor A, Stange EF, Travis SP. The second European evidence-based Consensus on the diagnosis and management of Crohn's disease: Current management. *J Crohns Colitis* 2010; **4**: 28-62 [PMID: 21122489 DOI: 10.1016/j.crohns.2009.12.002]

6 **Andoh A**, Tsujikawa T, Ban H, Hashimoto T, Bamba S, Ogawa A, Sasaki M, Saito Y, Fujiyama Y. Monitoring 6-thioguanine nucleotide concentrations in Japanese patients with inflammatory bowel disease. *J Gastroenterol Hepatol* 2008; **23**: 1373-1377 [PMID: 18662197 DOI: 10.1111/j.1440-1746.2008.05419.x]

7 **Komiyama T**, Yajima T, Kubota R, Iwao Y, Sakuraba A, Funakoshi S, Negishi K, Minami I, Tanaka Y, Mae H, Hibi T. Lower doses of 6-mercaptopurine/azathioprine bring enough clinical efficacy and therapeutic concentration of erythrocyte 6-mercaptopurine metabolite in Japanese IBD patients. *J Crohns Colitis* 2008; **2**: 315-321 [PMID: 21172230 DOI: 10.1016/j.crohns.2008.05.002]

8 **Ohtsuka Y**, Arai K, Aoyagi Y, Fujii T, Yamakawa Y, Ohtani K, Ikuse T, Baba Y, Inage E, Kudo T, Suzuki R, Nagata S, Shimizu T. Monitoring 6-thioguanine nucleotide concentrations in Japanese children and adolescents with inflammatory bowel disease. *J Gastroenterol Hepatol* 2010; **25**: 1626-1630 [PMID: 20880170 DOI: 10.1111/j.1440-1746.2010.06364.x]

9 **Seinen ML**, van Asseldonk DP, Mulder CJ, de Boer NK. Dosing 6-thioguanine in inflammatory bowel disease: expert-based guidelines for daily practice. *J Gastrointestin Liver Dis* 2010; **19**: 291-294 [PMID: 20922194]

10 **Derijks LJ**, Gilissen LP, de Boer NK, Mulder CJ. 6-Thioguanine-related hepatotoxicity in patients with inflammatory bowel disease: dose or level dependent? *J Hepatol* 2006; **44**: 821-822 [PMID: 16487623 DOI: 10.1016/j.jhep.2005.11.049]

11 **Gilissen LP**, Derijks LJ, Driessen A, Bos LP, Hooymans PM, Stockbrügger RW, Engels LG. Toxicity of 6-thioguanine: no hepatotoxicity in a series of IBD patients treated with long-term, low dose 6-thioguanine. Some evidence for dose or metabolite level dependent effects? *Dig Liver Dis* 2007; **39**: 156-159 [PMID: 17188950 DOI: 10.1016/j.dld.2006.10.007]

12 **Rogler G**. Gastrointestinal and liver adverse effects of drugs used for treating IBD. *Best Pract Res Clin Gastroenterol* 2010; **24**: 157-165 [PMID: 20227029 DOI: 10.1016/j.bpg.2009.10.011]

13 **de Boer NK**, Zondervan PE, Gilissen LP, den Hartog G, Westerveld BD, Derijks LJ, Bloemena E, Engels LG, van Bodegraven AA, Mulder CJ. Absence of nodular regenerative hyperplasia after low-dose 6-thioguanine maintenance therapy in inflammatory bowel disease patients. *Dig Liver Dis* 2008; **40**: 108-113 [PMID: 18083079 DOI: 10.1016/j.dld.2007.10.013]

14 **Jharap B,** van Asseldonk DP, de Boer NK, Colombel JF, Diebold J, Jonker AM, Leteurtre E, Reinisch W, Vernier-Massouille G, Wendum D, Wrba F, Zondervan PE, Mulder CJJ, van Bodegraven AA, Bloemena E. Low Inter-Observer Agreement on Nodular Regenerative Hyperplasia of the Liver: An European Inter-Observer Analysis. *Gastroenterology* 2010; **138:** S456 [DOI: 10.1016/S0016-5085(10)62112-9]

15 **Zech CJ**, Seiderer J, Reinisch W, Ochsenkuhn T, Schima W, Diebold J, Wrba F, Reiser MF, Schoenberg SO. Thioguanine-induced nodular regenerative hyperplasia of the liver-ROC analysis of different MR techniques. *Eur Radiol* 2007; **17**: 1898-1905 [PMID: 17221208 DOI: 10.1007/s00330-006-0544-3]

16 **Weinshilboum RM**, Otterness DM, Szumlanski CL. Methylation pharmacogenetics: catechol O-methyltransferase, thiopurine methyltransferase, and histamine N-methyltransferase. *Annu Rev Pharmacol Toxicol* 1999; **39**: 19-52 [PMID: 10331075 DOI: 10.1146/annurev.pharmtox.39.1.19]

17 **Kubota T**, Nishida A, Takeuchi K, Iida T, Yokota H, Higashi K, Nakahara K, Hanai H, Iga T. Frequency distribution of thiopurine S-methyltransferase activity in red blood cells of a healthy Japanese population. *Ther Drug Monit* 2004; **26**: 319-321 [PMID: 15167635 DOI: 10.1097/00007691-200406000-00017]

18 **Chouchana L**, Narjoz C, Beaune P, Loriot MA, Roblin X. Review article: the benefits of pharmacogenetics for improving thiopurine therapy in inflammatory bowel disease. *Aliment Pharmacol Ther* 2012; **35**: 15-36 [PMID: 22050052 DOI: 10.1111/j.1365-2036.2011.04905.x]

19 **Cuffari C**, Hunt S, Bayless TM. Enhanced bioavailability of azathioprine compared to 6-mercaptopurine therapy in inflammatory bowel disease: correlation with treatment efficacy. *Aliment Pharmacol Ther* 2000; **14**: 1009-1014 [PMID: 10930894 DOI: 10.1046/j.1365-2036.2000.00812.x]

20 **Osterman MT**, Kundu R, Lichtenstein GR, Lewis JD. Association of 6-thioguanine nucleotide levels and inflammatory bowel disease activity: a meta-analysis. *Gastroenterology* 2006; **130**: 1047-1053 [PMID: 16618398]

21 **Gilissen LP**, Wong DR, Engels LG, Bierau J, Bakker JA, Paulussen AD, Romberg-Camps MJ, Stronkhorst A, Bus P, Bos LP, Hooymans PM, Stockbrügger RW, Neef C, Masclee AA. Therapeutic drug monitoring of thiopurine metabolites in adult thiopurine tolerant IBD patients on maintenance therapy. *J Crohns Colitis* 2012; **6**: 698-707 [PMID: 22398098 DOI: 10.1016/j.crohns.2011.12.003]

22 **Rayner CK**, Hart AL, Hayward CM, Emmanuel AV, Kamm MA. Azathioprine dose escalation in inflammatory bowel disease. *Aliment Pharmacol Ther* 2004; **20**: 65-71 [PMID: 15225172 DOI: 10.1111/j.1365-2036.2004.02009.x]

23 **Bondesen S**, Nielsen OH, Schou JB, Jensen PH, Lassen LB, Binder V, Krasilnikoff PA, Danø P, Hansen SH, Rasmussen SN. Steady-state kinetics of 5-aminosalicylic acid and sulfapyridine during sulfasalazine prophylaxis in ulcerative colitis. *Scand J Gastroenterol* 1986; **21**: 693-700 [PMID: 2875518 DOI: 10.3109/00365528609011102]

24 **de Graaf P**, de Boer NK, Wong DR, Karner S, Jharap B, Hooymans PM, Veldkamp AI, Mulder CJ, van Bodegraven AA, Schwab M. Influence of 5-aminosalicylic acid on 6-thioguanosine phosphate metabolite levels: a prospective study in patients under steady thiopurine therapy. *Br J Pharmacol* 2010; **160**: 1083-1091 [PMID: 20590602 DOI: 10.1111/j.1476-5381.2010.00731.x]

25 **Hindorf U**, Appell ML. Genotyping should be considered the primary choice for pre-treatment evaluation of thiopurine methyltransferase function. *J Crohns Colitis* 2012; **6**: 655-659 [PMID: 22398041 DOI: 10.1016/j.crohns.2011.11.014]

26 **Booth RA**, Ansari MT, Loit E, Tricco AC, Weeks L, Doucette S, Skidmore B, Sears M, Sy R, Karsh J. Assessment of thiopurine S-methyltransferase activity in patients prescribed thiopurines: a systematic review. *Ann Intern Med* 2011; **154**: 814-23, W-295-8 [PMID: 21690596]

27 **Relling MV**, Gardner EE, Sandborn WJ, Schmiegelow K, Pui CH, Yee SW, Stein CM, Carrillo M, Evans WE, Klein TE. Clinical Pharmacogenetics Implementation Consortium guidelines for thiopurine methyltransferase genotype and thiopurine dosing. *Clin Pharmacol Ther* 2011; **89**: 387-391 [PMID: 21270794 DOI: 10.1038/clpt.2010.320]

28 **Colombel JF**, Ferrari N, Debuysere H, Marteau P, Gendre JP, Bonaz B, Soulé JC, Modigliani R, Touze Y, Catala P, Libersa C, Broly F. Genotypic analysis of thiopurine S-methyltransferase in patients with Crohn's disease and severe myelosuppression during azathioprine therapy. *Gastroenterology* 2000; **118**: 1025-1030 [PMID: 10833476]

29 **Colombel JF**, Loftus EV, Tremaine WJ, Pemberton JH, Wolff BG, Young-Fadok T, Harmsen WS, Schleck CD, Sandborn WJ. Early postoperative complications are not increased in patients with Crohn's disease treated perioperatively with infliximab or immunosuppressive therapy. *Am J Gastroenterol* 2004; **99**: 878-883 [PMID: 15128354 DOI: 10.1111/j.1572-0241.2004.04148.x]

30 **Sandborn WJ**. A review of immune modifier therapy for inflammatory bowel disease: azathioprine, 6-mercaptopurine, cyclosporine, and methotrexate. *Am J Gastroenterol* 1996; **91**: 423-433 [PMID: 8633486]

31 **Glück T**, Kiefmann B, Grohmann M, Falk W, Straub RH, Schölmerich J. Immune status and risk for infection in patients receiving chronic immunosuppressive therapy. *J Rheumatol* 2005; **32**: 1473-1480 [PMID: 16078322]

32 **Khan KJ**, Dubinsky MC, Ford AC, Ullman TA, Talley NJ, Moayyedi P. Efficacy of immunosuppressive therapy for inflammatory bowel disease: a systematic review and meta-analysis. *Am J Gastroenterol* 2011; **106**: 630-642 [PMID: 21407186 DOI: 10.1038/ajg.2011.64]

33 **Candy S**, Wright J, Gerber M, Adams G, Gerig M, Goodman R. A controlled double blind study of azathioprine in the management of Crohn's disease. *Gut* 1995; **37**: 674-678 [PMID: 8549944 DOI: 10.1136/gut.37.5.674]

34 **Ewe K**, Press AG, Singe CC, Stufler M, Ueberschaer B, Hommel G, Meyer zum Büschenfelde KH. Azathioprine combined with prednisolone or monotherapy with prednisolone in active Crohn's disease. *Gastroenterology* 1993; **105**: 367-372 [PMID: 8335191]

35 **Oren R**, Moshkowitz M, Odes S, Becker S, Keter D, Pomeranz I, Shirin C, Reisfeld I, Broide E, Lavy A, Fich A, Eliakim R, Patz J, Villa Y, Arber N, Gilat T. Methotrexate in chronic active Crohn's disease: a double-blind, randomized, Israeli multicenter trial. *Am J Gastroenterol* 1997; **92**: 2203-2209 [PMID: 9399753]

36 **Reinisch W**, Panés J, Lémann M, Schreiber S, Feagan B, Schmidt S, Sturniolo GC, Mikhailova T, Alexeeva O, Sanna L, Haas T, Korom S, Mayer H. A multicenter, randomized, double-blind trial of everolimus versus azathioprine and placebo to maintain steroid-induced remission in patients with moderate-to-severe active Crohn's disease. *Am J Gastroenterol* 2008; **103**: 2284-2292 [PMID: 18671816 DOI: 10.1111/j.1572-0241.2008.02024.x]

37 **Tremaine WJ**, Schroeder KW, Harrison JM, Zinsmeister AR. A randomized, double-blind, placebo-controlled trial of the oral mesalamine (5-ASA) preparation, Asacol, in the treatment of symptomatic Crohn's colitis and ileocolitis. *J Clin Gastroenterol* 1994; **19**: 278-282 [PMID: 7876505 DOI: 10.1097/00004836-199412000-00003]

38 **Jewell DP**, Truelove SC. Azathioprine in ulcerative colitis: final report on controlled therapeutic trial. *Br Med J* 1974; **4**: 627-630 [PMID: 4441827 DOI: 10.1136/bmj.4.5945.627]

39 **Sood A**, Midha V, Sood N, Kaushal V. Role of azathioprine in severe ulcerative colitis: one-year, placebo-controlled, randomized trial. *Indian J Gastroenterol* 2000; **19**: 14-16 [PMID: 10659481]

40 **Lémann M**, Mary JY, Colombel JF, Duclos B, Soule JC, Lerebours E, Modigliani R, Bouhnik Y. A randomized, double-blind, controlled withdrawal trial in Crohn's disease patients in long-term remission on azathioprine. *Gastroenterology* 2005; **128**: 1812-1818 [PMID: 15940616 DOI: 10.1053/j.gastro.2005.03.031]

41 **O'Donoghue DP**, Dawson AM, Powell-Tuck J, Bown RL, Lennard-Jones JE. Double-blind withdrawal trial of azathioprine as maintenance treatment for Crohn's disease. *Lancet* 1978; **2**: 955-957 [PMID: 81986 DOI: 10.1016/S0140-6736(78)92524-2]

42 **Vilien M**, Dahlerup JF, Munck LK, Nørregaard P, Grønbaek K, Fallingborg J. Randomized controlled azathioprine withdrawal after more than two years treatment in Crohn's disease: increased relapse rate the following year. *Aliment Pharmacol Ther* 2004; **19**: 1147-1152 [PMID: 15153167 DOI: 10.1111/j.1365-2036.2004.01944.x]

43 **Rosenberg JL**, Levin B, Wall AJ, Kirsner JB. A controlled trial of azathioprine in Crohn's disease. *Am J Dig Dis* 1975; **20**: 721-726 [PMID: 1098449 DOI: 10.1007/BF01070829]

44 **Sood A**, Kaushal V, Midha V, Bhatia KL, Sood N, Malhotra V. The beneficial effect of azathioprine on maintenance of remission in severe ulcerative colitis. *J Gastroenterol* 2002; **37**: 270-274 [PMID: 11993510 DOI: 10.1007/s005350200034]

45 **Hawthorne AB**, Logan RF, Hawkey CJ, Foster PN, Axon AT, Swarbrick ET, Scott BB, Lennard-Jones JE. Randomised controlled trial of azathioprine withdrawal in ulcerative colitis. *BMJ* 1992; **305**: 20-22 [PMID: 1638191 DOI: 10.1136/bmj.305.6844.20]

46 **D'Haens GR**, Vermeire S, Van Assche G, Noman M, Aerden I, Van Olmen G, Rutgeerts P. Therapy of metronidazole with azathioprine to prevent postoperative recurrence of Crohn's disease: a controlled randomized trial. *Gastroenterology* 2008; **135**: 1123-1129 [PMID: 18727929 DOI: 10.1053/j.gastro.2008.07.010]

47 **Hanauer SB**, Korelitz BI, Rutgeerts P, Peppercorn MA, Thisted RA, Cohen RD, Present DH. Postoperative maintenance of Crohn's disease remission with 6-mercaptopurine, mesalamine, or placebo: a 2-year trial. *Gastroenterology* 2004; **127**: 723-729 [PMID: 15362027 DOI: 10.1053/j.gastro.2004.06.002]

48 **Peyrin-Biroulet L**, Deltenre P, Ardizzone S, D'Haens G, Hanauer SB, Herfarth H, Lémann M, Colombel JF. Azathioprine and 6-mercaptopurine for the prevention of postoperative recurrence in Crohn's disease: a meta-analysis. *Am J Gastroenterol* 2009; **104**: 2089-2096 [PMID: 19568226 DOI: 10.1038/ajg.2009.301]

49 **Reinisch W**, Angelberger S, Petritsch W, Shonova O, Lukas M, Bar-Meir S, Teml A, Schaeffeler E, Schwab M, Dilger K, Greinwald R, Mueller R, Stange EF, Herrlinger KR. Azathioprine versus mesalazine for prevention of postoperative clinical recurrence in patients with Crohn's disease with endoscopic recurrence: efficacy and safety results of a randomised, double-blind, double-dummy, multicentre trial. *Gut* 2010; **59**: 752-759 [PMID: 20551460 DOI: 10.1136/gut.2009.194159]

50 **De Cruz P**, Kamm M, Hamilton AL, Ritchie K, Gorelik A, Liew D, Prideaux L, Lawrance I, Andrews JM, Bampton P, Sparrow M, Jakobovits S, Florin T, Gibson P, Debinski H, Gearry R, Macrae F, Leong R, I. K, Connor S, Pavli P, Radford-Smith G, Selby W, Johnston M, Brouwer R, Keck JO, Woods R, Connell W, Brown SJ, Bell SJ, Lust M, Elliott R, Desmond PV. Adalimumab prevents post-operative Crohn's disease recurrence, and is superior to thiopurines: Early results from the prospective POCER study. *J Crohns Colitis* 2012; **6** Suppl 1: S146 [DOI: 10.1016/S1873-9946(12)60361-4]

51 **Swoger JM**, Regueiro M. Postoperative Crohn's disease: how can we prevent it? *Expert Rev Clin Immunol* 2010; **6**: 501-504 [PMID: 20594119 DOI: 10.1586/eci.10.33]

52 **Teml A**, Schwab M, Hommes DW, Almer S, Lukas M, Feichtenschlager T, Florin T, Seiderer J, Petritsch W, Bokemeyer B, Kreisel W, Herrlinger KR, Knoflach P, Bonaz B, Klugmann T, Herfarth H, Pedarnig N, Reinisch W. A systematic survey evaluating 6-thioguanine-related hepatotoxicity in patients with inflammatory bowel disease. *Wien Klin Wochenschr* 2007; **119**: 519-526 [PMID: 17943403 DOI: 10.1007/s00508-007-0841-0]

53 **Nielsen OH**, Rogler G, Hahnloser D, Thomsen OØ. Diagnosis and management of fistulizing Crohn's disease. *Nat Clin Pract Gastroenterol Hepatol* 2009; **6**: 92-106 [PMID: 19153563 DOI: 10.1038/ncpgasthep1340]

54 **Jharap B**, Seinen ML, de Boer NK, van Ginkel JR, Linskens RK, Kneppelhout JC, Mulder CJ, van Bodegraven AA. Thiopurine therapy in inflammatory bowel disease patients: analyses of two 8-year intercept cohorts. *Inflamm Bowel Dis* 2010; **16**: 1541-1549 [PMID: 20155846 DOI: 10.1002/ibd.21221]

55 **Hindorf U**, Lindqvist M, Hildebrand H, Fagerberg U, Almer S. Adverse events leading to modification of therapy in a large cohort of patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2006; **24**: 331-342 [PMID: 16842460 DOI: 10.1111/j.1365-2036.2006.02977.x]

56 **Hindorf U**, Johansson M, Eriksson A, Kvifors E, Almer SH.

Mercaptopurine treatment should be considered in azathioprine intolerant patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2009; **29**: 654-661 [PMID: 19183142 DOI: 10.1111/j.1365-2036.2008.03925.x]
57 **Connell WR**, Kamm MA, Dickson M, Balkwill AM, Ritchie JK, Lennard-Jones JE. Long-term neoplasia risk after azathioprine treatment in inflammatory bowel disease. *Lancet* 1994; **343**: 1249-1252 [PMID: 7910274 DOI: 10.1016/S0140-6736(94)92150-4]
58 **Present DH**, Meltzer SJ, Krumholz MP, Wolke A, Korelitz BI. 6-Mercaptopurine in the management of inflammatory bowel disease: short- and long-term toxicity. *Ann Intern Med* 1989; **111**: 641-649 [PMID: 2802419]
59 **Glazier KD**, Palance AL, Griffel LH, Das KM. The ten-year single-center experience with 6-mercaptopurine in the treatment of inflammatory bowel disease. *J Clin Gastroenterol* 2005; **39**: 21-26 [PMID: 15599205]
60 **Warman JI**, Korelitz BI, Fleisher MR, Janardhanam R. Cumulative experience with short- and long-term toxicity to 6-mercaptopurine in the treatment of Crohn's disease and ulcerative colitis. *J Clin Gastroenterol* 2003; **37**: 220-225 [PMID: 12960720 DOI: 10.1097/00004836-200309000-00006]
61 **Vernier-Massouille G**, Cosnes J, Lemann M, Marteau P, Reinisch W, Laharie D, Cadiot G, Bouhnik Y, De Vos M, Boureille A, Duclos B, Seksik P, Mary JY, Colombel JF. Nodular regenerative hyperplasia in patients with inflammatory bowel disease treated with azathioprine. *Gut* 2007; **56**: 1404-1409 [PMID: 17504943 DOI: 10.1136/gut.2006.114363]
62 **De Boer NK**, Tuynman H, Bloemena E, Westerga J, Van Der Peet DL, Mulder CJ, Cuesta MA, Meuwissen SG, Van Nieuwkerk CM, Van Bodegraven AA. Histopathology of liver biopsies from a thiopurine-naïve inflammatory bowel disease cohort: prevalence of nodular regenerative hyperplasia. *Scand J Gastroenterol* 2008; **43**: 604-608 [PMID: 18415755 DOI: 10.1080/00365520701800266]
63 **Wright S**, Sanders DS, Lobo AJ, Lennard L. Clinical significance of azathioprine active metabolite concentrations in inflammatory bowel disease. *Gut* 2004; **53**: 1123-1128 [PMID: 15247179 DOI: 10.1136/gut.2003.032896]
64 **Lees CW**, Maan AK, Hansoti B, Satsangi J, Arnott ID. Tolerability and safety of mercaptopurine in azathioprine-intolerant patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2008; **27**: 220-227 [PMID: 17988235 DOI: 10.1111/j.1365-2036.2007.03570.x]
65 **de Boer NK**, van Bodegraven AA, Jharap B, de Graaf P, Mulder CJ. Drug Insight: pharmacology and toxicity of thiopurine therapy in patients with IBD. *Nat Clin Pract Gastroenterol Hepatol* 2007; **4**: 686-694 [PMID: 18043678 DOI: 10.1038/ncpgasthep1000]
66 **Grassia R**, Paolo Coppeta G, Staiano T. Severe muscular weakness as an isolated symptom of azathioprine hypersensitivity. *Inflamm Bowel Dis* 2011; **17**: E61 [PMID: 21472829 DOI: 10.1002/ibd.21715]
67 **Lee WM**. Drug-induced hepatotoxicity. *N Engl J Med* 2003; **349**: 474-485 [PMID: 12890847 DOI: 10.1056/NEJMra021844]
68 **Costantino G**, Furfaro F, Belvedere A, Alibrandi A, Fries W. Thiopurine treatment in inflammatory bowel disease: response predictors, safety, and withdrawal in follow-up. *J Crohns Colitis* 2012; **6**: 588-596 [PMID: 22398045 DOI: 10.1016/j.crohns.2011.11.007]
69 **Bradford K**, Shih DQ. Optimizing 6-mercaptopurine and azathioprine therapy in the management of inflammatory bowel disease. *World J Gastroenterol* 2011; **17**: 4166-4173 [PMID: 22072847 DOI: 10.3748/wjg.v17.i37.4166]
70 **Travis SP**, Stange EF, Lémann M, Oresland T, Chowers Y, Forbes A, D'Haens G, Kitis G, Cortot A, Prantera C, Marteau P, Colombel JF, Gionchetti P, Bouhnik Y, Tiret E, Kroesen J, Starlinger M, Mortensen NJ. European evidence based consensus on the diagnosis and management of Crohn's disease: current management. *Gut* 2006; **55** Suppl 1: i16-i35 [PMID: 16481629 DOI: 10.1136/gut.2005.081950b]
71 **Chocair P**, Duley J, Simmonds HA, Cameron JS, Ianhez L, Arap S, Sabbaga E. Low-dose allopurinol plus azathioprine/cyclosporin/prednisolone, a novel immunosuppressive regimen. *Lancet* 1993; **342**: 83-84 [PMID: 8100914 DOI: 10.1016/0140-6736(93)91287-V]
72 **Ansari A**, Patel N, Sanderson J, O'Donohue J, Duley JA, Florin TH. Low-dose azathioprine or mercaptopurine in combination with allopurinol can bypass many adverse drug reactions in patients with inflammatory bowel disease. *Aliment Pharmacol Ther* 2010; **31**: 640-647 [PMID: 20015102 DOI: 10.1111/j.1365-2036.2009.04221.x]
73 **Shih DQ**, Nguyen M, Ibanez P, Kwan LY, Targan SR, Vasiliauskas EA. Split-dose administration of 6MP/Azathioprine: a novel and effective strategy for IBD patients with preferential 6MMP metabolism. *Gastroenterology* 2009; **136**: A677-A678 [DOI: 10.1016/S0016-5085(09)63119-X]
74 **Kandiel A**, Fraser AG, Korelitz BI, Brensinger C, Lewis JD. Increased risk of lymphoma among inflammatory bowel disease patients treated with azathioprine and 6-mercaptopurine. *Gut* 2005; **54**: 1121-1125 [PMID: 16009685 DOI: 10.1136/gut.2004.049460]
75 **Kotlyar DS**, Osterman MT, Diamond RH, Porter D, Blonski WC, Wasik M, Sampat S, Mendizabal M, Lin MV, Lichtenstein GR. A systematic review of factors that contribute to hepatosplenic T-cell lymphoma in patients with inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2011; **9**: 36-41.e1 [PMID: 20888436]
76 **Sokol H**, Beaugerie L, Maynadié M, Laharie D, Dupas JL, Flourié B, Lerebours E, Peyrin-Biroulet L, Allez M, Simon T, Carrat F, Brousse N. Excess primary intestinal lymphoproliferative disorders in patients with inflammatory bowel disease. *Inflamm Bowel Dis* 2012; **18**: 2063-2071 [PMID: 22271569 DOI: 10.1002/ibd.22889]
77 **Armstrong RG**, West J, Card TR. Risk of cancer in inflammatory bowel disease treated with azathioprine: a UK population-based case-control study. *Am J Gastroenterol* 2010; **105**: 1604-1609 [PMID: 20104215 DOI: 10.1038/ajg.2009.745]
78 **Long MD**, Herfarth HH, Pipkin CA, Porter CQ, Sandler RS, Kappelman MD. Increased risk for non-melanoma skin cancer in patients with inflammatory bowel disease. *Clin Gastroenterol Hepatol* 2010; **8**: 268-274 [PMID: 20005977 DOI: 10.1016/j.cgh.2009.11.024]
79 **Long MD**, Martin CF, Pipkin CA, Herfarth HH, Sandler RS, Kappelman MD. Risk of melanoma and nonmelanoma skin cancer among patients with inflammatory bowel disease. *Gastroenterology* 2012; **143**: 390-399.e1 [PMID: 22584081]
80 **Peyrin-Biroulet L**, Khosrotehrani K, Carrat F, Bouvier AM, Chevaux JB, Simon T, Carbonnel F, Colombel JF, Dupas JL, Godeberge P, Hugot JP, Lémann M, Nahon S, Sabaté JM, Tucat G, Beaugerie L. Increased risk for nonmelanoma skin cancers in patients who receive thiopurines for inflammatory bowel disease. *Gastroenterology* 2011; **141**: 1621-28.e1-5 [PMID: 21708105]
81 **Clarke K**, Regueiro M. Stopping immunomodulators and biologics in inflammatory bowel disease patients in remission. *Inflamm Bowel Dis* 2012; **18**: 174-179 [PMID: 21674731 DOI: 10.1002/ibd.21792]
82 **Kim PS**, Zlatanic J, Korelitz BI, Gleim GW. Optimum duration of treatment with 6-mercaptopurine for Crohn's disease. *Am J Gastroenterol* 1999; **94**: 3254-3257 [PMID: 10566725 DOI: 10.1111/j.1572-0241.1999.01532.x]
83 **Treton X**, Bouhnik Y, Mary JY, Colombel JF, Duclos B, Soule JC, Lerebours E, Cosnes J, Lemann M. Azathioprine withdrawal in patients with Crohn's disease maintained on prolonged remission: a high risk of relapse. *Clin Gastroenterol Hepatol* 2009; **7**: 80-85 [PMID: 18849016 DOI: 10.1016/j.cgh.2008.08.028]
84 **Cassinotti A**, Actis GC, Duca P, Massari A, Colombo E, Gai E, Annese V, D'Albasio G, Manes G, Travis S, Porro GB,

Ardizzone S. Maintenance treatment with azathioprine in ulcerative colitis: outcome and predictive factors after drug withdrawal. *Am J Gastroenterol* 2009; **104**: 2760-2767 [PMID: 19623172 DOI: 10.1038/ajg.2009.410]

85 **Lobel EZ**, Korelitz BI, Xuereb MA, Panagopoulos G. A search for the optimal duration of treatment with 6-mercaptopurine for ulcerative colitis. *Am J Gastroenterol* 2004; **99**: 462-465 [PMID: 15056086 DOI: 10.1111/j.1572-0241.2004.04104.x]

86 **Colombel JF**, Sandborn WJ, Reinisch W, Mantzaris GJ, Kornbluth A, Rachmilewitz D, Lichtiger S, D'Haens G, Diamond RH, Broussard DL, Tang KL, van der Woude CJ, Rutgeerts P. Infliximab, azathioprine, or combination therapy for Crohn's disease. *N Engl J Med* 2010; **362**: 1383-1395 [PMID: 20393175 DOI: 10.1056/NEJMoa0904492]

87 **D'Haens G**, Baert F, van Assche G, Caenepeel P, Vergauwe P, Tuynman H, De Vos M, van Deventer S, Stitt L, Donner A, Vermeire S, Van de Mierop FJ, Coche JC, van der Woude J, Ochsenkühn T, van Bodegraven AA, Van Hootegem PP, Lambrecht GL, Mana F, Rutgeerts P, Feagan BG, Hommes D. Early combined immunosuppression or conventional management in patients with newly diagnosed Crohn's disease: an open randomised trial. *Lancet* 2008; **371**: 660-667 [PMID: 18295023]

88 **Lémann M**, Mary JY, Duclos B, Veyrac M, Dupas JL, Delchier JC, Laharie D, Moreau J, Cadiot G, Picon L, Bourreille A, Sobahni I, Colombel JF. Infliximab plus azathioprine for steroid-dependent Crohn's disease patients: a randomized placebo-controlled trial. *Gastroenterology* 2006; **130**: 1054-1061 [PMID: 16618399 DOI: 10.1053/j.gastro.2006.02.014]

89 **Reenaers C,** Louis E, Belaiche J, Keshav S, Travis S. Immunosuppressive co-treatment with adalimumab (ADA) may be more effective than ADA monotherapy for maintaining remission in Crohn's disease (CD). *J Crohns Colitis* 2012; **6** Suppl 1: S8 [DOI: 10.1016/S1873-9946(12)60016-6]

90 **Lichtenstein GR**, Diamond RH, Wagner CL, Fasanmade AA, Olson AD, Marano CW, Johanns J, Lang Y, Sandborn WJ. Clinical trial: benefits and risks of immunomodulators and maintenance infliximab for IBD-subgroup analyses across four randomized trials. *Aliment Pharmacol Ther* 2009; **30**: 210-226 [PMID: 19392858 DOI: 10.1111/j.1365-2036.2009.04027.x]

91 **Van Assche G**, Magdelaine-Beuzelin C, D'Haens G, Baert F, Noman M, Vermeire S, Ternant D, Watier H, Paintaud G, Rutgeerts P. Withdrawal of immunosuppression in Crohn's disease treated with scheduled infliximab maintenance: a randomized trial. *Gastroenterology* 2008; **134**: 1861-1868 [PMID: 18440315 DOI: 10.1053/j.gastro.2008.03.004]

92 **Louis E**, Mary JY, Vernier-Massouille G, Grimaud JC, Bouhnik Y, Laharie D, Dupas JL, Pillant H, Picon L, Veyrac M, Flamant M, Savoye G, Jian R, Devos M, Porcher R, Paintaud G, Piver E, Colombel JF, Lemann M. Maintenance of remission among patients with Crohn's disease on antimetabolite therapy after infliximab therapy is stopped. *Gastroenterology* 2012; **142**: 63-70.e5; quiz e31 [PMID: 21945953]

93 **Oussalah A**, Chevaux JB, Fay R, Sandborn WJ, Bigard MA, Peyrin-Biroulet L. Predictors of infliximab failure after azathioprine withdrawal in Crohn's disease treated with combination therapy. *Am J Gastroenterol* 2010; **105**: 1142-1149 [PMID: 20389296]

94 **Francella A**, Dyan A, Bodian C, Rubin P, Chapman M, Present DH. The safety of 6-mercaptopurine for childbearing patients with inflammatory bowel disease: a retrospective cohort study. *Gastroenterology* 2003; **124**: 9-17 [PMID: 12512024 DOI: 10.1053/gast.2003.50014]

95 **Goldstein LH**, Dolinsky G, Greenberg R, Schaefer C, Cohen-Kerem R, Diav-Citrin O, Malm H, Reuvers-Lodewijks ME, Rost van Tonningen-van Driel MM, Arnon J, Ornoy A, Clementi M, Di Gianantonio E, Koren G, Braunstein R, Berkovitch M. Pregnancy outcome of women exposed to azathioprine during pregnancy. *Birth Defects Res A Clin Mol Teratol* 2007; **79**: 696-701 [PMID: 17847119 DOI: 10.1002/bdra.20399]

96 **Cleary BJ**, Källén B. Early pregnancy azathioprine use and pregnancy outcomes. *Birth Defects Res A Clin Mol Teratol* 2009; **85**: 647-654 [PMID: 19343728 DOI: 10.1002/bdra.20583]

97 **Langagergaard V**, Pedersen L, Gislum M, Nørgard B, Sørensen HT. Birth outcome in women treated with azathioprine or mercaptopurine during pregnancy: A Danish nationwide cohort study. *Aliment Pharmacol Ther* 2007; **25**: 73-81 [PMID: 17229222 DOI: 10.1002/bdra.20583]

98 **Christensen LA**, Dahlerup JF, Nielsen MJ, Fallingborg JF, Schmiegelow K. Azathioprine treatment during lactation. *Aliment Pharmacol Ther* 2008; **28**: 1209-1213 [PMID: 18761704]

99 **Peyrin-Biroulet L**, Oussalah A, Roblin X, Sparrow MP. The use of azathioprine in Crohn's disease during pregnancy and in the post-operative setting: a worldwide survey of experts. *Aliment Pharmacol Ther* 2011; **33**: 707-713 [PMID: 21251032 DOI: 10.1111/j.1365-2036.2011.04577.x]

P- Reviewers Fries W, Cheon JH
S- Editor Shi ZF **L- Editor** A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i7.1049

World J Gastroenterol 2013 February 21; 19(7): 1049-1055
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

MINIREVIEW

Tailoring the area of hepatic resection using inflow and outflow modulation

Matteo Donadon, Fabio Procopio, Guido Torzilli

Matteo Donadon, Fabio Procopio, Guido Torzilli, Liver Surgery Unit, Department of General Surgery, University of Milan School of Medicine, Humanitas Clinical and Research Center, 20089 Milan, Italy

Author contributions: Donadon M designed and wrote the manuscript; Procopio F helped with the figures; Torzilli G reviewed and approved the manuscript.

Correspondence to: Guido Torzilli, MD, PhD, Associate Professor of Surgery, Chief, Liver Surgery Unit, Department of General Surgery, University of Milan School of Medicine, Humanitas Clinical and Research Center, Via Manzoni 56, Rozzano, 20089 Milan, Italy. guido.torzilli@humanitas.it

Telephone: +39-2-82244083 Fax: +39-2-82244590

Received: August 18, 2012 Revised: December 7, 2012
Accepted: December 15, 2012
Published online: February 21, 2013

Abstract

The performance of hepatic surgery without a parenchyma-sparing strategy carries significant risks for patient survival because of the not negligible occurrence of postoperative liver failure. The key factor of modern hepatic surgery is the use of the intraoperative ultrasound (IOUS), not only to stage the disease, but more importantly to guide resection with the specific aim to maximize the sparing of the functional parenchyma. Whether in patients with hepatocellular carcinoma and underlying liver cirrhosis, or in patients with colorectal liver metastasis, IOUS allows the performance of the so-called "radical but conservative surgery", which is the pivotal factor to offer a chance of cure to an increasing proportion of patients, who until few years ago were considered only for palliative care. Using some new IOUS-guided surgical maneuvers, which are based on the liver inflow and outflow modulations, more precise anatomically subsegmental- and segmental-oriented resections can be effectively performed. The present work describes the rationale and the surgical technique for a precise tailoring of the area of hepatic resection using the most recent attainments in IOUS. Such important technical achievements should be a fundamental part of the surgical armamentarium of the modern liver surgeon.

© 2013 Baishideng. All rights reserved.

Key words: Hepatic resection; Intraoperative ultrasound; Liver inflow; Liver outflow; Resection guidance

Donadon M, Procopio F, Torzilli G. Tailoring the area of hepatic resection using inflow and outflow modulation. *World J Gastroenterol* 2013; 19(7): 1049-1055 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i7/1049.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i7.1049

INTRODUCTION

The performance of hepatic surgery without a parenchyma-sparing strategy carries significant risks for patient survival because of the not negligible occurrence of postoperative liver failure, which is definitely related to the amount of the sacrificed parenchyma[1,2]. Indeed, major or extended hepatic resections are independent negative prognostic factors with regard to short- and long-term outcomes[2-7]. The key factor of modern hepatic surgery is the use of the intraoperative ultrasound (IOUS) not only to stage the disease, but more importantly to guide resection, with the specific aim of maximizing parenchyma-sparing, removing only the tumoral tissue[8]. Whether in patients with hepatocellular carcinoma (HCC) or in patients with colorectal liver metastasis (CLM), IOUS allows the performance of the so-called "conservative but radical surgery"[9], which is the pivotal factor to offer a chance of cure to an increasing proportion of patients, who until few years ago were considered only for palliative care. Indeed, in cases of HCC with cirrhosis the underlying liver function is generally marginal, and the prognosis of the patient might be more related to the residual liver function rather than to the pres-

ence of HCC. In such patients, the hepatectomy should always be tailored on the basis of both tumoral features and functional liver reserve. Similarly, in cases of CLM the rationale of the surgical approach described here is based on the need to minimize the rate of major or extended resections with the aim of reducing operative risk, and at the same time preserving the liver parenchyma, which could be the site for future hepatic recurrence potentially re-treated with curative intent.

OPERATIVE TECHNIQUE

The J-shaped laparotomy is the preferred incision for liver surgery, and the access into the right thoracic cavity following the 9th intercostal space is carried out to control the hepatocaval confluence. In particular, the thoracoabdominal approach is selected in obese patients, in patients with a deep chest, and during complex reoperations. Thus, the liver is partially mobilized by dividing the round and the falciform ligaments. Sometimes the coronary and triangular ligaments are also divided early to obtain enough space for IOUS. This should in fact be performed before complete mobilization of the liver to avoid any artifact made by the surgical maneuvers.

INTRAOPERATIVE ULTRASOUND

IOUS is the procedure of choice to stage disease in patients with liver tumors. It should be fully performed by the surgeon in charge for the operation rather than by the assistants, radiologists or technicians. This is because the information gathered during the exploration requires interpretation to have most impact on the surgical strategy. Thus, IOUS is mainly performed to plan the surgical strategy rather than to locate the lesions. Generally, high frequency probes (7.5-10 MHz) are recommended for IOUS, since they allow for a higher spatial resolution than those working at lower frequencies (3.5-5 MHz). However, those latter probes are very useful for the initial exploration providing a better panoramic view. Lower frequency probes are also useful for allowing contrast-enhanced IOUS. Different shapes of probes are available for intraoperative use: the linear T-shaped, the inter-digital, and micro-convex probes. The best probe is the one that ensures the optimal compromise between the volume of the probe itself, which should be minimal, the scanning windows, which should be the largest, and the stability once in contact with the liver surface. In this sense, a new micro-linear probe with trapezoid scanning windows probably represents the best compromise among all the aforementioned requirements; this probe is furthermore designed to meet the requirements for those surgical maneuvers discussed here (Figure 1). Of note, the performance of IOUS may take time, and it requires experience to be effective and beneficial[10].

RESECTION GUIDANCE

Apart from staging, IOUS is essential to guide resection. It is almost impossible to correctly define the hepatic segmental boundaries without IOUS, nor the boundaries of the tumor itself because of the existing wide variations in anatomy. The main advantage of IOUS-guided resection is modification of the traditional approach to liver tissue dissection, which involves dissection in vertical planes to avoid tumor exposure on the cut surface. With IOUS, the relationship between the dissection plane and the tumor edges can be followed in real time, and the direction of the dissection plane can be modified when needed. Versatile dissection planes around the tumors can avoid tumor exposure while sparing important vascular structures, thus sparing vital liver parenchyma. This approach has been recently redefined by the authors as the "radical but conservative approach", and should be applied in liver surgery to maximize the results[9]. Also, in patients in whom major resections should be required, IOUS allows better design of the dissection plane, leading to conservative surgery even in patients with complex tumoral presentations[11]. Specific, and original IOUS techniques have already been developed to help the surgeon during the operation[12-15]. The following paragraphs will focus on two crucial techniques for defining the area of resection using IOUS findings.

PLANNING OF THE SURGICAL STRATEGY

The information achieved from the preoperative imaging workup, which has an essential role in staging intra- and extra-hepatic disease, should be used to plan the surgical strategy. However, the surgical strategy should be intraoperatively defined only after IOUS exploration. The impact of IOUS on the operative decision-making, when compared with that of preoperative imaging techniques, is reported to be around 4%-7%[16,17]. These relatively low rates may be explained because of the different surgical policies applied by the different centers as well as the different tumor types considered. Indeed, IOUS, when used in a systematic and extensive way to map the tumor nodules, allows a 3-dimensional reconstruction of the relationships between the tumor and the main intrahepatic vascular structures [glissonian pedicles and hepatic veins (HVs)], which is pivotal in planning the individualized surgical strategy for each patient. Indeed, some experienced authors reported better results in terms of IOUS accuracy[18-21]. Some important tumor-vessel relationship rules have been developed by the authors, both for HCC and for CLM, with the aim of providing an intraoperative guide to individualize the surgical strategy and minimize parenchyma sacrifice.

Tumor in contact with a glissonian pedicle

The glissonian pedicle may be spared when in contact with an encapsulated HCC or with a CLM once the integrity of the vessel wall is confirmed at IOUS, without any sign of distal bile duct dilation. For CLM, the contact should extend for less than one-third of the pedicle circumference. In the presence of bile duct dilation, tu-

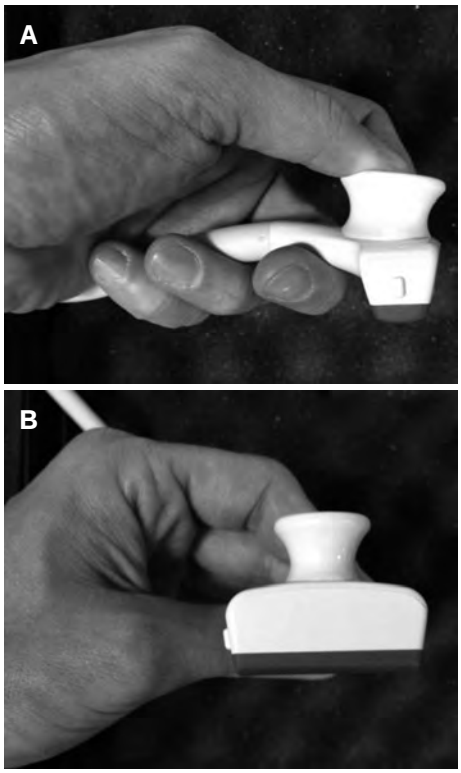


Figure 1 New probe for intraoperative ultrasound. This probe has a trapezoid scanning area, and an ergonomic shape, which help during intraoperative ultrasound-guided maneuvers. A: Lateral view; B: Front view.

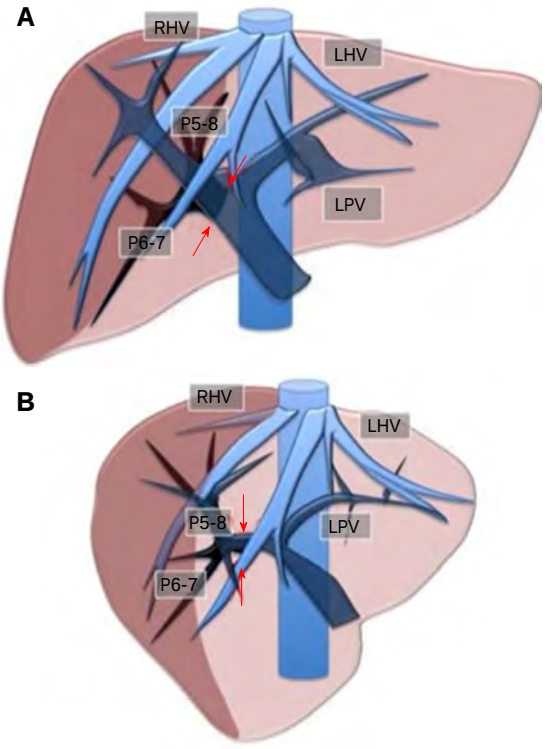


Figure 2 Layout of the liver for the inflow modulation. Ischemic demarcation of the right posterior sector by intraoperative ultrasound-guided finger compression at its origin of the right portal bifurcation. A: Front view; B: Lateral view. RHV: Right hepatic vein; LHV: Left hepatic vein; LPV: Left portal vein; P5-8: Right anterior portal braches; P6-7: Right posterior portal branches. The arrows indicate the point for the compression.

mor thrombus, or invasion of the vessel wall, the pedicle must be divided[9].

Tumor in contact with a HV

The HV may be spared when in contact with an encapsulated HCC or with CLM once the integrity of the vessel wall is confirmed at IOUS. For CLM, the contact should extent for less than two-thirds of the vessel circumference. Thus, in the presence of a tumor thrombus, invasion of the vessel wall, and wider contact the HV must be divided[15]. However, as described below, the extension of the hepatectomy to the portion of the liver theoretically drained by the resected HV is not systematically performed, but only when accessory HVs and/or communicating veins are missing or when inversion of the portal flow is demonstrated by IOUS[22].

INFLOW MODULATION

Initially used for tumors located in the left hemiliver[14], the inflow modulation technique has more recently been successfully extended to any liver segment[23], including segment 8, and even to sectional portions of the liver[24]. Once the feeding portal branch is identified at IOUS, it can be compressed using the IOUS probe by one side of the liver, and by the finger in the opposite side with the aim to induce a transient ischemia of the portion of the liver distal to the compression site. This portion can then be marked with the electrocautery, and when released, resection can be performed. This technique is simple, fast, non-invasive, and reversible. Also, the possibility of modifying the site of the compression, and then the corresponding resection volume allows tailoring of the resection according to tumor features, and more importantly to the status of the background liver. This is of paramount importance in patients with HCC and cirrhosis, in which the functional liver reserve may be marginal. Such a technique allows for precise anatomical resection of a subsegment, segment or section of the liver. As is well-established, anatomical resection of HCC is recommended to offer a higher chance of cure[25-29]. For segments such as segment 1 and 4 superior, for which direct compression of the feeding portal branch may not be feasible, the compression of the adjacent segmental branches allows definition of their segmental margins. Indeed, our technique can be used in a counter-compression perspective similar to the counter-staining technique reported by Takayama *et al*[30]. Figure 2 illustrates the layout of the liver with the compression technique applied to delineate the right posterior section, while Figures 3 and 4 show an actual case.

OUTFLOW MODULATION

The area of resection may be tailored not only using US-guided finger compression of the portal branch as described above, but also using IOUS outflow modulation. Indeed, we have already showed how to minimize

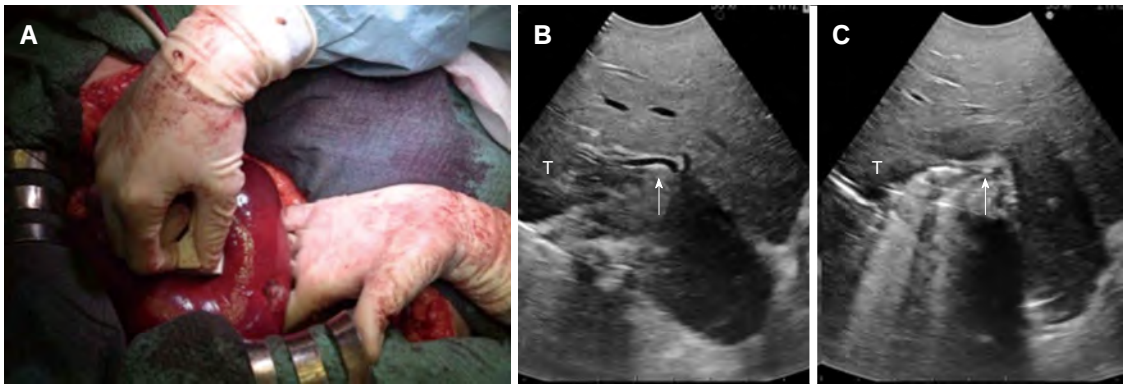


Figure 3 A case of intraoperative ultrasound-guided finger compression of segment 6. A: The portal pedicle for segment 6 is compressed by the probe in the right hand and by the finger in the left hand; B: Intraoperative ultrasound (IOUS) focused on the portal pedicle (arrow) for segment 6 before the compression; C: IOUS focused on the portal pedicle (arrow) for segment 6 during the compression. T: Tumor.

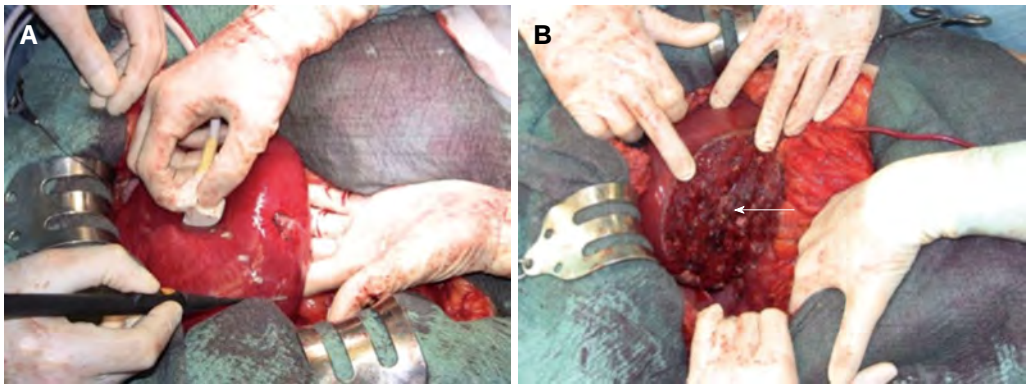


Figure 4 Demarcation of the compressed area by electrocautery. A: The operative field before the resection; B: The operative field at the end of the resection. The arrow indicates the stump of the portal pedicle for segment 6.

the sacrifice of liver parenchyma even in those patients with a tumor at the hepatocaval confluence, for which a standard major or extended hepatectomy should be indicated based on traditional criteria. In addition, we have introduced some new operations, such as mini-mesohepatectomy, and systematic extended right posterior sectionectomy[22-31], which simultaneously limit the need for formal major resection, and improve the chances of resection for those patients with complex tumoral presentation. The definition of the resection area using the outflow control is based on the extensive use of IOUS flow analyses, with the aim of checking the outflow modifications once the HV that should be resected is clamped. For this purpose rather than the direct closure of the vein by a vessel loop, the US-guided fingertip compression at the caval confluence might be adequate[32]. Certainly, the HV may already be closed by the tumor. At that case, the search is focused on at least one of the following criteria: reversal of flow direction in the peripheral portion of the compressed HV, which suggests drainage through the collateral circulation in adjacent HVs or inferior vena cava (IVC); direct detection of collaterals between the compressed HV and adjacent HV or IVC; or persistence of hepatopetal flow in the portal branches corresponding to the area drained by the compressed HV. In particular, in the case of hepatofugal flow direction in the portal branches, the resection should not be minimal but extended to the parenchyma fed by those portal branches. The presence of hepatofugal flow in the portal branches is a clear signal of insufficient drainage of the corresponding HV. Once at least one of the aforementioned criteria has been satisfied, full mobilization of the right and left hemiliver is performed, preserving most of the posterior short HVs to minimize the risk of congestion of the residual liver. Thus, the area of resection may be marked on the liver surface using electrocautery and IOUS to define the caudal, medial and lateral limits of the parenchyma to be removed, while the surgeon's left fingertip is visualized in the most cranial portion, and it is used to mark the dissection area. Parenchyma transection is then carried out with the surgeon's left hand behind the right hemiliver with the aim of guiding resection by the right hand in real time. Figure 5 illustrates the layout of the liver with the outflow modulation technique, while Figure 6 shows an actual case.

PROBLEM OF THE SURGICAL MARGIN

One of the main criticisms of this surgical approach is the problem of the surgical margin. Both for HCC and CLM the detachment of the tumor from a spared vessel

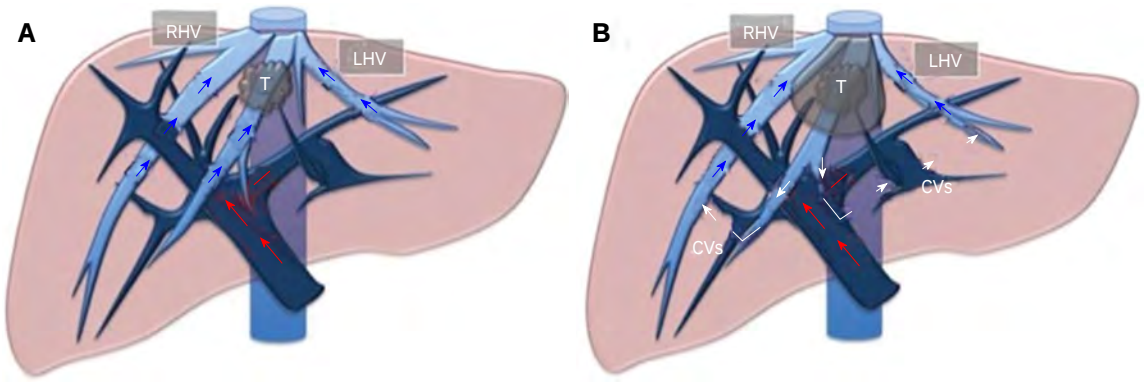


Figure 5 Layout of the liver for outflow modulation. A: A tumor in contact with the middle hepatic vein at the caval confluence; B: Once that vein is infiltrated and/or compressed, some collateral veins (CVs) shunting the flow from the middle hepatic vein territory to right hepatic vein (RHV) and/or left hepatic vein (LHV) territories can be detected. T: Tumor.

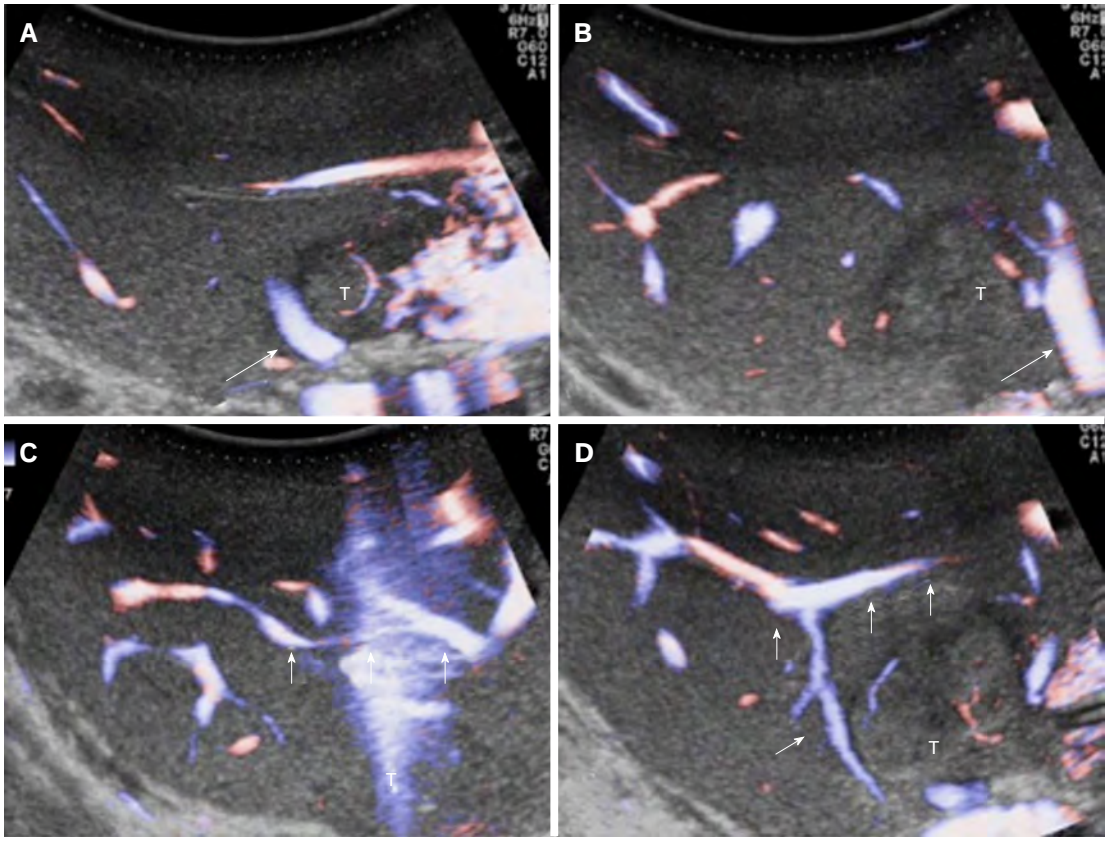


Figure 6 Intraoperative ultrasound study of communicating veins. A: A tumour located between the middle hepatic vein (MHV) (arrow) and the left hepatic vein (LHV) at their confluence into the inferior vena cava; B: The arrow indicates the LHV; C, D: Evidence of communicating veins (arrows) between the LHV and the MHV. T: Tumor.

may mean zero millimeters surgical margin, which traditionally is classified as R1 resection by the pathologist. Indeed, exposure of the tumor on the dissection plane is sometimes required to spare intrahepatic major vascular structures, which is the mainstay of our surgical policy. However, the effect of surgical margin status on survival of patients with HCC and CLM has been studied, but controversy still remains among surgeons. There is still debate about the real impact of the extent of the surgical margin once tumoral tissue is removed from the cut surface. For HCC, some authors reported that a margin smaller than 1 cm and even 2 cm plays a negative role in terms of long-term survival, while others authors found that a 0 mm margin is acceptable[33-37]. Also for CLM, there is no definitive agreement on the surgical margin[38,39]. It is well known that a positive margin is associated with increased risk of recurrence, but its width does not affect survival[40,41]. Moreover, it has been shown that patients with complex tumoral presentation treated with R1 resection may have the same long-term

survival of patients treated with R0 resection if aggressively treated with modern chemotherapy and repeated surgery[42]. Therefore, an anticipated minimal negative surgical margin should not be used as exclusion criterion for resection of HCC or CLM. The keystone is the performance of IOUS to guide the resection with the aim of achieving complete tumor clearance to minimize the risk of non-curative surgery.

In conclusions, IOUS is the best method for staging a liver tumor, and it is certainly the best method for the surgeon to understand in real-time the liver anatomy, and the relationships between tumors and intrahepatic vessels, thus allowing effective surgical operations. IOUS guidance allows for expanding indications offering the chance of cure to a greater proportion of patients, who would otherwise be excluded from the surgical program or submitted to more traditional but more risky operations. A precise tailoring of the area of hepatic resection using inflow and outflow modulation should be part of the surgical armamentarium of the modern liver surgeon.

REFERENCES

1 **Vauthey JN**, Pawlik TM, Abdalla EK, Arens JF, Nemr RA, Wei SH, Kennamer DL, Ellis LM, Curley SA. Is extended hepatectomy for hepatobiliary malignancy justified? *Ann Surg* 2004; **239**: 722-730; discussion 730-732 [PMID: 15082977 DOI: 10.1097/01.sla.0000124385.83887.d5]

2 **Schroeder RA**, Marroquin CE, Bute BP, Khuri S, Henderson WG, Kuo PC. Predictive indices of morbidity and mortality after liver resection. *Ann Surg* 2006; **243**: 373-379 [PMID: 16495703 DOI: 10.1097/01.sla.0000201483.95911.08]

3 **Balzan S**, Belghiti J, Farges O, Ogata S, Sauvanet A, Delefosse D, Durand F. The "50-50 criteria" on postoperative day 5: an accurate predictor of liver failure and death after hepatectomy. *Ann Surg* 2005; **242**: 824-828, discussion 828-829 [PMID: 16327492 DOI: 10.1097/01.sla.0000189131.90876.9e]

4 **Cucchetti A**, Ercolani G, Cescon M, Ravaioli M, Zanello M, Del Gaudio M, Lauro A, Vivarelli M, Grazi GL, Pinna AD. Recovery from liver failure after hepatectomy for hepatocellular carcinoma in cirrhosis: meaning of the model for end-stage liver disease. *J Am Coll Surg* 2006; **203**: 670-676 [PMID: 17084328 DOI: 10.1016/j.jamcollsurg.2006.06.018]

5 **Mullen JT**, Ribero D, Reddy SK, Donadon M, Zorzi D, Gautam S, Abdalla EK, Curley SA, Capussotti L, Clary BM, Vauthey JN. Hepatic insufficiency and mortality in 1,059 noncirrhotic patients undergoing major hepatectomy. *J Am Coll Surg* 2007; **204**: 854-62; discussion 862-864 [PMID: 17481498 DOI: 10.1016/j.jamcollsurg.2006.12.032]

6 **Wei AC**, Tung-Ping Poon R, Fan ST, Wong J. Risk factors for perioperative morbidity and mortality after extended hepatectomy for hepatocellular carcinoma. *Br J Surg* 2003; **90**: 33-41 [PMID: 12520572 DOI: 10.1002/bjs.4018]

7 **Karanjia ND**, Lordan JT, Quiney N, Fawcett WJ, Worthington TR, Remington J. A comparison of right and extended right hepatectomy with all other hepatic resections for colorectal liver metastases: a ten-year study. *Eur J Surg Oncol* 2009; **35**: 65-70 [PMID: 18222623 DOI: 10.1016/j.ejso.2007.12.002]

8 **Torzilli G**, Makuuchi M. Intraoperative ultrasonography in liver cancer. *Surg Oncol Clin N Am* 2003; **12**: 91-103 [PMID: 12735132 DOI: 10.1016/S1055-3207(02)00084-4]

9 **Torzilli G**, Montorsi M, Donadon M, Palmisano A, Del Fabbro D, Gambetti A, Olivari N, Makuuchi M. "Radical but conservative" is the main goal for ultrasonography-guided liver resection: prospective validation of this approach. *J Am Coll Surg* 2005; **201**: 517-528 [PMID: 16183489 DOI: 10.1016/j.jamcollsurg.2005.04.026]

10 **Donadon M**, Torzilli G. Intraoperative ultrasound of the liver. *AJR Am J Roentgenol* 2012; **198**: W398; author reply W399 [PMID: 22451581]

11 **Torzilli G**, Procopio F, Botea F, Marconi M, Del Fabbro D, Donadon M, Palmisano A, Spinelli A, Montorsi M. One-stage ultrasonographically guided hepatectomy for multiple bilobar colorectal metastases: a feasible and effective alternative to the 2-stage approach. *Surgery* 2009; **146**: 60-71 [PMID: 19541011 DOI: 10.1016/j.surg.2009.02.017]

12 **Makuuchi M**, Hasegawa H, Yamazaki S. Ultrasonically guided subsegmentectomy. *Surg Gynecol Obstet* 1985; **161**: 346-350 [PMID: 2996162]

13 **Makuuchi M**, Hasegawa H, Yamazaki S, Takayasu K. Four new hepatectomy procedures for resection of the right hepatic vein and preservation of the inferior right hepatic vein. *Surg Gynecol Obstet* 1987; **164**: 68-72 [PMID: 3026059]

14 **Torzilli G**, Makuuchi M. Ultrasound-guided finger compression in liver subsegmentectomy for hepatocellular carcinoma. *Surg Endosc* 2004; **18**: 136-139 [PMID: 14625736 DOI: 10.1007/s00464-003-9024-x]

15 **Torzilli G**, Montorsi M, Del Fabbro D, Palmisano A, Donadon M, Makuuchi M. Ultrasonographically guided surgical approach to liver tumours involving the hepatic veins close to the caval confluence. *Br J Surg* 2006; **93**: 1238-1246 [PMID: 16953487 DOI: 10.1002/bjs.5321]

16 **Cerwenka H**, Raith J, Bacher H, Werkgartner G, el-Shabrawi A, Kornprat P, Mischinger HJ. Is intraoperative ultrasonography during partial hepatectomy still necessary in the age of magnetic resonance imaging? *Hepatogastroenterology* 2003; **50**: 1539-1541 [PMID: 14571781]

17 **Jarnagin WR**, Bach AM, Winston CB, Hann LE, Heffernan N, Loumeau T, DeMatteo RP, Fong Y, Blumgart LH. What is the yield of intraoperative ultrasonography during partial hepatectomy for malignant disease? *J Am Coll Surg* 2001; **192**: 577-583 [PMID: 11333094 DOI: 10.1016/S1072-7515(01)00794-3]

18 **Machi J**, Sigel B, Zaren HA, Kurohiji T, Yamashita Y. Operative ultrasonography during hepatobiliary and pancreatic surgery. *World J Surg* 1993; **17**: 640-645; discussion 645-646 [PMID: 8273386 DOI: 10.1007/BF01659130]

19 **Takigawa Y**, Sugawara Y, Yamamoto J, Shimada K, Yamasaki S, Kosuge T, Makuuchi M. New lesions detected by intraoperative ultrasound during liver resection for hepatocellular carcinoma. *Ultrasound Med Biol* 2001; **27**: 151-156 [PMID: 11316523 DOI: 10.1016/S0301-5629(00)00346-X]

20 **Ozsunar Y**, Skjoldbye B, Court-Payen M, Karstrup S, Burcharth F. Impact of intraoperative ultrasonography on surgical treatment of liver tumours. *Acta Radiol* 2000; **41**: 97-101 [PMID: 10665881 DOI: 10.1034/j.1600-0455.2000.041001097.x]

21 **Torzilli G,** Botea F, Procopio F, Donadon M, Balzarini L, Lutman F, Calliada F, Montorsi M. Use of contrast-enhanced intraoperative ultrasonography during liver surgery for colorectal cancer liver metastases - Its impact on operative outcome. Analysis of a prospective cohort study. Eur J *Cancer* 2008; **6:** 16-23 [DOI: 10.1016/j.ejcsup.2008.06.004]

22 **Torzilli G**, Palmisano A, Procopio F, Cimino M, Botea F, Donadon M, Del Fabbro D, Montorsi M. A new systematic small for size resection for liver tumors invading the middle hepatic vein at its caval confluence: mini-mesohepatectomy. *Ann Surg* 2010; **251**: 33-39 [PMID: 19858707 DOI: 10.1097/SLA.0b013e3181b61db9]

23 **Torzilli G**, Procopio F, Cimino M, Del Fabbro D, Palmisano A, Donadon M, Montorsi M. Anatomical segmental and subsegmental resection of the liver for hepatocellular carcinoma: a new approach by means of ultrasound-guided vessel compression. *Ann Surg* 2010; **251**: 229-235 [PMID: 19838106 DOI: 10.1097/SLA.0b013e3181b7fdcd]

24 **Torzilli G**, Procopio F, Palmisano A, Cimino M, Del Fabbro

D, Donadon M, Montorsi M. New technique for defining the right anterior section intraoperatively using ultrasound-guided finger counter-compression. *J Am Coll Surg* 2009; **209**: e8-11 [PMID: 19632590 DOI: 10.1016/j.jamcollsurg.2009.05.014]

25 **Hasegawa K**, Kokudo N, Imamura H, Matsuyama Y, Aoki T, Minagawa M, Sano K, Sugawara Y, Takayama T, Makuuchi M. Prognostic impact of anatomic resection for hepatocellular carcinoma. *Ann Surg* 2005; **242**: 252-259 [PMID: 16041216 DOI: 10.1097/01.sla.0000171307.37401.db]

26 **Wakai T**, Shirai Y, Sakata J, Kaneko K, Cruz PV, Akazawa K, Hatakeyama K. Anatomic resection independently improves long-term survival in patients with T1-T2 hepatocellular carcinoma. *Ann Surg Oncol* 2007; **14**: 1356-1365 [PMID: 17252289 DOI: 10.1245/s10434-006-9318-z]

27 **Regimbeau JM**, Kianmanesh R, Farges O, Dondero F, Sauvanet A, Belghiti J. Extent of liver resection influences the outcome in patients with cirrhosis and small hepatocellular carcinoma. *Surgery* 2002; **131**: 311-317 [PMID: 11894036 DOI: 10.1067/msy.2002.121892]

28 **Cho YB**, Lee KU, Lee HW, Cho EH, Yang SH, Cho JY, Yi NJ, Suh KS. Anatomic versus non-anatomic resection for small single hepatocellular carcinomas. *Hepatogastroenterology* 2007; **54**: 1766-1769 [PMID: 18019714]

29 **Eguchi S**, Kanematsu T, Arii S, Okazaki M, Okita K, Omata M, Ikai I, Kudo M, Kojiro M, Makuuchi M, Monden M, Matsuyama Y, Nakanuma Y, Takayasu K. Comparison of the outcomes between an anatomical subsegmentectomy and a non-anatomical minor hepatectomy for single hepatocellular carcinomas based on a Japanese nationwide survey. *Surgery* 2008; **143**: 469-475 [PMID: 18374043 DOI: 10.1016/j.surg.2007.12.003]

30 **Takayama T**, Makuuchi M, Watanabe K, Kosuge T, Takayasu K, Yamazaki S, Hasegawa H. A new method for mapping hepatic subsegment: counterstaining identification technique. *Surgery* 1991; **109**: 226-229 [PMID: 1846986]

31 **Torzilli G**, Donadon M, Marconi M, Botea F, Palmisano A, Del Fabbro D, Procopio F, Montorsi M. Systematic extended right posterior sectionectomy: a safe and effective alternative to right hepatectomy. *Ann Surg* 2008; **247**: 603-611 [PMID: 18362622 DOI: 10.1097/SLA.0b013e31816387d7]

32 **Torzilli G**, Donadon M, Palmisano A, Del Fabbro D, Spinelli A, Makuuchi M, Montorsi M. Back-flow bleeding control during resection of right-sided liver tumors by means of ultrasound-guided finger compression of the right hepatic vein at its caval confluence. *Hepatogastroenterology* 2007; **54**: 1364-1367 [PMID: 17708255]

33 **Chau GY**, Lui WY, Tsay SH, King KL, Loong CC, Chiu JH, Wu CW, P'eng FK. Prognostic significance of surgical margin in hepatocellular carcinoma resection: an analysis of 165 Childs' A patients. *J Surg Oncol* 1997; **66**: 122-126 [PMID: 9354168 DOI: 10.1002/(SICI)1096-9098(199710)66]

34 **Shi M**, Guo RP, Lin XJ, Zhang YQ, Chen MS, Zhang CQ, Lau WY, Li JQ. Partial hepatectomy with wide versus narrow resection margin for solitary hepatocellular carcinoma: a prospective randomized trial. *Ann Surg* 2007; **245**: 36-43 [PMID: 17197963 DOI: 10.1097/01.sla.0000231758.07868.71]

35 **Ochiai T**, Takayama T, Inoue K, Yamamoto J, Shimada K, Kosuge T, Yamazaki S, Makuuchi M. Hepatic resection with and without surgical margins for hepatocellular carcinoma in patients with impaired liver function. *Hepatogastroenterology* 1999; **46**: 1885-1889 [PMID: 10430364]

36 **Poon RT**, Fan ST, Ng IO, Wong J. Significance of resection margin in hepatectomy for hepatocellular carcinoma: A critical reappraisal. *Ann Surg* 2000; **231**: 544-551 [PMID: 10749616 DOI: 10.1097/00000658-200004000-00014]

37 **Matsui Y**, Terakawa N, Satoi S, Kaibori M, Kitade H, Takai S, Kwon AH, Kamiyama Y. Postoperative outcomes in patients with hepatocellular carcinomas resected with exposure of the tumor surface: clinical role of the no-margin resection. *Arch Surg* 2007; **142**: 596-602; discussion 603 [PMID: 17638795 DOI: 10.1001/archsurg.142.7.596]

38 **Elias D**, Cavalcanti A, Sabourin JC, Pignon JP, Ducreux M, Lasser P. Results of 136 curative hepatectomies with a safety margin of less than 10 mm for colorectal metastases. *J Surg Oncol* 1998; **69**: 88-93 [PMID: 9808511 DOI: 10.1002/(SICI)1096-9098(199810)69]

39 **Nuzzo G**, Giuliante F, Ardito F, Vellone M, Giovannini I, Federico B, Vecchio FM. Influence of surgical margin on type of recurrence after liver resection for colorectal metastases: a single-center experience. *Surgery* 2008; **143**: 384-393 [PMID: 18291260 DOI: 10.1016/j.surg.2007.09.038]

40 **Pawlik TM**, Scoggins CR, Zorzi D, Abdalla EK, Andres A, Eng C, Curley SA, Loyer EM, Muratore A, Mentha G, Capussotti L, Vauthey JN. Effect of surgical margin status on survival and site of recurrence after hepatic resection for colorectal metastases. *Ann Surg* 2005; **241**: 715-722, discussion 722-724 [PMID: 15849507 DOI: 10.1097/01.sla.0000160703.75808.7d]

41 **Kokudo N**, Miki Y, Sugai S, Yanagisawa A, Kato Y, Sakamoto Y, Yamamoto J, Yamaguchi T, Muto T, Makuuchi M. Genetic and histological assessment of surgical margins in resected liver metastases from colorectal carcinoma: minimum surgical margins for successful resection. *Arch Surg* 2002; **137**: 833-840 [PMID: 12093342 DOI: 10.1001/archsurg.137.7.833]

42 **de Haas RJ**, Wicherts DA, Flores E, Azoulay D, Castaing D, Adam R. R1 resection by necessity for colorectal liver metastases: is it still a contraindication to surgery? *Ann Surg* 2008; **248**: 626-637 [PMID: 18936576 DOI: 10.1097/SLA.0b013e31818a07f1]

P- Reviewer Ooi LL **S- Editor** Gou SX
L- Editor Cant MR **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i7.1056

World J Gastroenterol 2013 February 21; 19(7): 1056-1067
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

ORIGINAL ARTICLE

Pseudomonas fluorescens-like bacteria from the stomach: A microbiological and molecular study

Saurabh Kumar Patel, Chandra Bhan Pratap, Ajay Kumar Verma, Ashok Kumar Jain, Vinod Kumar Dixit, Gopal Nath

Saurabh Kumar Patel, Chandra Bhan Pratap, Ajay Kumar Verma, Gopal Nath, Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Varanasi UP 221005, India
Ashok Kumar Jain, Vinod Kumar Dixit, Department of Gastroenterology, Institute of Medical Sciences, Banaras Hindu University, Varanasi UP 221005, India
Author contributions: Patel SK performed the major part of the experimental work, interpreted the results and prepared the manuscript; Pratap CB and Verma AK assisted in performing experiments; Jain AK and Dixit VK coordinated and helped in collection of human material, and also helped in shaping the manuscript; Nath G was responsible for genesis of the hypothesis, designing of the experiment, analysis of data and shaping of the manuscript; all authors read and approved the final draft.
Supported by Department of Biotechnology, Government of India, No. 102/IFD/SAN/PR1310/2006-07; Council of Scientific and Industrial Research, New Delhi, India, in the form of Senior Research Fellowship (to Patel SK)
Correspondence to: Dr. Gopal Nath, Department of Microbiology, Institute of Medical Sciences, Banaras Hindu University, Pandit Madan Mohan Malviya Rd, Varanasi UP 221005, India. gopalnath@gmail.com
Telephone: +91-542-6703484 Fax: +91-542-2367568
Received: July 19, 2012 Revised: September 13, 2012
Accepted: September 22, 2012
Published online: February 21, 2013

Abstract

AIM: To characterize oxidase- and urease-producing bacterial isolates, grown aerobically, that originated from antral biopsies of patients suffering from acid peptic diseases.

METHODS: A total of 258 antral biopsy specimens were subjected to isolation of bacteria followed by tests for oxidase and urease production, acid tolerance and aerobic growth. The selected isolates were further characterized by molecular techniques *viz.* amplifications for 16S rRNA using universal eubacterial and *HSP60* gene specific primers. The amplicons were subjected to restriction analysis and partial sequencing. A phylogenetic tree was generated using unweighted pair group method with arithmetic mean (UPGMA) from evolutionary distance computed with bootstrap test of phylogeny. Assessment of acidity tolerance of bacteria isolated from antrum was performed using hydrochloric acid from 10^{-7} mol/L to 10^{-1} mol/L.

RESULTS: Of the 258 antral biopsy specimens collected from patients, 179 (69.4%) were positive for urease production by rapid urease test and 31% (80/258) yielded typical *Helicobacter pylori* (*H. pylori*) after 5-7 d of incubation under a microaerophilic environment. A total of 240 (93%) antral biopsies yielded homogeneous semi-translucent and small colonies after overnight incubation. The partial 16S rRNA sequences revealed that the isolates had 99% similarity with *Pseudomonas* species. A phylogenetic tree on the basis of 16S rRNA sequences denoted that JQ927226 and JQ927227 were likely to be related to *Pseudomonas fluorescens* (*P. fluorescens*). On the basis of *HSP60* sequences applied to the UPGMA phylogenetic tree, it was observed that isolated strains in an aerobic environment were likely to be *P. fluorescens*, and *HSP60* sequences had more discriminatory potential rather than 16S rRNA sequences. Interestingly, this bacterium was acid tolerant for hours at low pH. Further, a total of 250 (96.9%) genomic DNA samples of 258 biopsy specimens and DNA from 240 bacterial isolates were positive for the 613 bp amplicons by targeting *P. fluorescens*-specific conserved putative outer membrane protein gene sequences.

CONCLUSION: This study indicates that bacterial isolates from antral biopsies grown aerobically were *P. fluorescens*, and thus acid-tolerant bacteria other than *H. pylori* can also colonize the stomach and may be implicated in pathogenesis/protection.

© 2013 Baishideng. All rights reserved.

Key words: Antral biopsy; *Helicobacter pylori*; *Pseudomonas fluorescens*; HSP60; Nested polymerase chain reaction; Acid-tolerant bacteria

Patel SK, Pratap CB, Verma AK, Jain AK, Dixit VK, Nath G. *Pseudomonas fluorescens*-like bacteria from the stomach: A microbiological and molecular study. *World J Gastroenterol* 2013; 19(7): 1056-1067 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i7/1056.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i7.1056

INTRODUCTION

Helicobacter pylori (*H. pylori*) is a gram-negative, microaerophilic bacterium found primarily in the stomach. It has been implicated in chronic gastritis, gastric ulcers, duodenal ulcers and stomach cancers that were previously believed to be of non-microbial origin[1-3]. There was a misconception that no bacteria could live in the stomach because of its highly acidic environment. For the first time, Steer and Colin-Jones[4] published their results regarding the presence of gram-negative, oxidase- and urease-producing bacteria, but they proposed that it was *Pseudomonas* (*P.*), a contaminant and not related to peptic ulcer. In an effort to grow *H. pylori* from an antral biopsy, we could see that a peculiar type of bacterial colony was growing consistently after overnight incubation while *H. pylori* was taking 3-5 d to grow. These colonies were also oxidase and urease producers, but growing in an aerobic environment. There are reports that show the presence of a variety of bacteria in the stomach by isolation, DNA profiling and polymerase chain reaction (PCR)-based analysis, and some of them are urease producers hindering the specificity of the urea breath test[5-7]. Therefore, we aimed to characterize this type of bacterial isolate and to analyze whether they are colonizers or contaminants.

MATERIALS AND METHODS

Collection of specimens

The study subjects were patients attending inpatient services of the Department of Gastroenterology, University Hospital of Banaras Hindu University, Varanasi, Uttar Pradesh, India. This hospital provides tertiary-level health services for the eastern part of Northern India. The culture isolation, phenotypic and molecular characterizations were carried out in the Department of Microbiology, Institute of Medical Sciences.

Patients and samples

A total of 258 patients suffering from upper gastrointestinal (UGI) diseases like non-ulcer dyspepsia (NUD), peptic ulcer diseases (PUD) including gastric ulcer and duodenal ulcer, and gastric carcinoma were enrolled during a period of 3 years (2007-2010) and three antral biopsy pieces from each patient were collected. Before taking a biopsy, the endoscope was rinsed with detergent followed by water, and disinfected with 2% alkaline glutaraldehyde for 30 min then rinsed with sterile water. In a similar way, biopsy forceps were washed and sterilized and one biopsy forceps was used for one patient exclusively. The biopsy specimens were collected by endoscopic forceps from each individual with full aseptic precautions after taking well-informed consent. The work was approved by the Ethics Committee of the Institute of Medical Sciences, Banaras Hindu University. Patients with mucosal breaks greater than 5 mm in size with apparent depth were diagnosed as having ulcer and those with ulcerato-infiltrative lesions with positive histology/brush cytology were considered as having stomach carcinoma. The patients not having ulcerative lesions but suffering from dyspeptic symptoms were diagnosed as NUD. Individuals with normal endoscopic findings without gastroduodenal symptoms but having other gut problems were treated as healthy controls. In the present study, those patients were excluded who had a history of previous gastric surgery, active UGI bleeding, chronic alcoholism, intake of antibiotics and proton pump inhibitors during the last 4 wk or those taking non-steroidal anti-inflammatory drugs. Further individuals less than 18 years of age, pregnant or lactating mothers or those having illnesses like cirrhosis, chronic renal failure or ischemic heart disease were also excluded.

Microbiological processing

The three biopsy pieces were pooled and homogenized into phosphate saline buffer together in an all glass disposable homogenizer and were divided into three aliquots. The first aliquot of the tissue homogenate was transferred immediately into a rapid urease test (RUT) medium and the second was plated within 30 min of collection onto the media used for bacterial culture [Mueller Hinton agar without supplement and media containing brain heart infusion agar (Difco, Becton Dickinson, Sparks, MD, United States), supplemented with 7% sheep blood, 0.4% IsoVitaleX, and Skirrow selective supplement (vancomycin 10 μg/mL; polymixin B sulfate 2.5 IU/mL; trimethoprim lactate 5 μg/mL) (Difco, Becton Dickinson, Sparks, MD, United States)]. The non-enriched plate was incubated at 37 ℃ in an aerobic atmosphere while the other was incubated in the presence of 5% O_2, 10% CO_2, and 85% N_2 for 3-7 d. Several small colonies could be seen after overnight incubation in the first plate. The other plate was examined every alternate day after 3-7 d to see if colonies other than those observed after overnight incubation developed. Small translucent colonies developed after 5-7 d of incubation other than those originally after observed overnight incubation on non-enriched nonselective medium. These colonies were further subjected to morphological and biochemical tests *viz.* motility, oxidase, catalase, and urease. All the isolates were divided into two groups on the basis of the incubation period, *i.e.*, those isolates obtained after overnight incubation designated as group A and the other group B possessing those strains isolated after 5-7 d of incubation. The third aliquot was subjected to genomic DNA extraction.

Table 1 Primers used to study *Helicobacter pylori* and *Pseudomonas fluorescens* isolates

Target gene	Primer name	Primer sequence (5′-3′)	PCR condition (number of cycles, size of product)
16S rRNA	16S F	TTGGAGAGTTTGATCCTGGCC	94 ℃, 30 s; 59 ℃, 30 s; 72 ℃, 30 s (30, 1155 bp)
	16S R	ACGTCATCCCACCTTCTC	
HSP60			
Primary	HSP1	AAGGCATGCAATTTGATAGAGGCT	94 ℃, 30 s; 56 ℃, 30 s; 72 ℃, 30 s (35, 594 bp)
	HSP2	CTTTTTTCTCTTTCATTTCCACTT	
Nested	HSPN1	TTGATAGAGGCTACCTCTCC	94 ℃, 30 s; 56 ℃, 30 s; 72 ℃, 30 s (35, 501 bp)
	HSPN2	TGTCATAATCGCTTGTCGTGC	
Putative membrane-bound protein (PFMP)			
Primary	*PFMPF*	TCTKRYCRMGAATCRARACWRYC	94 ℃, 1 min; 50 ℃, 1 min; 72 ℃, 1 min (35, 704 bp)
	PFMPR	GKTWYTGCKCRWWKCSYTSMMC	
Nested	*PFMPNF*	TGCGYWMMWCCYWRWCCWTGA	94 ℃, 1 min; 50 ℃, 1 min; 72 ℃, 1 min (35, 613 bp)
	PFMPNR	AKCABGGTSCWGCMVRCCRBGC	
mupV			
Primary	mupVF	TGAGTTCGATGTGACCTGCCTG	94 ℃, 1 min; 55 ℃, 1 min; 72 ℃, 1 min (35 cycles, 722 bp)
	mupVR	AACTCGCCAGATTGTCGTACAC	
Nested	mupVnF	CAGCATTATCCTGCCACTGAC	94 ℃, 1 min; 55 ℃, 1 min; 72 ℃, 1 min (35, 611 bp)
	mupVnR	ATGATGTCCTGGCACACCTGATC	

PCR: Polymerase chain reaction.

RUT

For the RUT, biopsy specimens were inoculated into 1 mL of 10% urea in deionized water (pH 6.8), to which two drops of 1% phenol red solution was added, and incubated at 37 ℃ for 24 h. A positive result was recorded when the color changed from yellow to pink within 30 min. If there was mild color change within 30 min, the RUT tubes were incubated for a further 24 h.

Subculture at different temperatures

The organisms which appeared after overnight incubation were tested for growth at 4 ℃, 35 ℃ and 42 ℃ onto Muller Hinton agar; incubation was maintained for 14 d to distinguish *Pseudomonas* spp.[8,9]. Those organisms showing growth at 4 ℃ were sub cultured twice and incubated at the appropriate temperature for more than 10 d each time.

Assessment of acidity tolerance of bacteria isolated from gastric niche

Five isolates were suspended into different molar concentrations of hydrochloric acid (10^{-1} to 10^{-7} mol/L corresponding to pH 1.0 to 7.0) and a CFU was maintained as 10^6 CFU/mL. After the intervals of 0 min, 5 min, 10 min, 20 min, 30 min and 60 min, 100 μL acidic suspension (10^5 CFU) were transferred into 3 mL BHI broth. After overnight incubation optical density was recorded with the help of a spectrophotometer at 600 nm wavelength and bacterial count was expressed in CFU/mL. This experiment was repeated twice.

Preparation of genomic DNA for PCR assay

Extraction of genomic DNA from both types of bacterial isolates (A total of 320 strains including groups A + B) as well as from tissue homogenate was performed using a standard proteinase K and phenol-chloroform method[10]. To exclude the possibility of cross contamination of DNA during DNA extraction, one set of double distilled was included in each batch of DNA extraction.

Detection of H. pylori by nested PCR

Confirmation of *H. pylori* was done at a molecular level by nested PCR targeting the conserved *HSP60* gene and its restriction fragment length polymorphism. The reaction was performed in 25 μL final volume containing 10 ng of DNA, 1 U of Taq polymerase (Bangalore Genie, India), 200 mmol/L (each) deoxynucleotide triphosphate (MBI, Fermentas) and 1.5 mmol/L $MgCl_2$ in standard PCR buffer and 10 pmol of each primer as described by Singh *et al*[11]. Primer sequences and PCR conditions are displayed in Table 1. For the internal amplification, the PCR product from the primary cycle was diluted 1/50 and 1 μL was used as the template in the nested PCR. The conditions for the PCR amplification, first and nested reactions were the same. DNA from *H. pylori* reference J99 and a tube containing water in place of DNA were assayed in each PCR run as positive and negative controls, respectively.

After amplification, the PCR products (501 bp) were precipitated with 2.5 volumes of ethanol. The pellets were washed twice with 70% ethanol and dissolved in Tris-EDTA buffer (pH 8.0). A 10 μL precipitated amplified DNA sample was then digested with 10 U of restriction enzyme *Hind*Ⅲ in appropriate buffered solution recommended by the manufacturer (Bangalore Genie, India) and incubated for 3 h at 37 ℃. The digested DNA fragments were analyzed by electrophoresis on 2% agarose gels (Bangalore Genie, India) containing 0.5 μg of ethidium bromide per mL. The gel was run at 70 V with TBE (Tris Boric acid EDTA) buffer for 3 h and was examined by a transilluminator and photographed. The sizes of digested DNA fragments were estimated from distances of molecular weight standards and compared with in silico restriction digestion.

Amplified rDNA restriction analysis

For each group of isolates, the 16S rRNA from 8 randomly selected isolates was amplified using forward primer-16SF and reverse primer-16SR[12]. PCR amplification was performed in a thermocycler (Biometra, Germany) according to standard procedures (Table 1). After amplification by universal eubacterial primers, the PCR products (1155 bp) were precipitated with 2.5 volumes of ethanol. The pellets were washed twice with 70% ethanol and dissolved in Tris-EDTA buffer (pH 8.0). A 10 μL precipitated amplified DNA sample was then digested with 10 U of restriction enzyme *Eco*R1, in appropriate buffered solution recommended by the manufacturer (Bangalore Genie) and incubated for 3 h at 37 ℃. The visualization of the restriction fragment was done by the same method as described in the previous paragraph.

Sequencing

The amplified PCR products were purified from salts and primers using QIA quick PCR purification kit (Qiagen, United States). A total of 8 purified amplicons generated targeting *HSP60* and 2 amplicon 16S rRNA genes were outsourced for partial sequencing to Bangalore Genei, India. Sequences were analyzed using BLASTN (http://www.ncbi.nlm.nih.gov/BLAST) to verify the identity of the sequences: whether *H. pylori* or some other microorganism.

Sequence analyses

Reference sequences of *Pseudomonas* group and other enteric pathogens used for phylogenetic analyses were retrieved from Genbank. The partial 16S rRNA sequences and HSP60 sequence for the strains were aligned with reference sequences using Clustal X version 1.81, with default parameters[13]. Phylogenetic and molecular evolutionary analyses were performed using *MEGA* version 4[14]. The phylogenetic tree was generated using the unweighted pair group method with arithmetic mean (UPGMA) from evolutionary distance computed with bootstrap test of phylogeny. The degree of statistical support for branches was determined with 500 bootstrap replicates.

Primers designed for Pseudomonas fluorescens targeting putative membrane-bound protein

The putative membrane-bound protein coding gene present in all the strains of *P. fluorescens* is available in NCBI Genbank. Due to a similarity of sequences of about 55% in *P. fluorescens,* we therefore planned to design nested degenerate primers to amplify the partial sequence of putative membrane bound protein so that it was able to amplify all strains of *P. fluorescens*. Forward and reverse oligo-nucleotide degenerate primers derived from the region located between bases 92072 and 92775 of the *Pseudomonas fluorescens* (*Pseudomonas fluorescens* Pf0-1; GenBank Accession number CP000094.2 and GI: 253992019) were synthesized. Internal primers were derived from the region between bases 92114-92726 for nested PCR. DNA extracted from *P. putida* and *P. aeruginosa* was used to monitor sensitivity against *P. putida* and *P. aeruginosa*. During the PCR assay in each batch, Mili Q water was used as a template to ensure that there was no contamination by water and PCR reagent[15].

Pseudomonas fluorescens NCIMB 10586 mupirocin biosynthetic gene

Forward and reverse oligonucleotides were derived from the conserved region located between bases 68101 and 68822 of the mupirocin biosynthetic gene cluster of *Pseudomonas fluorescens* (NCIMB 10586). An internal primer was derived from the region between bases 68796 and 68796 (GenBank Accession number AF318063.2; gene GI: 20150006). PCR amplification was similar to amplification of *HSP60* gene and conditions are described in Table 1.

Randomly amplified polymorphic DNA PCR

Fingerprinting of 71 randomly selected strains from group A was performed based on randomly amplified polymorphic DNA (RAPD) PCR methods by using primers RAPD3 5'-TACAGCTCG-3'and RAPD5 5'AGCACTGCCT-3' (this study). PCR was carried out in 25 μL volume using 10 ng of genomic DNA, 1 U of Taq polymerase (Bangalore Genie, India), and 15 pmol of each primer (Bangalore Genie), 200 mmol/L (each) deoxynucleotide triphosphate (Bangalore Genie, India) and 2 mmol/L $MgCl_2$ in standard PCR buffer. Amplification reactions were carried out in a thermal cycler (Biometra, Goettingen, Germany).

The gel images were analyzed under ultraviolet light in a gel documentation system (Alpha Innotech, United States). Cluster analysis of all the isolates was done on the basis of the fingerprint generated. Based on the presence or absence of different DNA fragments in the fingerprints of the *P. fluorescens* strains, a binary data matrix was created. Overall similarity between the pair of strains was calculated from the binary data matrix using the simple matching-dice coefficient. The resulting similarity matrix was used as the input data for cluster analysis by NTSYS pc2.0 programme of UPGMA[16].

Statistical analysis

The level of significance between the two proportions, *i.e.*, culture rates and molecular detection rates, was calculated by Fischer's Exact Probability test.

RESULTS

Bacteriological findings

Of the 258 antral biopsy specimens collected from patients, 69.4% (179/258) were found to be positive by RUT and 31% (80/258) by culture for typical *H. pylori,* after 5-7 d of incubation under a microaerophilic environment; these were gram-negative curved rods and were positive for oxidase, catalase and urease. However, 258 antral biopsies yielded 240 (93%) homogeneous semi-translucent and small colonies after overnight incubation. These isolates also grew aerobically but the colonies had

Table 2 Comparative isolation rates and prevalence of *Helicobacter pylori* and *Pseudomonas fluorescens* against rapid urease test in antral biopsies by nested polymerase chain reaction targeting *HSP60* gene and membrane bound protein *n* (%)

Diseases	Antral biopsies	RUT, positivity	*H. pylori HSP60*, positivity	*P* value[1]	*P. fluorescens* putative outer membrane protein, positivity	*P* value[2]	Isolation of different types of bacteria, positivity		*P* value[3]
							Group A	Group B	
PUD	65	51 (78.5)	59 (90.8)	< 0.001	63 (96.9)	< 0.010	61 (93.8)	23 (35.4)	< 0.001
NUD	123	92 (74.8)	109 (88.6)	< 0.001	121 (98.4)	< 0.001	119 (96.7)	39 (31.7)	< 0.001
CA	49	23 (46.9)	24 (48.9)	< 0.050	46 (93.9)	< 0.001	43 (87.5)	12 (24.5)	< 0.001
Normal	21	13 (61.9)	19 (90.4)	< 0.001	20 (95.2)	0.001	17 (80.9)	6 (28.6)	< 0.001
Total	258	179 (69.4)	211 (81.8)	< 0.001	250 (96.9)	< 0.001	240 (93.0)	80 (31.0)	< 0.001

[1]$P < 0.001$ between *Helicobacter pylori* (*H. pylori*) and nested polymerase chain reaction (PCR); [2]$P < 0.001$ between *H. pylori* and *Pseudomonas fluorescens* (*P. fluorescens*); [3]$P < 0.001$ between colonies appearing after overnight incubation group (group A) and colonies appearing after 3-7 d of incubation group (group B). Group A: Non-ulcer dyspepsia (NUD) *vs* gastric carcinoma (CA), $P = 0.02$; NUD *vs* normal, $P < 0.02$; *H. pylori*: Peptic ulcer diseases (PUD) *vs* CA, $P < 0.001$; NUD *vs* CA, $P < 0.001$; CA *vs* Normal, $P = 0.001$; Overall: Culture of *P. fluorescens* *vs* nested PCR, $P < 0.001$. Normal: Patients whose endoscopic findings were normal; RUT: Rapid urease test; RUT: Rapid urease test.

an opaque, small character contrary to the translucent one which is typical for *H. pylori* (Table 2).

Biochemical characterization

A total of 100 isolates randomly selected from group A were subjected to extensive phenotypic characterization. All of them were gram-negative, oxidase-, catalase- and urease-positive. All were non-fermenters, showed variable nitrate reduction and failed to utilize simple sugars (glucose, lactose, sucrose, mannitol and maltose). Citrate was utilized by all of them. All the tested strains were Methyl Red negative and Voges-Praskauer negative or equivocal. All the strains were oxidase- and catalase-positive. Indole test was negative but on mixing with the Kovac's reagent a typical greenish color developed. All these isolates were able to multiply at 4 ℃. These findings intimated that those isolates which appeared after overnight incubation were *P. fluorescens*.

Acid tolerance assay

The bacterial count of *P. fluorescens* was 7.3×10^8, 6.9×10^8, 7×10^8, 6.1×10^8, 7.6×10^8, 6.5×10^8 and 9.8×10^8 CFU/mL for 0 min, 5 min, 10 min, 15 min, 20 min, 30 min and 60 min after acid exposure (pH 1.0). Average bacterial counts were 9.3×10^8, 9.8×10^8, 1.0×10^9, 9.5×10^8 and 9.8×10^8 CFU/mL for acid tolerance of low pH 2, pH 3, pH 4, pH 5, pH 6 and pH 7, respectively, for different time intervals (0 min, 5 min, 10 min, 15 min, 20 min, 30 min and 60 min). Similarly, bacterial growth was approximately the same in control experiments where acidic solution was replaced by LB Broth. The exposure to acidic pH showed that *P. fluorescens* growth was not killed by exposure to lower pH 1.0 for an hour.

Amplification and RLFP of HSP60 gene

Isolates from group B, which grew under microaerophilic environment, were subjected to amplification by primers specific for *HSP60* gene of *H. pylori*. All the 80 isolates from 258 patients were positive for the 501 bp of amplicon for the corresponding gene. However, 211 (81.8%) of 258 antral biopsies were positive for *H. pylori* DNA as the 501 bp amplicon was produced by nested PCR. All nested amplicons of 501 bp were restricted into two fragments of 310 bp and 191 bp by the *Hind*Ⅲ restriction enzyme (Figures 1 and 2). Although colonies grown after overnight incubation could give amplification with first round of PCR primers, nested PCR did not generate 501 bp amplicon specific to *H. pylori*. Similarly, 240 aerobic isolates could not amplify in second round PCR.

16S rRNA amplified rDNA restriction analysis

The isolates analyzed by using *Eco*R1 restriction endonuclease enzyme on amplicons generated by 16S rRNA specific primers fell into two groups: group A isolates could not be restricted, while group B isolates which were restricted into two fragments of 635 bp and 520 bp which were similar to *in silico* restriction of *H. pylori* J99 (Figure 3).

Identification on the basis of 16S rRNA and HSP60 gene sequence

The partial nucleotide sequence of 16S rRNA of 2 isolates (GenBank accession number JQ927226 and JQ927227) from group A represented no restriction site for *Eco*R1 restriction enzyme, and comparison of the nucleotide sequences with the NCBI database showed similarity of 99% with *P. fluorescens*. Similarly, partial nucleotide sequence of unrestricted amplified *HSP60* gene (590 bp) of the isolates that grew after overnight incubation aerobically also showed 96% similarity with *P. fluorescens*.

Phylogenetic sequence analysis

The partial sequence of the 16S rRNA gene of two *P. fluorescens*-like isolated strains and sequences of *16S rRNA* gene from 34 reference strains representative of the principal *Pseudomonas* phyla and 12 other bacterial pathogens were used for comparison of a phylogenetic tree. Similarly, a partial sequence of *HSP60* gene of 8 *P. fluorescens*-like isolates and sequence of *HSP60* gene of 5 *P. fluorescens* reference strains and 6 other reference strains representative of the principal *Pseudomonas* phyla and 11 other clinical pathogens were used to prepare a phylogenetic tree. To simplify the comparisons for the resulting phylogenetic tree by the UPGMA method, we named clusters based on *16S rRNA* gene data, r-clusters, and those based on

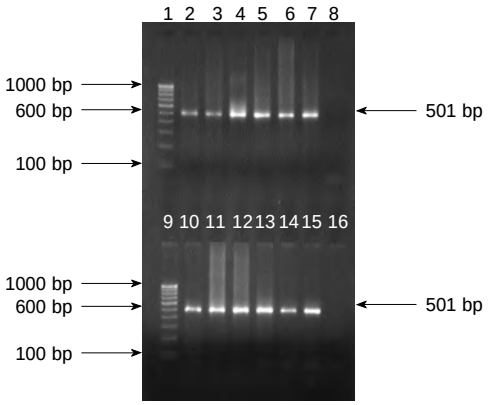


Figure 1 Amplification of partial 501 bp *HSP60* gene with specific nested primer for *Helicobacter pylori* in antral biopsies and culture isolates. Lanes 1 and 9: Molecular marker (100 bp); Lane 2: Positive control; Lanes 3 to 7: gDNA from antral biopsies; Lanes 8 and 16: Negative control; and Lanes 10 to 15: Bacterial gDNA from culture isolates.

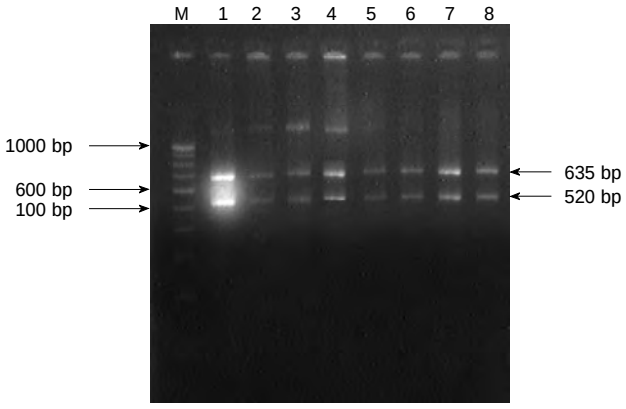


Figure 3 Gel picture of amplified rDNA restriction analysis. Lane M: 100 bp molecular marker; Lanes 1 to 8: Digested 1155 bp 16S rRNA amplicon into two fragments (635 bp and 520 bp) of culture isolates of *Helicobacter pylori*.

HSP60 data, h-clusters. The tree showed, on the basis of 16S rRNA sequences, it can be grouped clearly into three r-clusters on the basis of number of bootstraps *i.e.*, 90, 78 and 60. Cluster r-1 represents a fluorescens group along with two sequences submitted to gene bank (accession number as JQ927226 and JQ927227 for RCa25 and RCa24 respectively). Cluster r-2 represents *Pseudomonas aeruginosa, Pseudomonas syringae, Pseudomonas stuzeri, Pseudomonas chlororaphis, Pseudomonas putida, Pseudomonas pertucinogena* group and 4 *P. fluorescens* reference strains, and r-3 cluster represents other microbes and enteric pathogens rather than non-*Pseudomonas* spp. From cluster analysis it is clear that JQ927226 and JQ927227 are related to *P. fluorescens* (Figure 4).

The tree shows that, on the basis of *HSP60* sequences, three h-clusters could be observed (bootstraps number, 99, 63 and 71): the sequences of seven isolated strains from the gastric niche could be grouped in h-cluster I along with *P. fluorescens* strains while cluster II represents non-fluorescens pseudomonas species with the exception of *P. fluorescens* F113 (CP003150). The h-cluster III grouped non-*Pseudomonas* sp. including enteric pathogens along with *H. pylori*.

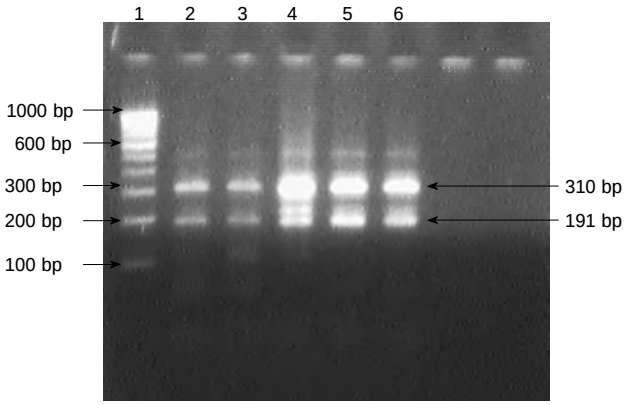


Figure 2 Electrophotograph of restriction digestion of *HSP60* gene of *Helicobacter pylori* isolates with *Hind*Ⅲ. Lane 1: 100 bp molecular marker; Lanes 2 to 6: Restricted 501 bp *HSP60* gene amplicon into two fragments (310 and 191 bp) of culture isolates of *Helicobacter pylori*.

Interestingly, one strain of *P. fluorescens* RCa 24 fell into this III cluster with closeness to *Stenotrophomonas maltophilia* and *Bordetella pertusis* (Figure 5).

Pseudomonas fluorescens specific PCR

One specific pair of primers targeting putative outer membrane protein was used to identify *P. fluorescens*. The other specific primers targeting mupirocin biosynthetic gene were used to screened out whether any *P. fluorescens*-like isolate was producing mupirocin. Genomic DNA extracted from group-A isolates and all biopsies was used as template for PCR amplification. A total of 250 (96.9%) out of 258 biopsy specimen genomic DNA samples and 240 bacterial isolates were positive for the amplification of the corresponding gene, *i.e.*, the putative outer membrane protein gene sequences (Table 2 and Figure 6). Although putative outer membrane protein gene targeting primers were degenerate, they were unable to produce the 613 bp amplicon from *Pseudomonas aeruginosa* and *Pseudomonas putida* at similar PCR conditions. The mupirocin biosynthetic gene targeting primers were unable to produce amplification of the 722 bp or 611 bp amplicon either in primary or secondary round PCR, respectively, from any of the isolates (data not shown).

RAPD

All the 71 strains tested from group-A yielded significant PCR products with RAPD primers. The strains generated approximately 3-13 well-defined bands between 150 bp to 2.5 kb sizes (9 bands on average) with each isolate yielding a unique profile of products. Cluster analysis with a RAPD-PCR based method showed that only a few isolates exhibited an identical profile. Nearly all the isolates appeared as dissimilar from each strain, but 5 strains isolated from cancer patients showed similar banding pattern at 0.0 coefficient (Figures 7 and 8).

DISCUSSION

Traditionally, the human stomach has been viewed as an

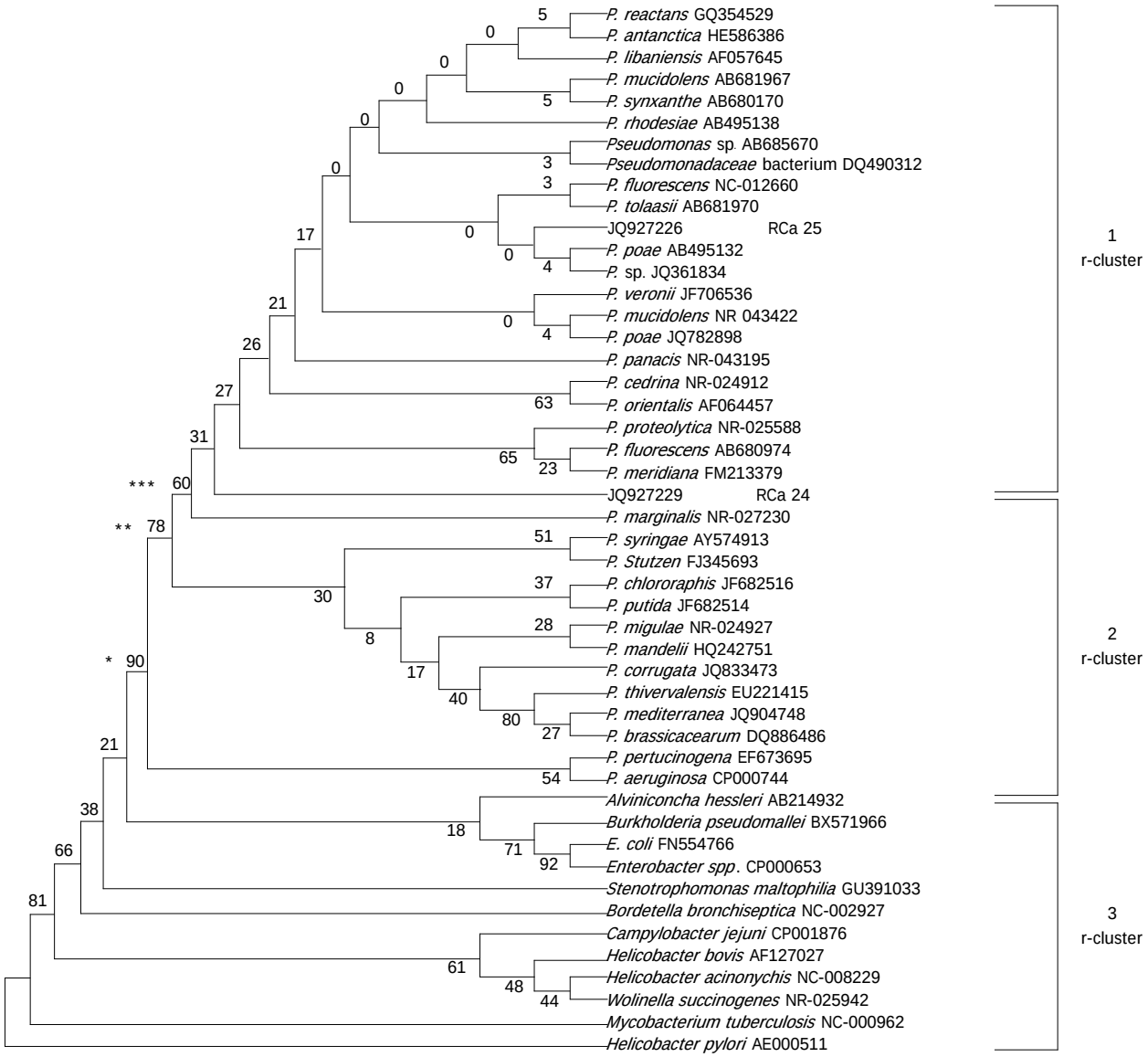


Figure 4 Phylogenetic affiliation of the *Pseudomonas fluorescens*-like isolates (*n* = 2; JQ927226 and JQ927227). The unrooted tree was generated using unweighted pair group method with arithmetic mean from evolutionary distance computed with bootstrap test of phylogeny using *MEGA* version 4 by aligning published sequences from Genbank of *16S rRNA* genes from 34 reference strains representative of the principal *Pseudomonas* (*P.*) *phyla* (accession number followed by species name in parentheses). For checking relatedness with other genera, we included *16S rRNA* gene sequences from GenBank of 12 bacterial pathogens namely *Stenotrophomonas maltophilia*, *Bordetella bronchiseptica*, *Wolinella succinogenes*, *Helicobacter pylori*, *Helicobacter acinonychis*, *Escherichia coli* (*E. coli*), *Enterobacter* spp., *Campylobacter jejuni*, *Burkholderia pseudomallei*, *Alviniconcha hessleri*, *Helicobacter bovis* and *Mycobacterium tuberculosis*. Branches found by maximum likelihood are labeled with asterisks: one asterisk if bootstrap values = 90%, two asterisks if = 78% and three asterisks if = 60%.

inhospitable environment for microorganisms because of its acidic environment along with several other antimicrobial factors. With the discovery of *H. pylori* and other gastric helicobacters, and subsequent insight into the mechanisms by which these organisms adapt to the gastric environment[17], the existence of a bacterial community adapted to this human niche seems quite plausible. This is the first report of its kind showing the presence of a *P. fluorescens*-like bacterium in the human stomach. We isolated the bacteria from NUD, gastric ulcer, duodenal ulcer and gastric carcinoma patients with the belief that only *H. pylori* is associated with acid peptic diseases. However, *P. fluorescens* grew on a simple medium like Mueller-Hinton agar without an antibiotic supplement. The small white colonies had Gram-negative slightly curved rods and produced oxidase, urease and catalase enzymes. These colonies appeared after overnight incubation in an aerobic environment. These isolates exhibited growth at 4 ℃, which is one of the key characteristics of *P. fluorescens*[9]. When these isolates were exposed to acidic pH as low as pH 1.0 for 1 h, the subsequent growth of the bacteria was not affected, indicating that they were acid tolerant. However, due to deviation from the classical *H. pylori* growth characteristics, we were prompted to consider them as non-*H. pylori* and submit them for further characterization. For the purpose, *H. pylori* specific primers for amplifying *HSP60* gene-specific sequences were applied[11]. However, an approximate 600 bp sized amplicon

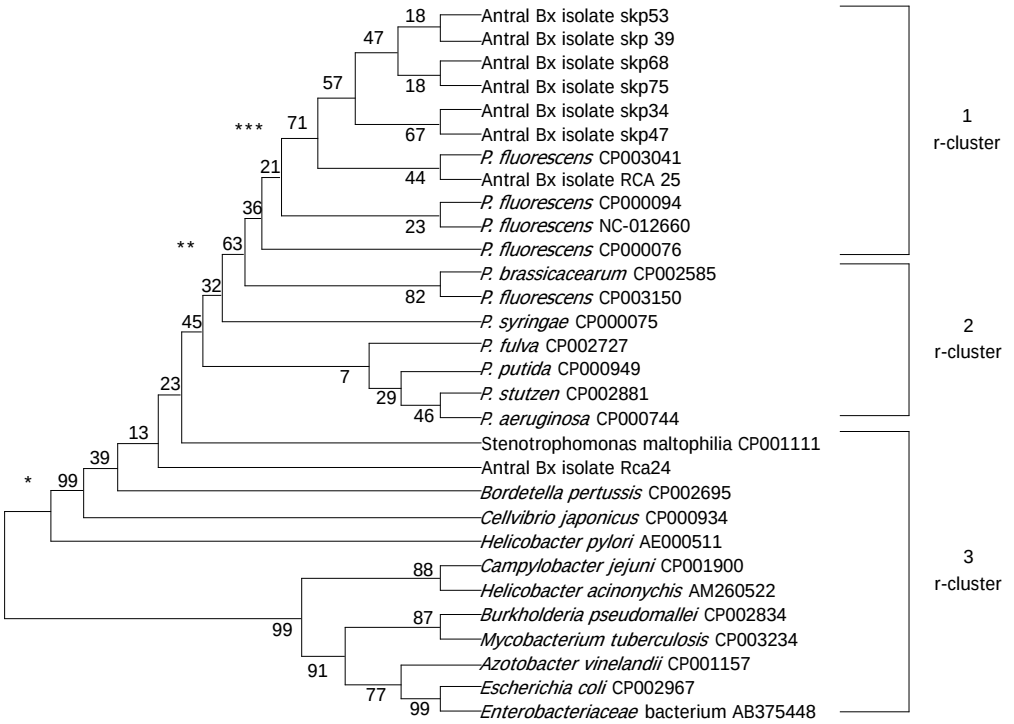


Figure 5 Phylogenetic affiliation of the *Pseudomonas fluorescens*-like isolates (*n* = 8; partial sequences of *HSP60*) on the basis of *HSP60* gene sequences. The unrooted tree was generated using unweighted pair group method with arithmetic mean from evolutionary distance computed with bootstrap test of phylogeny using *MEGA* version 4 by aligning published sequences from Genbank of heat shock protein (*HSP60*) genes from 5 reference strains of *Pseudomonas fluorescens* (*P. fluorescens*), 6 other reference strains representative of the principal *Pseudomonas phyla* (accession number followed by species name in parentheses). For checking relatedness with other genera, we included *HSP60* gene sequences from GenBank of 11 bacterial pathogens, namely *Helicobacter pylori*, *Helicobacter acinonychis*, *Campylobacter jejuni*, *Enterobacter* spp., *Escherichia coli*, *Bordetella pertussis*, *Burkholderia pseudomallei*, *Stenotrophomonas maltophilia*, *Cellvibrio japonicus*, *Azotobacter vinelandii* and *Mycobacterium tuberculosis*. Branches found by maximum likelihood are labeled with asterisks: one asterisk if bootstrap values = 99%, two asterisks if = 63% and three asterisks if = 71%.

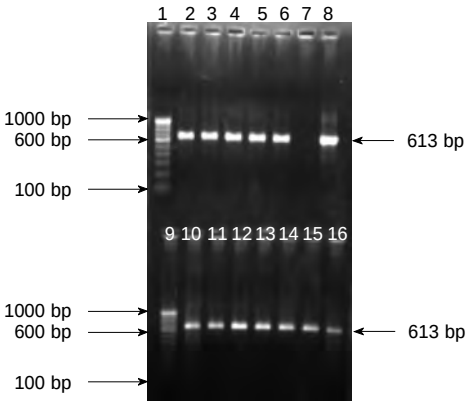


Figure 6 Amplification of putative outer membrane protein gene with specific nested primer generating 613 bp amplicon for *Pseudomonas fluorescens* of antral biopsies and culture isolates. Lanes 1 and 9: Molecular marker (100 bp); Lanes 2 to 6: gDNA from antral biopsy specimens; Lane 7: Negative control; Lane 8: Positive control; and Lanes 10 to 16: Bacterial gDNA from culture isolates.

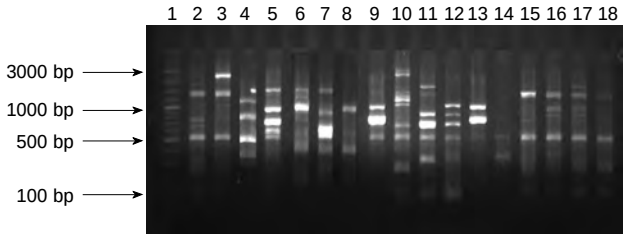


Figure 7 Representative gel showing amplification of *Pseudomonas fluorescens* genomic sequences by randomly amplified polymorphic DNA primers. Randomly amplified polymorphic DNA (RAPD) 3 5'-TACAGCTCG-3' and RAPD5 5'-AGCACTGCCT-3'. Lane 1: 100 bp molecular marker; Lanes 2 to 18: *Pseudomonas fluorescens* isolates isolated from stomach.

was produced by the first round of PCR, but a restriction site specific for *H. pylori* for *Hind*Ⅲ enzyme was absent in these amplicons. Further, none of these isolates could yield a 501 bp amplicon for *H. pylori* by nested PCR primers. Thus, the possibility of these isolates being *H. pylori* was excluded. On the other hand, the typical *H. pylori* isolates which grew after 5-7 d of incubation yielded 501 bp amplicon by nested PCR protocol targeting the *HSP60* gene.

Identification based on partial nucleotide sequencing of 16S rRNA of the representative isolates showed 99% sequence similarity with γ-*protobacteria*. On the basis of enzyme restriction analysis as well as partial nucleotide sequencing of 16S rRNA, the strains isolated from the acidic environment of the stomach either could be grouped in the genus *Pseudomonas* or with a closely related new genus. Further, on the basis of blasting of partial nucleotide sequences of *HSP60* gene sequences on the NCBI gene data bank, isolated strains could be grouped with *P. fluorescens*. On the basis of h cluster based on the

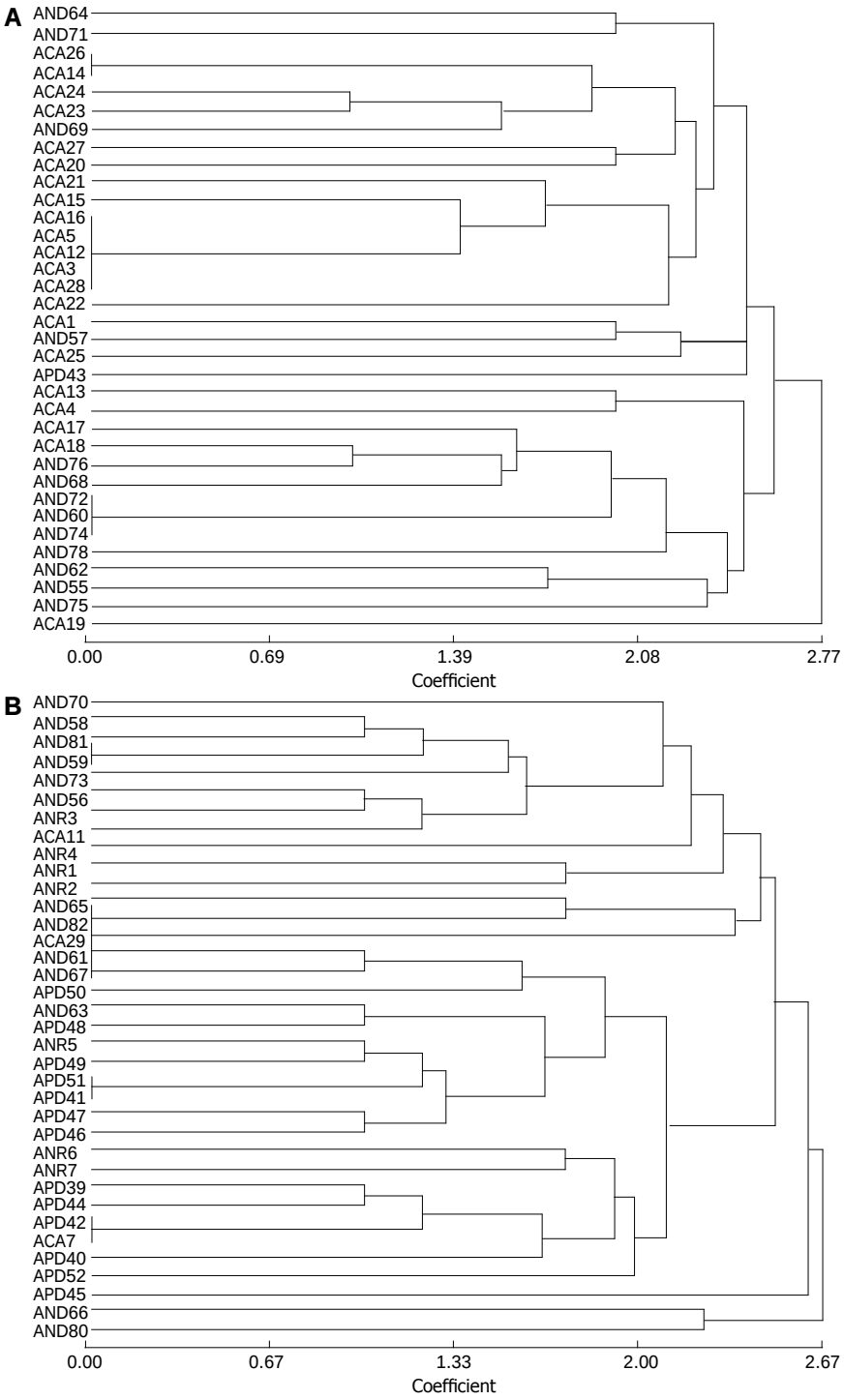


Figure 8 Dendrogram generated by unweighted pair-group method, arithmetic mean on basis of banding pattern of randomly amplified polymorphic DNA for *Pseudomonas fluorescens*. A: 1-35 out of 71 randomly selected isolates of *Pseudomonas fluorescens* from group A (n = 35); B: 36-71 out of 71 randomly selected isolates of *Pseudomonas fluorescens* from group A (n = 36).

UPGMA phylogenetic tree, it may be concluded that the *HSP60* gene sequence has better discriminatory power than 16S rRNA. Further, as one of the isolates could be grouped with *Stenotrophomonas maltophilia* and *Bordetella pertusis,* the possibility of the presence of bacteria other than *P. fluorescens* may not be denied.

The relatively conserved *16S rRNA* gene of *P. fluorescens* has been targeted for PCR-based amplification in culture isolates, but with variable specificity[18]. Therefore, we decided to target a conserved putative membrane-bound protein gene of *P. fluorescens*. The putative membrane-bound protein specific primer screened in the present study was found to be specific for *P. fluorescens* and it yielded an amplicon of 613 bp, confirming the isolates as being *P. fluorescens*. Mupirocin is known for its antibacterial activity and has been reported in only one strain of *P. fluorescens* (NCIMB 10586). A mupirocin-producing gene cluster could not be traced in any of the *P. fluorescens*

isolates despite confirmation by 3 genes, *i.e.*, 16S rRNA, *HSP60* and putative membrane bound protein genes. None of our isolates showed the presence of *mup*V (mupirocin). It is quite possible that this gene might be either absent in all the strains or have too much polymorphism at the loci of primer annealing[19]. We could isolate the *P. fluorescens* strain from 93% of antral biopsies of the patients suffering from gastric diseases. These isolates were further confirmed by PCR-based amplification targeting 3 conserved genes. In DNA of antral biopsy tissue also, 98.4% of NUD and 93.9% of gastric cancer patients were found to be positive for the *P. fluorescens* DNA by nested PCR, while 95.2% of normal stomach had *P. fluorescens* and 96.9% of patients with PUD were found to have the bacterium. Thus our observation indicates a high prevalence and density of these non-*H. pylori* bacteria in the gastric mucosa of patients. Further, to ascertain that the isolated *P. fluorescens* were not contaminants, we carried out whole genome fingerprinting of the 71 randomly selected isolates by using RAPD[20,21] with two RAPD primers. Cluster analysis indicated that almost all the strains had different banding patterns with only a few exceptions, confirming that the isolates were of different clones and thus ruling out the possibility of cross contamination. Moreover, from time to time, we did specific PCR amplification for the *P. fluorescens* on DNA extracted from samples of tap water and washouts of the endoscope, which never yielded the required amplicon even by nested protocol for putative outer membrane protein.

It is interesting to mention that before the implication of *H. pylori* in acid peptic diseases and stomach cancer, Steer and Collin-Jones[4] reported that 80% of antral biopsies have *Pseudomonas* spp. There are reports showing the presence of non-*Helicobacter* bacteria in gastric biopsies of patients suffering from gastric atrophy[22,23]. In cases of reduced gastric acidity due to antacids, it also has been reported that there is presence of several other bacteria[24-26]. Two more studies based on profiling of bacterial flora by temperature gradient gel electrophoresis, 16S rRNA sequence analysis[7] and molecular analysis of the bacterial microbiota[27] have shown presence of several bacterial species including *Pseudomonas* in the stomach. This bacterium seems to be able to colonize the stomach due to its ability to produce urease enzyme. Similarly, it was reported that a non-*H. pylori* bacterium, *Ochrobacrum anthropi,* could be implicated in causation of gastritis in the Squirrel monkey[6].

Such a high prevalence of *P. fluorescens* in human stomach raises many questions: Is it prevalent in the stomach of patients of other subcontinents and continents? Does it have any pathogenic role? Does it have some protective role? Is it simply a part of commensal flora of human stomach? There is a study from Venezuela[5] reporting that *Pseudomonas* strains may interfere with the identification of *H. pylori*. They suggested that one should not rely on rapid urease, catalase and oxidase tests for identification of *H. pylori*. However, studies are needed to observe and identify the presence of *P. fluorescens* in other parts of the world also. With regard to its pathogenic potential, *P. fluorescens* is known as an unusual pathogen of humans. It has been reported as causing septicemia in humans, especially associated with transfusion and cancer[28-30]. Moreover, there is a report indicating that *P. fluorescens* encodes the Crohn's disease-associated I^2 sequence and T cell super antigen, thus implicating it in the pathogenesis of Crohn's disease[31].

However, its commensal state in the stomach may be speculated strongly due to its prevalence in stomach at such a high level and density (it could be isolated in the majority of the antral biopsy). Assumptions may be made that *P. fluorescens* might be producing some antibacterial substances, such as is produced by *P. aeruginosa*, *P. aeruginosa* is known for producing 4-hydroxy-2-alkylquinoline which is inhibitory *in vitro* to *H. pylori*[32]. Moreover, one of the *P. fluorescens* strains is already known for production of mupirocin which is very effective against methicillin-resistant *Staphylococcus aureus*. This bacterium has been stated to have a probiotic role in the gills of fish[33]. Furthermore, low isolation of *H. pylori* and fewer incidences of acid peptic diseases including gastric cancer in North Indians may also be speculated on the basis of the probiotic activity of *P. fluorescens* in the stomach. In addition, there are reports of the unique property of *P. fluorescens* to inhibit the growth of other bacteria, fungi and nematodes causing plant pathology[34-40].

In view of the suggestions made by previous studies[41-43], there is a strong need to explore the exact role of *H. pylori* in stomach diseases because the commensal role of *H. pylori* cannot be rejected outright. In a similar way, the observations made in the present study strongly indicate that further exploration of the different aspects of associations of *P. fluorescens* with human disease and health should be carried out. The pathogenic potential may be explored in animal models like gerbils.

This study concludes that *P. fluorescens* is as common as *H. pylori* in the stomach of humans. Colonies that appeared on enriched BHI agar after 72 h of strictly microaerophilic incubation were *H. pylori* while those growing faster in an aerobic atmosphere were different. These different growths could be identified as *P. fluorescens*. The activity of *P. fluorescens* in the stomach may be speculated to be either pathogenic or probiotic.

COMMENTS

Background

Although *Helicobacter pylori* (*H. pylori*) has been implicated in acid peptic diseases along with stomach cancer, there are reports indicating the presence of several other bacterial species in the stomach. Antral biopsies from North Indian subjects frequently yielded a bacterial growth on selective, non-enriched simple medium in an aerobic environment at 37 ℃ of small, low convex, and pinhead-size translucent colonies. Presence of these types of growth provoked questions about its characterization and its status: whether it was a contaminant from the environment during antral biopsy collection.

Research frontiers

The big question to be answered is further characterization of these isolates and to ensure that they are actual colonizers of the stomach. Do these acid tolerant isolates have pathogenic potential if they are real colonizers?

Innovations and breakthroughs

Pseudomonas fluorescens (*P. fluorescens*)-like bacteria colonize the stomach quite frequently of North Indian patients at high density, as polymerase chain reaction (PCR)-based detection and isolation rates were both comparable. In contrast, the density of *H. pylori* seems to be quite low as nested PCR-based detection is significantly high as compared to the isolation rate of the bacterium. *P. fluorescens* isolates are urease producers and acid tolerant. Although the mupirocin gene could not be detected in any of the *P. fluorescens* isolates, the probiotic (inhibitory to *H. pylori*) role of the bacterium in the stomach may be speculated.

Applications

The potential of *P. fluorescens* as a probiotic may be explored because despite very high prevalence of *H.pylori* in India the incidences of acid peptic diseases and stomach cancer are quite low.

Terminology

Nested polymerase chain reaction is a modified technique of PCR intended to increase sensitivity and specificity of primer and to reduce the contamination in amplicons due to the amplification of undesired primer annealing sites. Phylogenetics is the study of evolutionary relations among groups of organisms such as strains or species, which are based on molecular sequencing data matrices. Probiotics are live microorganisms which when administered in adequate amounts confer a health benefit on the host, *e.g.*, lactic acid bacteria.

Peer review

The study is well carried out from the methodological perspective. This manuscript characterized the bacterial isolates growing aerobically from antral biopsies were *P. fluorescens*, which was the acid tolerant bacteria other than *H. pylori*. On the basis of 16S rRNA and HSP60 sequence and from phylogenetic sequence analysis these organisms were closely related to *P. fluorescens*. The authors also reconfirmed this unknown bacterium as *P. fluorescens* by PCR positivity of *P. fluorescens* specific conserved putative outer membrane protein gene. Finally, the authors concluded that *P. fluorescens* is as common as *H. pylori* in the human stomach.

REFERENCES

1 **Marshall BJ**, Warren JR. Unidentified curved bacilli in the stomach of patients with gastritis and peptic ulceration. *Lancet* 1984; **1**: 1311-1315 [PMID: 6145023 DOI: 10.1016/S0140-6736(84)91816-6]

2 **Cover TL**, Blaser MJ. Helicobacter pylori and gastroduodenal disease. *Annu Rev Med* 1992; **43**: 135-145 [PMID: 1580578 DOI: 10.1146/annurev.me.43.020192.001031]

3 **McGowan CC**, Cover TL, Blaser MJ. Helicobacter pylori and gastric acid: biological and therapeutic implications. *Gastroenterology* 1996; **110**: 926-938 [PMID: 8608904 DOI: 10.1053/gast.1996.v110.pm8608904]

4 **Steer HW**, Colin-Jones DG. Mucosal changes in gastric ulceration and their response to carbenoxolone sodium. *Gut* 1975; **16**: 590-597 [PMID: 810394 DOI: 10.1136/gut.16.8.590]

5 **Domínguez-Bello MG**, Reyes N, Teppa-Garrán A, Romero R. Interference of Pseudomonas strains in the identification of Helicobacter pylori. *J Clin Microbiol* 2000; **38**: 937 [PMID: 10722321]

6 **Khanolkar-Gaitonde SS**, Reubish GK, Lee CK, Stadtländer CT. Isolation of bacteria other than Helicobacter pylori from stomachs of squirrel monkeys (Saimiri spp.) with gastritis. *Dig Dis Sci* 2000; **45**: 272-280 [PMID: 10711437]

7 **Monstein HJ**, Tiveljung A, Kraft CH, Borch K, Jonasson J. Profiling of bacterial flora in gastric biopsies from patients with Helicobacter pylori-associated gastritis and histologically normal control individuals by temperature gradient gel electrophoresis and 16S rDNA sequence analysis. *J Med Microbiol* 2000; **49**: 817-822 [PMID: 10966230]

8 **Blazevic DJ**, Koepcke MH, Matsen JM. Incidence and identification of Pseudomonas fluorescens and Pseudomonas putida in the clinical laboratory. *Appl Microbiol* 1973; **25**: 107-110 [PMID: 4631431]

9 **Gilligan PH.** Pseudomonas and Burkholderia. 6th ed. In: Murray PR, Baron EJ, Pfaller MA, Tenover FC, Yolken RH. Manual of clinical microbiology. Washington: ASM Press, 1995: 509-519

10 **Ho SA**, Hoyle JA, Lewis FA, Secker AD, Cross D, Mapstone NP, Dixon MF, Wyatt JI, Tompkins DS, Taylor GR. Direct polymerase chain reaction test for detection of Helicobacter pylori in humans and animals. *J Clin Microbiol* 1991; **29**: 2543-2549 [PMID: 1723072]

11 **Singh V**, Mishra S, Rao GR, Jain AK, Dixit VK, Gulati AK, Mahajan D, McClelland M, Nath G. Evaluation of nested PCR in detection of Helicobacter pylori targeting a highly conserved gene: HSP60. *Helicobacter* 2008; **13**: 30-34 [PMID: 18205663 DOI: 10.1111/j.1523-5378.2008.00573.x]

12 **Carroll NM**, Jaeger EE, Choudhury S, Dunlop AA, Matheson MM, Adamson P, Okhravi N, Lightman S. Detection of and discrimination between gram-positive and gram-negative bacteria in intraocular samples by using nested PCR. *J Clin Microbiol* 2000; **38**: 1753-1757 [PMID: 10790093]

13 **Thompson JD**, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. The CLUSTAL_X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. *Nucleic Acids Res* 1997; **25**: 4876-4882 [PMID: 9396791 DOI: 10.1093/nar/25.24.4876]

14 **Tamura K**, Dudley J, Nei M, Kumar S. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. *Mol Biol Evol* 2007; **24**: 1596-1599 [PMID: 17488738 DOI: 10.1093/molbev/msm092]

15 **Grahn N**, Olofsson M, Ellnebo-Svedlund K, Monstein HJ, Jonasson J. Identification of mixed bacterial DNA contamination in broad-range PCR amplification of 16S rDNA V1 and V3 variable regions by pyrosequencing of cloned amplicons. *FEMS Microbiol Lett* 2003; **219**: 87-91 [PMID: 12594028 DOI: 10.1016/S03781097(02)01190-4]

16 **Romesbrug HC.** Cluster Analysis for Researchers. Belmont (CA): Lifetime Learning Publications, 1984

17 **Merrell DS**, Goodrich ML, Otto G, Tompkins LS, Falkow S. pH-regulated gene expression of the gastric pathogen Helicobacter pylori. *Infect Immun* 2003; **71**: 3529-3539 [PMID: 12761138 DOI: 10.1128/IAI.71.6.3529-3539.2003]

18 **Kumar NR,** Thirumalai V Arasu, Gunasekaran P. Genotyping of antifungal compounds producing plant growth promoting rhizobacteria, Pseudomonas fluorescens. *Curr Sci* 2002; **82:** 1463-1468

19 **Fuller AT,** Mellows G, Woolford M, Banks GT, Barrow KD, Chain EB. Pseudomonic acid: an antibiotic produced by Pseudomonas fluorescens. *Nature* 1971; **234**: 416-417 [PMID: 5003547]

20 **Charan AR,** Reddy VP, Reddy PN, Reddy SS. Assessment of genetic diversity in Pseudomonas fluorescens using PCR-based methods. *Biorem Biodiv Bioavail* 2011; **5**: 10–16

21 **Manceau C**, Horvais A. Assessment of genetic diversity among strains of Pseudomonas syringae by PCR-restriction fragment length polymorphism analysis of rRNA operons with special emphasis on P. syringae pv. tomato. *Appl Environ Microbiol* 1997; **63**: 498-505 [PMID: 9023928]

22 **Dolby JM**, Webster AD, Borriello SP, Barclay FE, Bartholomew BA, Hill MJ. Bacterial colonization and nitrite concentration in the achlorhydric stomachs of patients with primary hypogammaglobulinaemia or classical pernicious anaemia. *Scand J Gastroenterol* 1984; **19**: 105-110 [PMID: 6710072]

23 **Jonkers D**, van den Broek E, van Dooren I, Thijs C, Dorant E, Hageman G, Stobberingh E. Antibacterial effect of garlic and omeprazole on Helicobacter pylori. *J Antimicrob Chemother* 1999; **43**: 837-839 [PMID: 10404325 DOI: 10.1093/jac/43.6.837]

24 **Mauritz W**, Graninger W, Schindler I, Karner J, Zadrobilek E, Sporn P. [Pathogenic flora in the gastric juice and bronchial secretion of long-term ventilated intensive-care patients]. *Anaesthesist* 1985; **34**: 203-207 [PMID: 4003748]

25 **Howden CW**, Hunt RH. Relationship between gastric secretion and infection. *Gut* 1987; **28**: 96-107 [PMID: 3546004]

26 **Houben GM**, Hooi J, Hameeteman W, Stockbrügger RW.

Twenty-four-hour intragastric acidity: 300 mg ranitidine b.d., 20 mg omeprazole o.m., 40 mg omeprazole o.m. vs. placebo. *Aliment Pharmacol Ther* 1995; **9**: 649-654 [PMID: 8824652 DOI: 10.1111/j.1365-2036.1995.tb00434.x]

27 **Bik EM**, Eckburg PB, Gill SR, Nelson KE, Purdom EA, Francois F, Perez-Perez G, Blaser MJ, Relman DA. Molecular analysis of the bacterial microbiota in the human stomach. *Proc Natl Acad Sci USA* 2006; **103**: 732-737 [PMID: 16407106 DOI: 10.1073/pnas.0506655103]

28 **Murray AE**, Bartzokas CA, Shepherd AJ, Roberts FM. Blood transfusion-associated Pseudomonas fluorescens septicaemia: is this an increasing problem? *J Hosp Infect* 1987; **9**: 243-248 [PMID: 2886528 DOI: 10.1016/0195-6701(87)90120-4]

29 **Hsueh PR**, Teng LJ, Pan HJ, Chen YC, Sun CC, Ho SW, Luh KT. Outbreak of Pseudomonas fluorescens bacteremia among oncology patients. *J Clin Microbiol* 1998; **36**: 2914-2917 [PMID: 9738043]

30 **Gershman MD**, Kennedy DJ, Noble-Wang J, Kim C, Gullion J, Kacica M, Jensen B, Pascoe N, Saiman L, McHale J, Wilkins M, Schoonmaker-Bopp D, Clayton J, Arduino M, Srinivasan A. Multistate outbreak of Pseudomonas fluorescens bloodstream infection after exposure to contaminated heparinized saline flush prepared by a compounding pharmacy. *Clin Infect Dis* 2008; **47**: 1372-1379 [PMID: 18937575 DOI: 10.1086/592968]

31 **Wei B**, Huang T, Dalwadi H, Sutton CL, Bruckner D, Braun J. Pseudomonas fluorescens encodes the Crohn's disease-associated I2 sequence and T-cell superantigen. *Infect Immun* 2002; **70**: 6567-6575 [PMID: 12438326 DOI: 10.1128/IAI.70.12.6567-6575.2002]

32 **Lacey SL**, Mehmet S, Taylor GW. Inhibition of Helicobacter pylori growth by 4-hydroxy-2-alkyl-quinolines produced by Pseudomonas aeruginosa. *J Antimicrob Chemother* 1995; **36**: 827-831 [PMID: 8626264 DOI: 10.1093/jac/36.5.827]

33 **Gram L,** Melchiorsen J, Spanggaard B, Huber I, Nielsen TF. Inhibition of vibrio anguillarum by Pseudomonas fluorescens AH2, a possible probiotic treatment of fish. *Appl Environ Microbiol* 1999; **65**: 969-973 [PMID: 10049849]

34 **Haas D,** Défago G. Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nat Rev Microbiol* 2005; **3**: 307-319 [PMID: 15759041 DOI: 10.1038/nrmicro1129]

35 **Haas D,** Keel C. Regulation of antibiotic production in root-colonizing Peudomonas spp. and relevance for biological control of plant disease. *Annu Rev Phytopathol* 2003; **41**: 117-153 [PMID: 12730389 DOI: 10.1146/annurev.phyto.41.052002.095656]

36 **Sharifi-Tehrani A,** Zala M, Natsch A, Loccoz YM, Defago G. Biocontrol of soil-borne fungal plant diseases by 2,4-diacetylphloroglucinol-producing fluorescent pseudomonads with different restriction profiles of amplified 16S rDNA. *Er J Plant Pathol* 1998; **104**: 631–643

37 **Thomashow LS,** Weller D. Current concepts in the use of introduced bacteria for biological disease control: mechanisms and antifungal metabolites. In: Stacey G, Keen NT, editors. Plant-microbe interactions 1996. United Kingdom: Chapman and Hall, Ltd.: 187-236 [DOI: 10.1007/978-1-4613-1213-0_6]

38 **Toyoda H,** Katsuragi K, Tamari T and Ouchi S. DNA sequence of genes for detoxification of fusaric acid a wilt-inducing agent produced by Fusarium spp. *Journal of Phytopathology* 1991; **133**: 265-277 [DOI: 10.1111/j.14390434.1991.tb00162.x]

39 **Loper JE.** Role of fluorescent siderophore production in biological control of Pythium ultimum by a Pseudomonas fluorescens strain. *Phytopathology* 1988; **78**: 166-172 [DOI: 10.1094/Phyto-78-166]

40 **Neilands JB,** Leong SA. Siderophores in relation to plant growth and disease. *Ann Rev Plant Physiol* 1986; **37**: 187-208 [DOI: 10.1146/annurev.pp.37.060186.001155]

41 **Blaser MJ.** Not all Helicobacter pylori strains are created equal: should all be eliminated? *Lancet* 1997; **349**: 1020-1022 [PMID: 9100641 DOI: 10.1016/S0140-6736(96)09133-7]

42 **Blaser MJ.** Helicobacters are indigenous to the human stomach: duodenal ulceration is due to changes in gastric microecology in the modern era. *Gut* 1998; **43**: 721-727 [PMID: 9824358 DOI: 10.1136/gut.43.5.721]

43 **Blaser MJ.** Hypothesis: the changing relationships of Helicobacter pylori and humans: implications for health and disease. *J Infect Dis* 1999; **179**: 1523-1530 [PMID: 10228075 DOI: 10.1086/314785]

P- Reviewers Slomiany BL, Wang JT
S- Editor Gou SX **L- Editor** Logan S **E- Editor** Lu YJ

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i7.1068

World J Gastroenterol 2013 February 21; 19(7): 1068-1078
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

ORIGINAL ARTICLE

Long interspersed nuclear element ORF-1 protein promotes proliferation and resistance to chemotherapy in hepatocellular carcinoma

Fan Feng, Yin-Ying Lu, Fan Zhang, Xu-Dong Gao, Chuan-Fu Zhang, Alex Meredith, Zhong-Xian Xu, Yu-Tao Yang, Xiu-Juan Chang, Hong Wang, Jian-Hui Qu, Zhen Zeng, Jun-Lan Yang, Chun-Ping Wang, Yun-Feng Zhu, Jia-Jun Cui, Yong-Ping Yang

Fan Feng, Yin-Ying Lu, Xu-Dong Gao, Zhong-Xian Xu, Xiu-Juan Chang, Hong Wang, Jian-Hui Qu, Zhen Zeng, Chun-Ping Wang, Yong-Ping Yang, Clinical Research Center of Hepatocarcinoma, 302 Military Hospital, Beijing 100039, China
Fan Feng, Institute of Toxicology and Pharmacology, Chinese Academy of Military Medical Sciences, Beijing 100850, China
Fan Zhang, Jun-Lan Yang, Yun-Feng Zhu, Tumor Center, PLA General Hospital, Beijing 100853, China
Chuan-Fu Zhang, Jia-Jun Cui, Institute of Disease Control and Prevention, Chinese Academy of Military Medical Sciences, Beijing 100071, China
Alex Meredith, Jia-Jun Cui, Department of Cancer and Cell Biology, University of Cincinnati College of Medicine, Cincinnati, OH 45267, United States
Yu-Tao Yang, Beijing Institute for Neuroscience, Capital Medical University, Beijing 100069, China
Author contributions: Feng F, Lu YY and Zhang F contributed equally to this work; Lu YY, Zhang F, Gao XD, Xu ZX, Yang YT, Qu JH, Zeng Z and Chang XJ performed the majority of experiments; Zhang CF, Wang H, Yang JL, Wang CP and Zhu YF provided some reagents and analytical tools and were also involved in revising the manuscript; Feng F, Cui JJ and Yang YP designed the study; Feng F and Cui JJ wrote the manuscript; Meredith A refined the manuscript and the experiment.
Supported by The Key Scientific and Technological Research Foundation of the National Special Purpose Program, No. 2008ZX10002-018
Correspondence to: Yong-Ping Yang, Professor, Clinical Research Center of Hepatocarcinoma, 302 Military Hospital, No. 100, the 4th West Ring Road, Beijing 100039, China. yongpingyang@hotmail.com
Telephone: +86-10-63879193 Fax: +86-10-63879193
Received: July 20, 2012 Revised: December 4, 2012
Accepted: December 27, 2012
Published online: February 21, 2013

Abstract

AIM: To clarify the specific roles and mechanisms of long interspersed nuclear element-1 ORF-1 protein [human long interspersed nuclear element-1 (LINE-1), ORF-1p] in chemotherapeutic drug resistance and cell proliferation regulation in hepatocellular carcinoma (HCC) cells.

METHODS: MTT assays were performed to identify the effect of the chemotherapeutic drug toxicity on HepG2 cells. Cell proliferation inhibition and the IC_{50} were calculated by the Origin 8.0 software. Western blotting assays were performed to investigate whether LINE-1 ORF-1p modulates the expression of some important genes, including *p53*, *p27*, *p15*, *Bcl-2*, *mdr*, and *p-gp*. To corroborate the proliferation and anchor-independent growth results, the HepG2 cells were analyzed by flow cytometry to investigate the effect of LINE-1 ORF-1p on the apoptosis regulation.

RESULTS: LINE-1 ORF-1p contributed to the resistance to several chemotherapeutic drugs (cisplatin and epirubicin) in HepG2 cells. The IC_{50} of the epirubicin and cisplatin increased from 36.04 nmol/L to 59.11 nmol/L or from 37.94 nmol/L to 119.32 nmol/L. Repression of LINE-1 ORF-1p expression by the siRNA could markedly enhance the response of HepG2 cells to the epirubicin and cisplatin. The IC_{50} correspondingly decreased from 28.06 nmol/L to 3.83 nmol/L or from 32.04 nmol/L to 2.89 nmol/L. Interestingly, down-regulation of LINE-1 ORF-1p level by siRNA could promote the response of HepG2 cells to the paclitaxel. The IC_{50} decreased from 35.90 nmol/L to 7.36 nmol/L. However, overexpression of LINE-1 ORF-1p did not modulate the paclitaxel toxicity in HepG2 cells. Further Western blotting revealed that LINE-1 ORF-1p enhanced *mdr* and *p-gp* gene expression. As a protein arrested in the nucleus, LINE-1 ORF-1p may function through modulating transcriptional activity of some important transcription factors. Indeed, LINE-1 ORF-1p promoted HepG2 cell prolifera-

tion, anchor-independent growth and protected the cells against apoptosis through modulating the expression of *p15*, *p21*, *p53*, and *Bcl-2* genes.

CONCLUSION: LINE-1 ORF-1p promotes HepG2 cell proliferation and plays an important role in the resistance of chemotherapeutic drugs. By establishing novel roles and defining the mechanisms of LINE-1 ORF-1p in HCC chemotherapeutic drug resistance and cell proliferation regulation, this study indicates that LINE-1 ORF-1p is a potential target for overcoming HCC chemotherapeutic resistance.

© 2013 Baishideng. All rights reserved.

Key words: Long interspersed nuclear element-1 ORF-1 protein; Hepatocellular carcinoma; Chemotherapeutic drugs; Multi-drug resistance

Feng F, Lu YY, Zhang F, Gao XD, Zhang CF, Meredith A, Xu ZX, Yang YT, Chang XJ, Wang H, Qu JH, Zeng Z, Yang JL, Wang CP, Zhu YF, Cui JJ, Yang YP. Long interspersed nuclear element ORF-1 protein promotes proliferation and resistance to chemotherapy in hepatocellular carcinoma. *World J Gastroenterol* 2013; 19(7): 1068-1078 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i7/1068.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i7.1068

INTRODUCTION

Hepatocellular carcinoma (HCC) which is the most common tumor worldwide, and the third most common cause of cancer death, is often accompanied by a poor prognosis[1]. The majority of HCC patients are unresectable at the time of first diagnosis, and even in patients who are suitable for surgery, the risk of recurrence is high. Consequently, chemotherapy is an important alternative strategy for most HCC patients[2]. Cisplatin, paclitaxel and epirubicin are common chemotherapeutic agents used in the treatment of HCC. However, chemotherapy is often ineffective in HCC patients due to potent multidrug resistance (MDR)[3]. Many studies have indicated that alterations in target gene expression are correlated with drug resistance and may provide a new strategy for sensitizing cancer cells to chemotherapeutic drugs and to reverse MDR in cancer cells.

The human long interspersed nuclear element-1 (*LINE-1*) gene encodes two proteins: LINE-1 ORF-1p and LINE-1 ORF-2p[4]. It is reported that LINE-1 ORF-2p is mainly involved in the retrotransposition process through its endonuclease activity[4]. In human cancer cells, LINE-1 ORF-2p can be regulated by LINE-1 ORF-1p[4,5]. The expression of the LINE-1 ORF-1 protein is almost 1000-fold greater than the LINE-1 ORF-2 protein[5], which indicates that LINE-1 ORF-1p has many roles. Our previous results suggested that LINE-1 ORF-1p enhances the proliferation of several cancer cell lines[6]. LINE-1 ORF-1p also increased the proliferation of hepatocellular carcinoma cells Bel-7402, SMMC-7721, HepG2, and LO2 cells and was associated with a significant risk of HCC progression[7]. However, the detailed mechanisms and roles of LINE-1 ORF-1p in HCC development are still largely unknown.

Based on our previous study[7], we hypothesized that LINE-1 ORF-1p might also be involved in HCC development and chemotherapy resistance regulation. To address this hypothesis, we performed MTT assays and found that LINE-1 ORF-1p protects HepG2 cells against chemotherapy. More importantly, we also found that LINE-1 ORF-1p enhances HepG2 cell proliferation through the modulation of genes involved in cell proliferation and apoptosis such as *p53* and *Bcl-2*.

MATERIALS AND METHODS

Plasmids

The full length of LINE-1 ORF-1p cDNA was cloned into a pcDNA3.1 vector linked with fludarabine + high-dose cytarabine + G-CSF (FLAG) at the amino terminus by polymerase chain reaction using a cDNA library as the template[6,7]. The siRNA targeted to LINE-1 ORF-1p was cloned into the psilencer-2.1-U6 (neo) vector (Ambion). The sequence of siRNA is AAGGAGGTGCACTATA-AGAAC[6,7]. The luciferase reporters, Luc-p21 (containing p53 binding element), Luc-Smad4 binding element (SBE), Luc-SP1 and Luc-androgen response element (ARE) were gifts from Dr. Cui JJ and Yang YT.

Antibodies

Antibodies against *p27*, *p53*, *Bcl-2*, and *p15* were from Santa Cruz Biotechnology Inc., United States, and antibodies against MDR, p-gp and GAPDH were from Sigma-Aldrich, St. Louis, United States. The antibody against LINE-1 ORF-1p was described previously[6,7]. Polyclonal anti-rabbit IgG was from Qiagen, Beijing, China.

Chemotherapeutic agents and cell culture

Epirubicin (Pfizer, NY, United States), Cisplatin (QILU Pharmaceutical Co., Jinan, China), paclitaxel (Roche, Basel, Swiss), Lipofectamine 2000 (Invitrogen, Carlsbad, CA, United States) and other agents (*Amersham Biosciences*, Piscataway, NJ, United States) were used in this study. HepG2 cells (American Type Culture Collection, ATCC) were cultured in DMEM (GIBCO, Grand Island, NY, United States) medium containing 10% FBS.

Cell proliferation assays

HepG2 cells were transfected with FLAG-LINE-1 ORF-1p or siRNA plasmid, and cultured for 24 h at 37 ℃ and 5% CO_2. Then, 10 μL CCK-8 reagent was added to each well and cultured at 37 ℃ and 5% CO_2 for 4 h. The absorbance of the inhibition rate was measured using a multifunctional microplate reader at 490 nm. The inhibition rate = (*A* control group - *A* administration group)/(*A* control group - *A* blank group) × 100%. The assays were performed three times with similar results.

Table 1 Effect of long interspersed nuclear element ORF-1 protein on the cytotoxic activity of chemotherapeutic drugs *in vitro*

Drugs	IC_{50} (nmol/L)			
	FLAG	FLAG-ORF-1p	Control	siRNA
Epirubicin	36.04 ± 4.48	59.11 ± 3.37	28.06 ± 5.31	3.84 ± 0.48
Cisplatin	37.94 ± 2.90	119.32 ± 21.99	32.04 ± 3.71	2.89 ± 0.44
Paclitaxel	27.01 ± 3.91	29.01 ± 2.95	35.90 ± 7.03	7.36 ± 0.69

FLAG: Fludarabine + high-dose cytarabine + G-CSF; siRNA: Small interfering RNA.

Anchorage-independent growth assay

HepG2 cells which stably expressed FLAG-LINE-1 ORF-1p or siRNA were placed in 6-well plates, with a bottom layer of 0.7% low-melting-temperature agar in DMEM and a top layer of 0.25% agar in DMEM. Colonies were scored after 3 wk of growth. The assays were performed in three independent experiments with similar results.

Luciferase assay

HepG2 cells were seeded in 24-well plates. After 24 h, the cells were transfected with FLAG-LINE-1 ORF-1p and the indicated reporter gene using Lipofectamine 2000 (Invitrogen). Twenty-four hours later, the cells were harvested and analyzed for luciferase following the manual protocol (Promega Corp., Madison, WI, United States).

Flow cytometry and apoptosis analysis

Assays were performed following the protocol provided by the apoptosis assay kit (Qiagen, Beijing, China). In brief, cells were treated with dehydrated alcohol for flow cytometry analysis. Before analysis, cells were treated with 0.5% RNase at 65 ℃ for 30 min. The assays were performed three times with similar results.

Cell transfection and stable transfection

Plasmids were transfected into HepG2 cells using Lipofectamine 2000 (Invitrogen). Forty-eight hours later, transfected cells were cultured in 500 μg/mL G418 (Invitrogen) for approximately 2 mo. Individual clones were screened by Western blotting using anti-FLAG or anti-LINE-1 ORF-1p antibodies. Similar results were observed with stable transfection or transient transfection with individual clones or pool clones.

Immunoblotting analysis

Total protein in the samples was measured by SDS-PAGE and trans-printed to a nitrocellulose (NC) membrane. The NC membranes were blocked with 5% BSA in TBST buffer and incubated with the antibodies. The membranes were then incubated with horseradish peroxidase-conjugated secondary antibodies after washing with TBST buffer. Membranes were visualized using the appropriate kit (Qiagen).

RESULTS

LINE ORF-1 protein modulates the cytotoxic effects of chemotherapeutic agents

To determine whether LINE-1 ORF-1p modulates the cytotoxic effects of chemotherapeutic agents, MTT assays were performed. The results showed that overexpression of LINE-1 ORF-1p significantly reduced the cytotoxicity of epirubicin and cisplatin on HepG2 cells (Figure 1) and the corresponding IC_{50} values increased significantly (Table 1). Reduction of LINE-1 ORF-1p expression by siRNA markedly promoted the sensitivity of HepG2 cells to epirubicin and cisplatin (Figure 1). The IC_{50} values correspondingly decreased significantly (Table 1).

Interestingly, our data also showed that neither overexpression of LINE-1 ORF-1p plasmids nor the empty vector protected HepG2 cells from the cytotoxicity of paclitaxel (Figure 1E). Knockdown of LINE-1 ORF-1p increased the cytotoxic effect of paclitaxel on HepG2 cells (Figure 1F). The IC_{50} values correspondingly decreased from 35.90 nmol/L to 7.36 nmol/L (Table 1). Taken together, these results suggest that LINE-1 ORF-1p mediates chemotherapeutic drug resistance in HepG2 cells.

LINE ORF-1 protein promotes HepG2 cell proliferation

To determine whether LINE-1 ORF-1p affected HepG2 cell proliferation, MTT assays were performed. HepG2 cells transfected with FLAG-LINE-1 ORF1-p had an increased proliferation rate compared with those transfected with FLAG empty vector (Figure 2A); whereas cells transfected with LINE-1 ORF-1p siRNA, grew more slowly than those transfected with the control siRNA (Figure 2B). In addition, LINE-1 ORF-1p also promoted HCC SMMC-7721 (Figure 2C and D) and BEL-7402 cell growth (Figure 2E and F). These results indicated that LINE-1 ORF-1p affects HCC cell proliferation.

LINE ORF-1 protein protects HepG2 cells against apoptosis

To investigate the role of LINE-1 ORF-1p in HepG2 cell apoptosis, apoptosis assays were performed. Overexpression of LINE-1 ORF-1p protected HepG2 cells from apoptosis (Figure 3), whereas knockdown of LINE-1 ORF-1p significantly promoted the apoptosis of HepG2 cells (Figure 3).

LINE ORF-1 protein increases HepG2 anchor-independent growth

The effects of LINE-1 ORF1-p on anchorage-independent growth were tested in stable HepG2 cell lines expressing either FLAG-LINE-1 ORF1-p or siRNA. Overexpression of LINE-1 ORF-1p significantly increased the anchorage-independent growth of HepG2 cells (Figure 3); whereas knockdown of endogenous LINE-1 ORF1-p decreased anchorage-independent growth of HepG2 cells (Figure 3). These results suggest that LINE-1 ORF-1p also strongly promotes HCC cell growth.

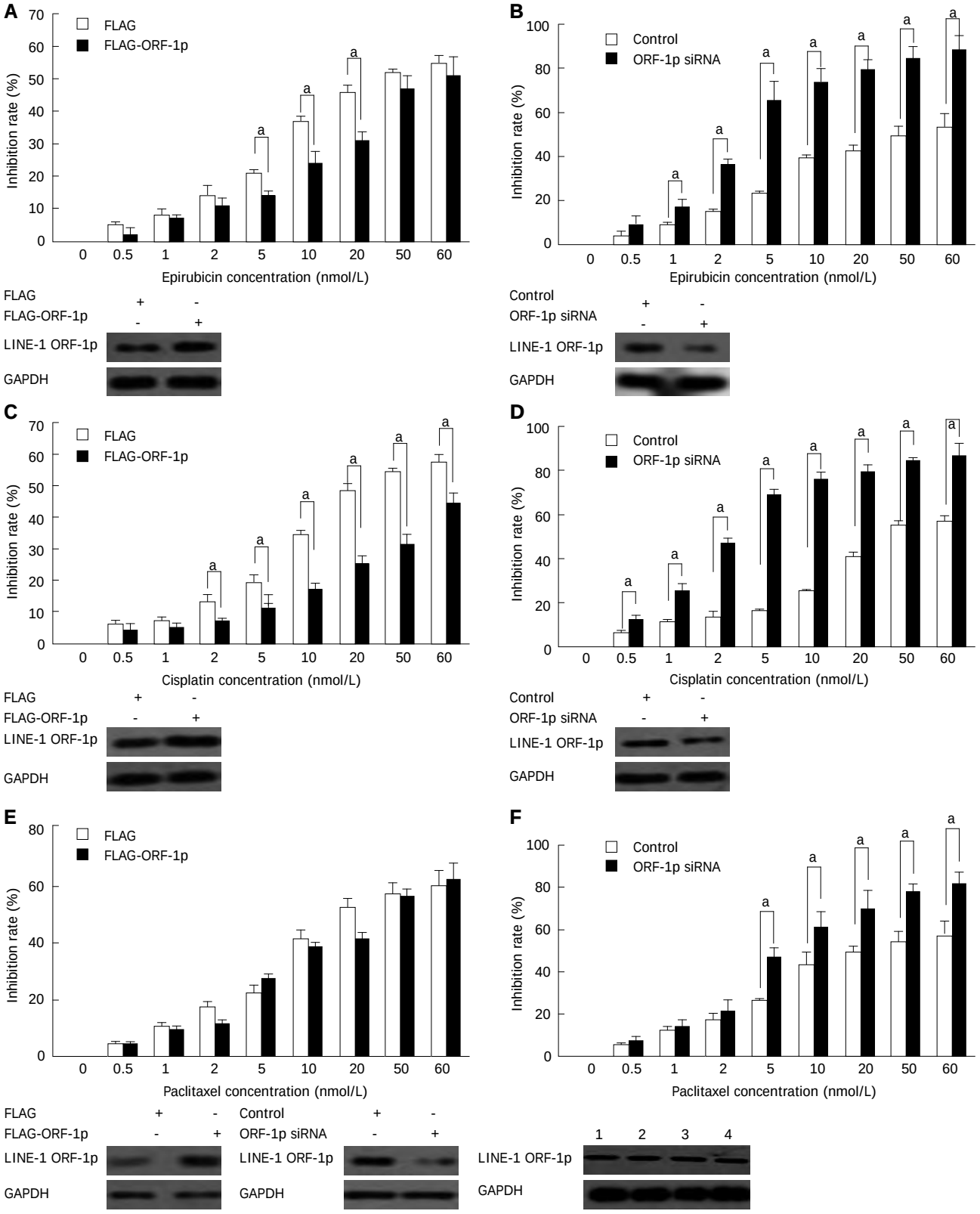


Figure 1 Effect of long interspersed nuclear element ORF-1 protein on epirubicin, cisplatin and paclitaxel cytotoxicity. HepG2 cells were treated with the indicated amounts of epirubicin (A, B), cisplatin (C, D) or paclitaxel (E, F): 0 nmol/L, 0.5 nmol/L, 1 nmol/L, 2 nmol/L, 5 nmol/L, 10 nmol/L, 20 nmol/L, 50 nmol/L, 60 nmol/L. A: HepG2 cells were transfected with the fludarabine + high-dose cytarabine + G-CSF human long interspersed nuclear element-1 (FLAG-LINE-1) ORF-1p expression vector, or the empty vector; B: HepG2 cells were transfected with the control siRNA vector or the LINE-1 ORF-1p siRNA vector; C: HepG2 cells were transfected with the FLAG-LINE-1 ORF-1p expression vector, or the empty vector; D: HepG2 cells were transfected with the control siRNA vector or the LINE-1 ORF-1p siRNA vector; E: HepG2 cells were transfected with the FLAG-LINE-1 ORF-1p expression vector, or the empty vector; F: HepG2 cells were transfected with the control siRNA vector or the LINE-1 ORF-1p siRNA vector. The assays were performed three times with similar results. $^{a}P < 0.05$ *vs* control. 1: Control; 2: Epirubicin; 3: Cisplatin; 4: Paclitaxel. GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

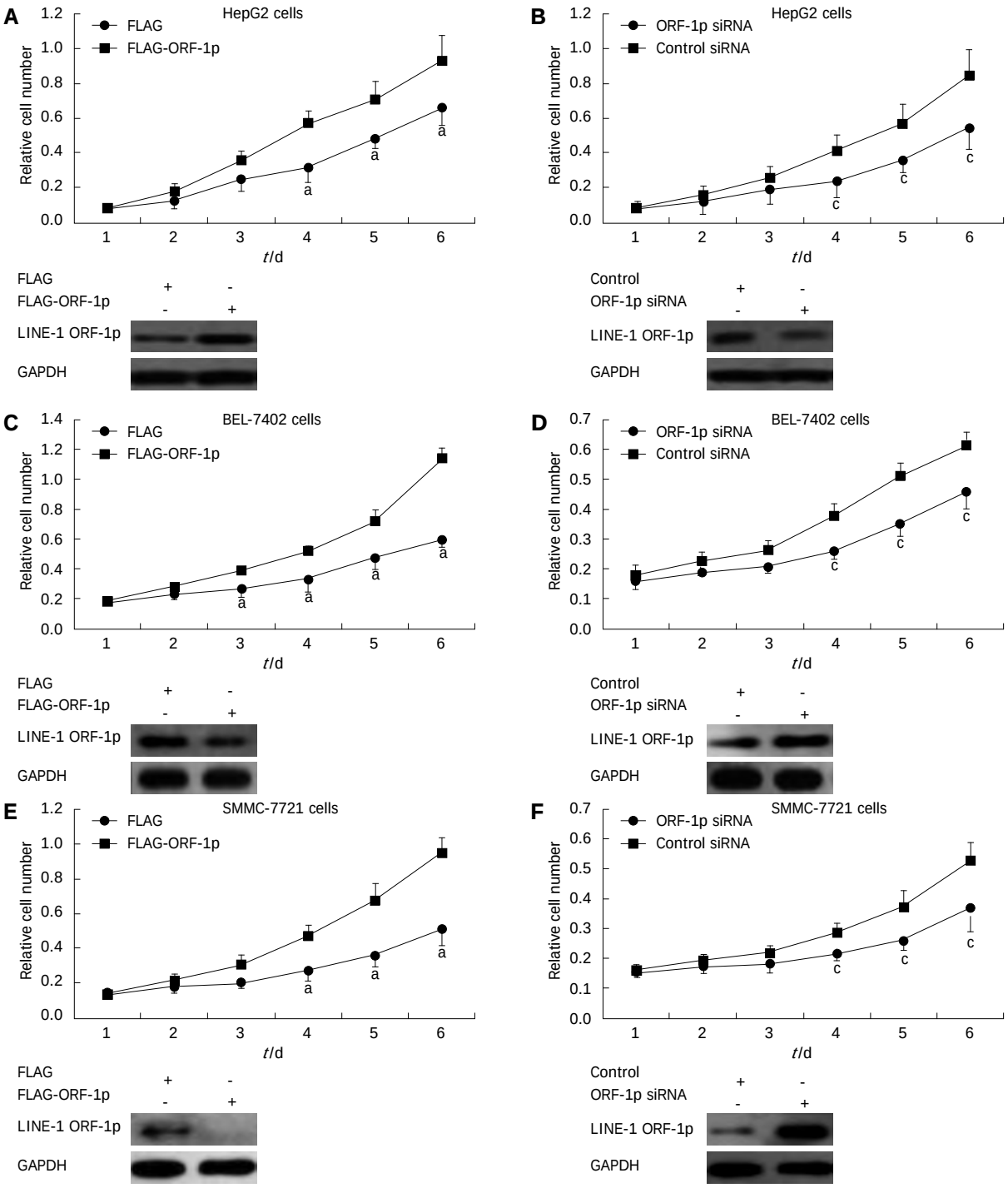


Figure 2 Long interspersed nuclear element ORF-1 protein promotes the proliferation of hepatocellular carcinoma cells. A: HepG2 cells were stably transfected with the fludarabine + high-dose cytarabine + G-CSF human long interspersed nuclear element-1 (FLAG-LINE-1) ORF-1p or the empty vector; B: HepG2 cells were stably transfected with the control siRNA or LINE-1 ORF-1p siRNA; C: BEL-7402 cells were stably transfected with the FLAG-LINE-1 ORF-1p or the empty vector; D: BEL-7402 cells were stably transfected with the control siRNA or LINE-1 ORF-1p siRNA; E: SMMC-7721 cells were stably transfected with the FLAG-LINE-1 ORF-1p or the empty vector; F: SMMC-7721 cells were stably transfected with the control siRNA or LINE-1 ORF-1p siRNA. The relative cell numbers ($A_{490\,nm}$) were determined by MTT assays, relative cell numbers (A, B) with mean ± SD (n = 3). $^{a}P < 0.05$ *vs* FLAG; $^{c}P < 0.05$ *vs* control siRNA. GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

LINE ORF-1 protein regulates the reporter gene activities of p21, SBE and Sp1

To determine the mechanism of LINE-1 ORF1-p, luciferase assays were performed. The results showed that overexpression of LINE-1 ORF-1p decreased Luc-p21, Luc-SBE and Luc-Sp1 activity. Knock-down of LINE-1 ORF-1p expression enhanced the activities of Luc-p21 (Figure 4A), Luc-SBE (Figure 4B) and Luc-Sp1 (Figure 4C).

In addition, overexpression of LINE-1 ORF-1p promoted the transcription of Luc-ARE reporter (Figure 4D). Down-regulation of LINE-1 ORF1-p expression reduced the activity of Luc-ARE (Figure 4D). Taken to-

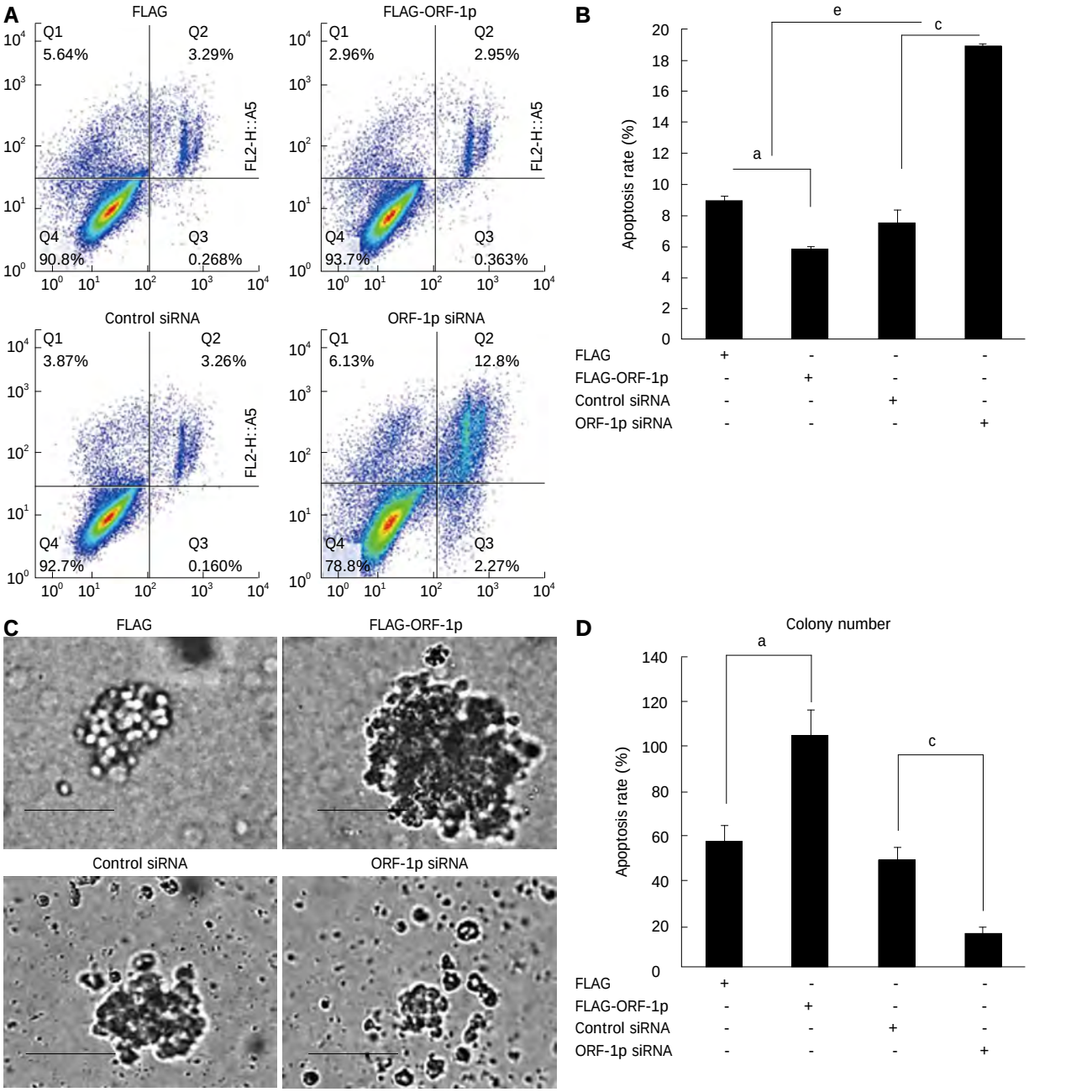


Figure 3 Long interspersed nuclear element ORF-1 protein promotes apoptosis and anchor-independent growth of HepG2 cells. HepG2 cells were transfected with the empty vector or f ludarabine + high-dose cytarabine + G-CSF human long interspersed nuclear element-1 (FLAG-LINE-1) ORF-1p, or with the control siRNA or LINE-1 ORF-1p siRNA. A, B: The rates of apoptosis are shown in the figures (A). The assays were performed three times with similar results (B); C, D: Colony numbers shown in the photographs (C), or mean ± SD (n = 3) of triplicate independent experiments (D). $^{a}P < 0.05$ *vs* FLAG; $^{c}P < 0.05$ *vs* control siRNA; $^{e}P < 0.05$ *vs* FLAG-LINE-1 ORF-1p. GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

gether, these results suggest that LINE-1 ORF-1p may regulate cell proliferation by modulating the transcription activities of these genes

LINE ORF-1 protein modulates the expression of genes involved in cell proliferation and apoptosis

To corroborate the results of the luciferase assay, it is necessary to investigate whether LINE-1 ORF-1p regulates the protein levels of the genes related to cell proliferation and apoptosis. The results showed that overexpression of LINE-1 ORF-1p decreased the expression of *p15*, *p53* and *p27* genes (Figure 5A) and increased the expression of Bcl-2 (Figure 5A). However, reduction of LINE-1 ORF-1p expression with LINE-1 ORF1-p siRNA increased the protein level of *p15*, *p53* and *p27* (Figure 5B), and reduced the protein level of Bcl-2 (Figure 5B). These findings indicated that LINE-1 ORF-1p promotes cell proliferation by regulating the expression of these genes.

LINE ORF-1 protein enhances MDR and p-gp gene expression

In order to investigate the mechanism of LINE-1 ORF-1p-mediated drug resistance, the effects of LINE-1

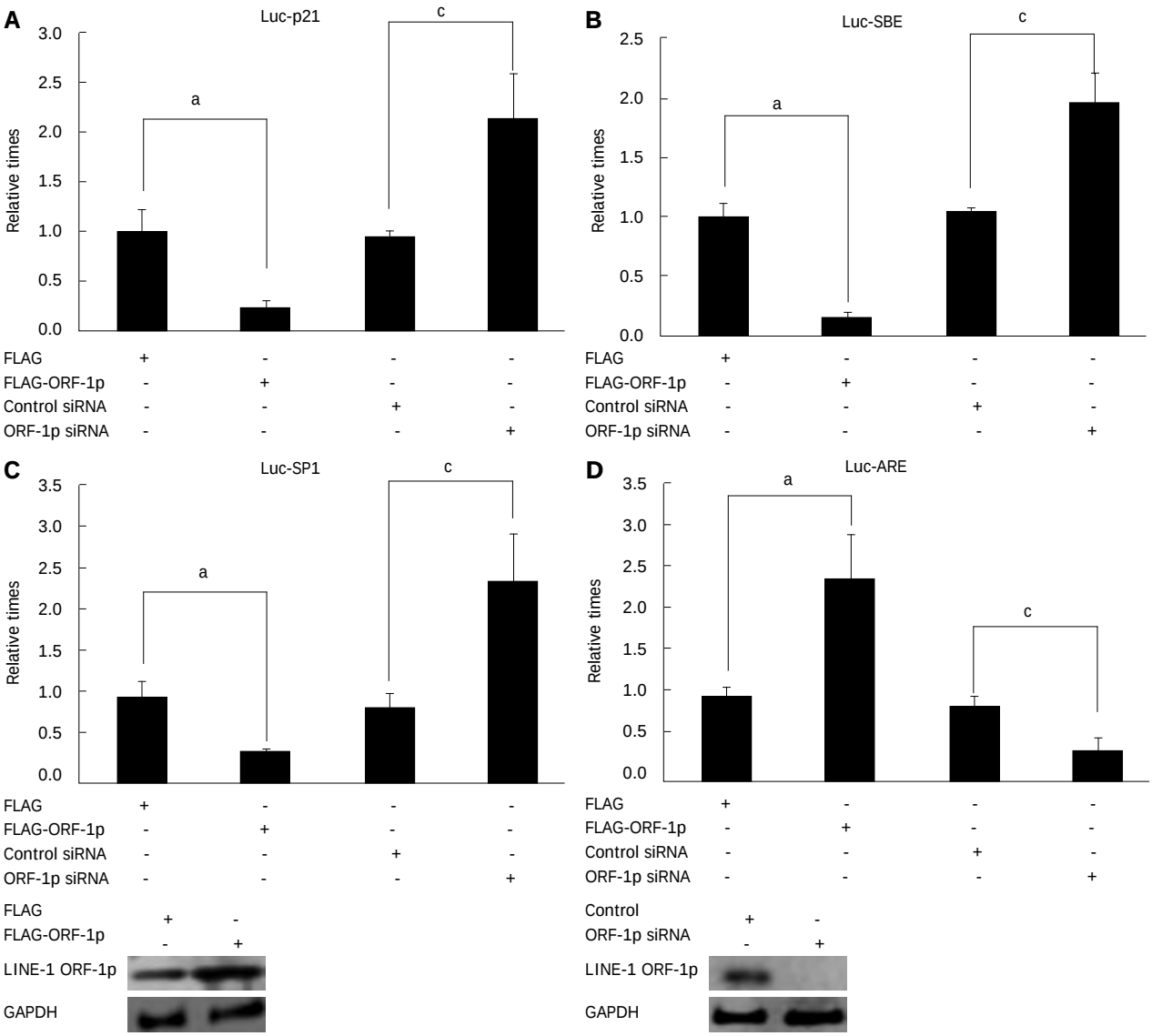


Figure 4 Long interspersed nuclear element ORF-1 protein regulates the transcription of Luciferase reporters. HepG2 cells were transfected with the fludarabine + high-dose cytarabine + G-CSF human long interspersed nuclear element-1 (FLAG-LINE-1) ORF-1p or FLAG empty vector, LINE-1 ORF-1p siRNA or control siRNA. HepG2 cells were co-transfected with Luc-p21 (A), Luc-SBE (B), Luc-Sp1 (C), or Luc-ARE (D). The assays were performed three times with similar results. [a]*P* < 0.05 *vs* FLAG; [c]*P* < 0.05 *vs* control siRNA. GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

ORF-1p on the expression of genes involved in drug resistance were examined. The results showed that over-expression of LINE-1 ORF1-p increased MDR and p-gp protein levels (Figure 6A). Repression of LINE-1 ORF-1p expression through siRNA strongly reduced MDR and p-gp protein levels (Figure 6B). These results indicated that LINE-1 ORF-1p mediates drug resistance by modulating the expression of MDR and p-gp.

LINE ORF-1 protein is expressed in clinical specimens and HepG2 cells

Next, we used immunohistochemistry and immunoblot assays to detect LINE-1 ORF-1p protein expression in clinical specimens and the HepG2 cell line. In most cases, the HepG2 cell line and liver tumors expressed high protein levels of LINE-1 ORF-1p (Figure 7).

DISCUSSION

In this study, we provide evidence of the novel roles of LINE-1 ORF-1p in chemotherapeutic drug response and proliferation of HepG2 cells. The results demonstrated that over-expreesion of LINE-1 ORF-1p significantly inhibited the cytotoxic effects of epirubicin and cisplatin on HepG2 cells. However, knockdown of LINE-1 ORF-1p with epirubicin, cisplatin or paclitaxel had a synergistic effect on HepG2 cells. Moreover, LINE-1 ORF-1p regulated the proliferation and anchor-independent growth of HepG2 cells. In addition, LINE-1 ORF-1p repressed Luc-SBE, Luc-SP1, and Luc-p21 transcription, and enhanced Luc-ARE transcription. More importantly, LINE-1 ORF-1p modulated the expression of important genes: *p15*, *p27*, *p53* and *Bcl-2*, which are related to

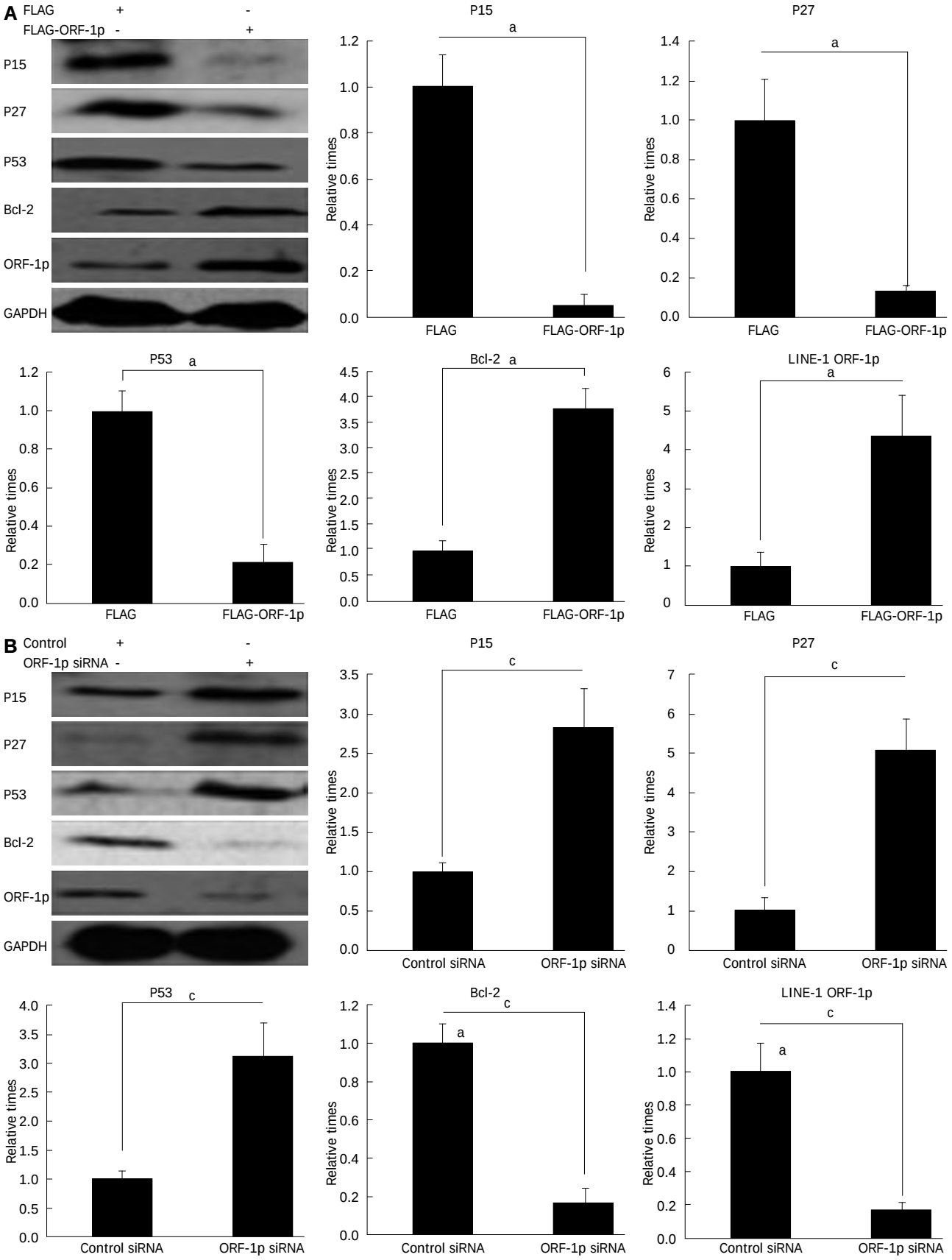


Figure 5 Long interspersed nuclear element ORF-1 protein modulates the expression of genes related to cell proliferation and apoptosis regulation. A: HepG2 cells were transfected with the fludarabine + high-dose cytarabine + G-CSF human long interspersed nuclear element-1 (FLAG-LINE-1) ORF-1p vector, or the empty vector. The assays were performed three times with similar results. [a]*P* < 0.05 *vs* FLAG; B: HepG2 cells were transfected with control siRNA or LINE-1 ORF-1p siRNA. The bar graph shows the relative protein expression level of each protein compared with GAPDH. The assays were performed three times with similar results. [a]*P* < 0.05 *vs* FLAG; [c]*P* < 0.05 *vs* control siRNA. GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

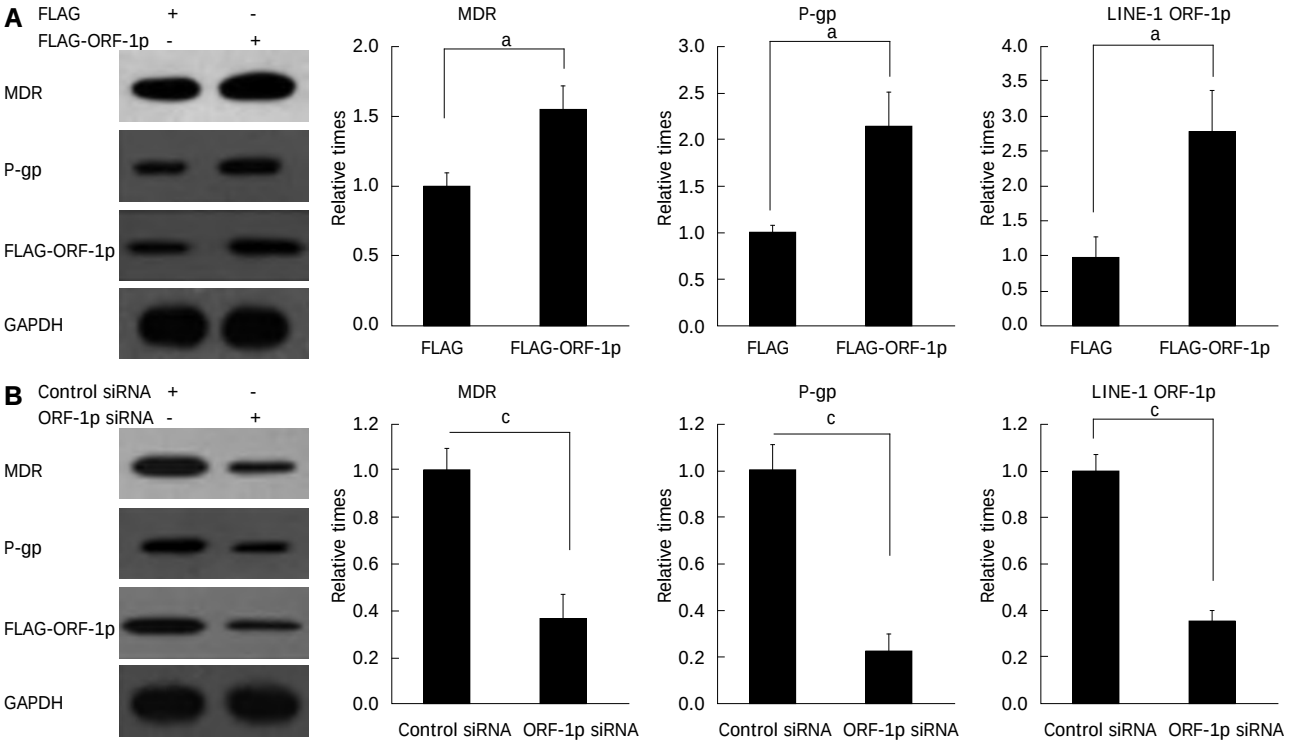


Figure 6 Long interspersed nuclear element ORF-1 protein modulates the expression of genes related to drug resistance. A: HepG2 cells were transfected with the fludarabine + high-dose cytarabine + G-CSF human long interspersed nuclear element-1 (FLAG-LINE-1) ORF-1p vector, or the empty vector; B: HepG2 cells were transfected with control siRNA or LINE-1 ORF-1p siRNA. The bar graph shows the relative protein expression level of each protein compared with GAPDH. The assays were performed three times with similar results. $^{a}P < 0.05$ *vs* FLAG; $^{c}P < 0.05$ *vs* control siRNA. MDR: Multidrug resistance; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

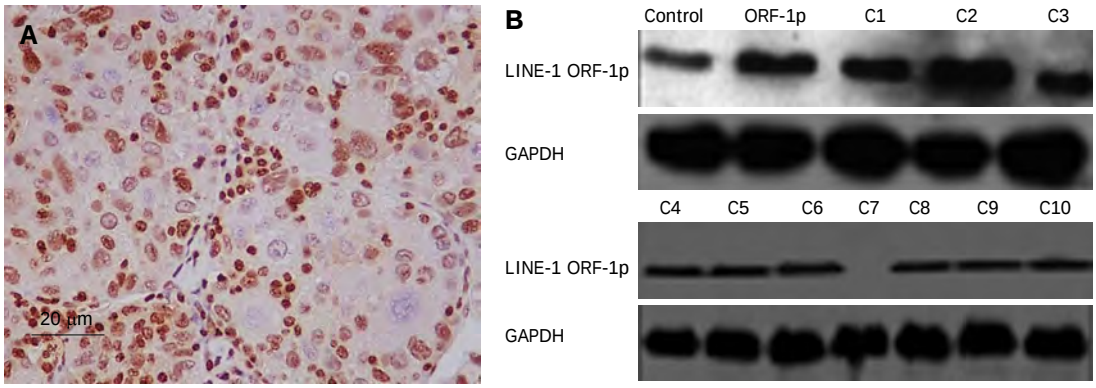


Figure 7 Long interspersed nuclear element ORF-1 protein expressed in clinical specimens and HepG2 cells. Representative immunohistochemical staining (A) and immunoblots (B) of human long interspersed nuclear element-1 (LINE-1) ORF-1p proteins in HepG2 cells, which were stably transfected with empty vector or LINE-1 expression vector, and in human cancerous liver tissues (C1-C10). Original magnification: × 40. GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

HepG2 cell proliferation and apoptosis. Finally, LINE-1 ORF-1p also affected *mdr* and *p-gp* gene expression, which is related to chemotherapeutic drug resistance in cancer cells.

LINE-1 comprises at least 17% of the human genome sequence and may significantly affect human genome stability and chromosome structure after de-methylation. LINE-1 displays a silenced state in normal adult tissues with high methylation[8]. It is activated by the de-methylation mechanism, and then frequently retrotransposes[9]. Frequent transposition of LINE-1 may lead to significant changes in the genome, eventually inducing tumorigenesis[10]. It was reported that genome hypomethylation was significantly increased in patients with HCC. The levels of serum LINE-1 hypomethylation at initial presentation correlated significantly with tumor size, tumor number and alpha-fetoprotein level. Moreover, high serum LINE-1 hypomethylation correlates significantly with poor survival[11,12]. Therefore, it is thought that overexpression of LINE-1-associated protein promotes the progression of HCC and affects the outcome of systemic chemotherapy.

Our previous studies indicated that LINE-1 ORF-1p was involved in the regulation of transformation, devel-

opment and proliferation of several cancer cells[13,14]. We also established several tumor cell models of LINE-1 ORF-1p in human lung cancer, gastric cancer and breast cancer[15]. It is reported that LINE-1 ORF-1p interacts with its own DNA[16]. Thus, LINE-1ORF-1p may regulate important transcription factor activity by changing the structure of chromatin, and in turn promote the growth of cancer cells by modulating the expression of genes related to proliferation, cell cycling and apoptosis regulation[17-19]. LINE-1 ORF-1p may play a role in the regulation of cancer cell proliferation and chemotherapy resistance through various mechanisms.

In this study, we found that overexpression of LINE-1 ORF-1p significantly inhibited the cytotoxic effect of epirubicin and cisplatin on HepG2 cells, without affecting the role of paclitaxel, these results were expected. LINE-1 ORF-1p siRNA with epirubicin, cisplatin or paclitaxel had a synergistic effect on HepG2 cells. In HCC, the rapid growth of cells is caused by high levels of DNA replication and expression, and a high rate of metabolism. In addition, in HCC cells, chromatins are generally unfolded and not agglutinated[17-19]. Therefore, the DNA is more vulnerable than in normal somatic cells[12]. Some chemotherapeutic agents such as cisplatin and epirubicin target bio-macromolecules, such as DNA, and are naturally cytotoxic to HepG2 cells[12]. While LINE-1 ORF-1p binds to DNA, it is likely to play a protective role and act as a barrier to DNA. It is possible to protect DNA against the cytotoxic effects of epirubicin and cisplatin. Our previous study showed that LINE-1 ORF-1p promotes the cell cycling program. These previous results also confirmed the conclusions in this study. In addition, LINE-1 ORF-1p enhanced the protein level of *mdr* and *p-gp* genes[20-22]. Thus, LINE-1 ORF-1p also modulates the genes involved in multi-drug resistance.

We then found that LINE-1 ORF-1p promoted *Bcl-2* gene expression which is involved in resistance to apoptosis, and inhibited the expression of the genes, *p15*, *p27* and *p53*[13]. These genes promote apoptosis and arrest cell growth or cell cycling. These results were consistent with those from the luciferase assays. LINE-1 ORF-1p reduced the activity of Luc-p21, Luc-SBE, and Luc-Sp1 reporters and enhanced Luc-ARE reporter transcription. p53, Smad4, Sp1 and AR are important transcription factors, which modulate the transcription of many genes and play a role in the regulation of cancer progression. LINE-1 ORF-1p may interact with these transcriptional factors which are recruited to specific regulatory elements such as SP1 and p53 binding sites. They then interfere with their function through conformational alteration or by affecting their co-repressor recruitment. Further studies are required to address this hypothesis.

Paclitaxel targets microtubules. Overexpression of LINE-1 ORF-1p did not antagonize the cytotoxic effect of paclitaxel, however, knockdown of LINE-1 ORF-1p and paclitaxel had a synergistic effect and reduced its expression level and slowed cell growth by modulating *p53*, *p15*, *p27*, and *Bcl-2* expression.

Taken together, these findings suggest that LINE-1 ORF-1p may have a role in the development of HCC, and is likely to be involved in the prediction of chemotherapeutic effect. In addition, these findings significantly advance our understanding of the functions and mechanisms of LINE-1 ORF-1p in cancer cell proliferation and may provide a novel potential therapeutic target in HCC.

COMMENTS

Background

Hepatocellular carcinoma (HCC) is the commonest form of cancer worldwide, and the third most common cause of cancer death. Chemotherapy is currently the most commonly adopted strategy for treating HCC patients; however, it also has shortcomings as HCC patients can exhibit robust multidrug resistance.

Research frontiers

Long interspersed nuclear element-1 ORF-1 protein (LINE-1 ORF-1p) plays an important role in the development of several types of carcinoma. However, the involvement of LINE-1 ORF-1p in the pathogenesis of hepatocellular carcinoma and whether it is responsible for making these tumors resistant to chemotherapy has not been addressed. In this study, the authors demonstrate that the overexpression of LINE-1 ORF-1p, commonly observed in HCC patients, could be a potential mechanism for both promoting cell proliferation and for resistance to chemotherapy.

Innovations and breakthroughs

Recent reports have highlighted the importance of LINE-1 ORF-1p in different types of cancers. This is the first study to report that LINE-1 ORF-1p mediates resistance to chemotherapy in HCC patients. Furthermore, our *in vitro* studies suggest that LINE-1 ORF-1p mediates the regulation of cellular apoptotic and proliferative pathways, and aids in the metastatic progression of HCC.

Applications

By understanding the mechanism of LINE-1 ORF-1p-mediated induction of resistance to chemotherapy, this may represent a future strategy for therapeutic intervention in the treatment of patients with hepatocellular carcinoma. In addition, the expression levels of LINE-1 ORF-1p in hepatocellular carcinoma patients may serve as a predictive marker of chemotherapy outcome.

Terminology

LINE-1 is an autonomous non-LTR retrotransposon in mammals. Retrotransposition requires the function of the two L1-encoded polypeptides, ORF1p and ORF2p. LINE-1 ORF-1p, which comprises at least 17% of the human genome sequence, is silenced (non-active) in normal adult tissues. However, in cancer cells, LINE-1 ORF-1p is active and affects genomic stability and chromosome structure through its retrotransposonal activity, eventually leading to tumorigenesis.

Peer review

In this work, the authors investigated the roles of LINE-1 ORF-1p in the drug resistance and proliferation of HCC with interesting findings.

REFERENCES

1 **Guo XL**, Li D, Hu F, Song JR, Zhang SS, Deng WJ, Sun K, Zhao QD, Xie XQ, Song YJ, Wu MC, Wei LX. Targeting autophagy potentiates chemotherapy-induced apoptosis and proliferation inhibition in hepatocarcinoma cells. *Cancer Lett* 2012; **320**: 171-179 [PMID: 22406827 DOI: 10.1016/j.canlet.2012.03.002]

2 **Lee JH**, Chung YH, Kim JA, Shim JH, Lee D, Lee HC, Shin ES, Yoon JH, Kim BI, Bae SH, Koh KC, Park NH. Genetic predisposition of hand-foot skin reaction after sorafenib therapy in patients with hepatocellular carcinoma. *Cancer* 2013; **119**: 136-142 [PMID: 22736425 DOI: 10.1002/cncr.27705]

3 **Sauer G**, Kafka A, Grundmann R, Kreienberg R, Zeillinger R, Deissler H. Basal expression of the multidrug resistance gene 1 (MDR-1) is associated with the TT genotype at the polymorphic site C3435T in mammary and ovarian carcinoma cell lines. *Cancer Lett* 2002; **185**: 79-85 [PMID: 12142082 DOI: 10.1016/S0304-3835(02)00232-X]

4 **Goodier JL**, Zhang L, Vetter MR, Kazazian HH. LINE-1 ORF1 protein localizes in stress granules with other RNA-binding proteins, including components of RNA interference RNA-induced silencing complex. *Mol Cell Biol* 2007; **27**: 6469-6483 [PMID: 17562864 DOI: 10.1128/MCB.00332-07]

5 **Evans JD**, Peddigari S, Chaurasiya KR, Williams MC, Martin SL. Paired mutations abolish and restore the balanced annealing and melting activities of ORF1p that are required for LINE-1 retrotransposition. *Nucleic Acids Res* 2011; **39**: 5611-5621 [PMID: 21441536 DOI: 10.1093/nar/gkr171]

6 **Gao XD,** Hu M, Zhu Y. The down-regulation of LINE-1 encoded ORF-1p impacts the characters of A549 cells. *Zhongguo Shengwu Kexue Zazhi* 2010; **30**: 14-20

7 **Fan F,** Gao XD, Lu YY, Zhang F, Wang B, Chang XJ, Qu JH, Wang CP, Yang YP. Regulatory effect of LINE-1 ORF-1p on hepatocellular carcinoma cells and proliferation of immortalized hepatocellular cells. *Jiefangjun Yixue Zazhi* 2012; **37**: 213-216

8 **Sunami E**, de Maat M, Vu A, Turner RR, Hoon DS. LINE-1 hypomethylation during primary colon cancer progression. *PLoS One* 2011; **6**: e18884 [PMID: 21533144 DOI: 10.1371/journal.pone.0018884]

9 **Beck CR**, Garcia-Perez JL, Badge RM, Moran JV. LINE-1 elements in structural variation and disease. *Annu Rev Genomics Hum Genet* 2011; **12**: 187-215 [PMID: 21801021 DOI: 10.1146/annurev-genom-082509-141802]

10 **Ohka F**, Natsume A, Motomura K, Kishida Y, Kondo Y, Abe T, Nakasu Y, Namba H, Wakai K, Fukui T, Momota H, Iwami K, Kinjo S, Ito M, Fujii M, Wakabayashi T. The global DNA methylation surrogate LINE-1 methylation is correlated with MGMT promoter methylation and is a better prognostic factor for glioma. *PLoS One* 2011; **6**: e23332 [PMID: 21829728 DOI: 10.1371/journal.pone.0023332]

11 **Ramzy II,** Omran DA, Hamad O, Shaker O, Abboud A. Evaluation of serum LINE-1 hypomethylation as a prognostic marker for hepatocellular carcinoma. *Arab J Gastroenterol* 2011; **12**: 139-142 [PMID: 22055592]

12 **Hu MM,** Zhu YF, Wang Y, Wang Y, Zhang L, Gao XD, Dong J, Yu JY. Prokaryotic expression of LINE-1 encoded protein LINE-1 ORF-1p and preparation of anti-LINE-1 ORF-1p antibody. *Zhongguo Shengwu Kexue Zazhi* 2010; **30**: 7-10

13 **Pivonková H**, Pecinka P, Cesková P, Fojta M. DNA modification with cisplatin affects sequence-specific DNA binding of p53 and p73 proteins in a target site-dependent manner. *FEBS J* 2006; **273**: 4693-4706 [PMID: 16981908 DOI: 10.1111/j.1742-4658.2006.05472.x]

14 **Gao XD**. Master degree thesis, Academy of Military Medical Science, PR China, 2010

15 **Gao XD,** Lu YY, Feng F, Wang CP, Wang H, Chang XJ, Yang YP . Effects of LINE-1 ORF-1p overexpression on proliferation and anchor- independent growth of SMMC7721 hepatoma cell line. *Shiyong Ganzangbing Zazhi* 2011; **14**: 323-326

16 **Chanel-Vos C**, Giannakakou P. CENP-E checks in microtubule-drug resistance. *Cell Cycle* 2010; **9**: 1456 [PMID: 20421712 DOI: 10.4161/cc.9.8.11382]

17 **Yan J**, Fang Y, Ding L, Zhu J, Lu Q, Huang C, Yang X, Ye Q. Regulation of large-scale chromatin unfolding by Smad4. *Biochem Biophys Res Commun* 2004; **315**: 330-335 [PMID: 14766211 DOI: 10.1016/j.bbrc.2004.01.051]

18 **Zhang F**, Feng F, Yang PX, Li ZJ, You JH, Xie WX, Gao XD, Yang JL. Four-and-a-half-LIM protein 1 down-regulates estrogen receptor α activity through repression of AKT phosphorylation in human breast cancer cell. *Int J Biochem Cell Biol* 2012; **44**: 320-326 [PMID: 22094188]

19 **Tolomeo M**, Grimaudo S, Di Cristina A, Roberti M, Pizzirani D, Meli M, Dusonchet L, Gebbia N, Abbadessa V, Crosta L, Barucchello R, Grisolia G, Invidiata F, Simoni D. Pterostilbene and 3′-hydroxypterostilbene are effective apoptosis-inducing agents in MDR and BCR-ABL-expressing leukemia cells. *Int J Biochem Cell Biol* 2005; **37**: 1709-1726 [PMID: 15878840 DOI: 10.1016/j.biocel.2005.03.004]

20 **Qin Q**, Chen XP, Yang ZS, Xiao YH, Min H, Li YJ. Neferine increases STI571 chemosensitivity via inhibition of P-gp expression in STI571-resistant K562 cells. *Leuk Lymphoma* 2011; **52**: 694-700 [PMID: 21261505 DOI: 10.3109/10428194.2010.550075]

21 **Choi WI**, Jeon BN, Yun CO, Kim PH, Kim SE, Choi KY, Kim SH, Hur MW. Proto-oncogene FBI-1 represses transcription of p21CIP1 by inhibition of transcription activation by p53 and Sp1. *J Biol Chem* 2009; **284**: 12633-12644 [PMID: 19244234 DOI: 10.1074/jbc.M809794200]

22 **Cui J**, Germer K, Wu T, Wang J, Luo J, Wang SC, Wang Q, Zhang X. Cross-talk between HER2 and MED1 regulates tamoxifen resistance of human breast cancer cells. *Cancer Res* 2012; **72**: 5625-5634 [PMID: 22964581 DOI: 10.1158/0008-5472.CAN-12-1305]

P- Reviewer Tong QS **S- Editor** Zhai HH
L- Editor Ma JY **E- Editor** Lu YJ



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i7.1079

World J Gastroenterol 2013 February 21; 19(7): 1079-1084
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Papillary thyroid cancer and inflammatory bowel disease: Is there a relationship?

Irene S Sonu, Wojciech Blonski, Ming Valerie Lin, James Lewis, Faten Aberra, Gary R Lichtenstein

Irene S Sonu, Division of Internal Medicine, Stanford University Hospital and Clinics, Stanford, CA 94305, United States
Wojciech Blonski, Division of Internal Medicine, UHS Wilson Medical Center, Johnson City, NY 13790, United States
Ming Valerie Lin, Division of Digestive Diseases, University of Cincinnati, Cincinnati, OH 45229, United States
James Lewis, Faten Aberra, Gary R Lichtenstein, Division of Gastroenterology, Hospital of the University of Pennsylvania, University of Pennsylvania School of Medicine, Philadelphia, PA 19103, United States
Author contributions: Sonu IS was responsible for the study concept and design, acquisition of data, analysis and interpretation of data and drafting of the manuscript; Blonski W was responsible for the study concept and design, drafting of the manuscript, and critical revision of the manuscript for important intellectual content; Lin MV was responsible for the study concept and design and critical revision of the manuscript for important intellectual content; Lewis J and Aberra F were responsible for study concept and design, statistical analysis, and critical revision of the manuscript for important intellectual content; Lichtenstein GR was responsible for the study concept and design, critical revision of the manuscript for important intellectual content, study supervision, and guarantor of the manuscript.
Correspondence to: Gary R Lichtenstein, MD, Professor of Medicine, Division of Gastroenterology, Hospital of the University of Pennsylvania, University of Pennsylvania School of Medicine, 9th Floor Penn Tower, One Convention Avenue, Philadelphia, PA 19103, United States. grl@uphs.upenn.edu
Telephone: +1-215-3498222 Fax: +1-215-3495915
Received: July 1, 2012 Revised: September 28, 2012
Accepted: October 22, 2012
Published online: February 21, 2013

Abstract

AIM: To formally study age of diagnosis of papillary thyroid cancer (PTC) in inflammatory bowel disease (IBD) patients and evaluate the prevalence of PTC in IBD patients compared to a control population.

METHODS: We were interested in testing the hypothesis that patients with IBD are more likely to be diagnosed with PTC than a control population. A retrospective cohort analysis was performed using the University of Pennsylvania Health System's electronic database. Outpatients from 1998-2009 were included in the search, and patients in the cohort were selected based on ICD-9 codes. Inclusion criteria included the diagnosis of Crohn's disease (CD) or ulcerative colitis (UC) and the concurrent diagnosis of thyroid cancer in comparison to a control population. Using these methods 912 patients with CD and 1774 with UC were compared to 1638 diverticulitis and 19 447 asthma controls. Statistics were performed using corrected chi-square analysis. The primary outcome for this study was the diagnosis of PTC. Approval to conduct this study was obtained by the Institutional Review Board at the University of Pennsylvania.

RESULTS: The mean age was 47.5 years (range: 18-102 years) and 66% patients were female. An analysis of variance model was used to compare the age of PTC diagnosis between the CD, UC, asthma and diverticulitis groups, and a statistically significant difference in age at PTC diagnosis was noted across all groups (F = 6.35, df = 3, P = 0.0006). The age of PTC diagnosis in CD patients was statistically significantly lower than UC, asthma, and diverticulitis patients (average PTC diagnosis age for CD 25, UC 49, asthma 45, diverticulitis 63). After covarying for sex and age in 2009, the difference in age at PTC diagnosis remained statistically significant (F = 4.13, df = 3, P = 0.0089). A total of 86 patients were diagnosed with PTC. Nine patients (0.5%) with UC were diagnosed with PTC. Patients with UC were not shown to be more likely to develop PTC [odds ratio (OR): 1.544, 95%CI 0.767-3.108] compared to asthma controls. Four patients (0.4%) with CD were diagnosed with PTC. Patients with CD were not shown to be more likely to develop PTC (OR: 1.334, 95%CI 0.485-3.672) compared to a control population with asthma. Nine patients (0.5%) with a history of diverticulitis were diagnosed with PTC. Pa-

tients with diverticulitis were not shown to be more likely to develop PTC (OR: 1.673, 95%CI 0.831-3.368) compared to asthma controls. Patients with CD or UC were not less likely to develop PTC compared to those with diverticulitis (CD OR: 0.80, 95%CI 0.25-2.60; UC OR: 0.92, 95%CI 0.37-2.33). None of the patients used immunosuppressant medications prior to the diagnosis of PTC (azathioprine, 6-mercaptopurine, and methotrexate).

CONCLUSION: There is a significant difference in age of diagnosis of PTC in patients with CD compared to patients with UC and the control populations studied.

© 2013 Baishideng. All rights reserved.

Key words: Papillary thyroid cancer; Inflammatory bowel disease; Crohn disease; Ulcerative colitis

Sonu IS, Blonski W, Lin MV, Lewis J, Aberra F, Lichtenstein GR. Papillary thyroid cancer and inflammatory bowel disease: Is there a relationship? *World J Gastroenterol* 2013; 19(7): 1079-1084 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i7/1079.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i7.1079

INTRODUCTION

Inflammatory bowel diseases (IBD), specifically Crohn's disease (CD) and ulcerative colitis (UC), are idiopathic chronic inflammatory disorders resulting from defects in the barrier function of the intestinal epithelium and inappropriate activation of the mucosal immune system. IBD is also associated with several other extra-intestinal disease states, including ankylosing spondylitis, arthritis, pyoderma gangrenosum, and uveitis[1]. Less is known about the association of IBD with diseases of the thyroid.

Early studies have suggested a relationship between thyroid abnormalities and IBD[2]. For example, CD has been associated with Graves' disease and Hashimoto' s thyroiditis[3]. However, the association with IBD and thyroid cancer has been less critically analyzed. Thyroid cancer is the most common endocrine cancer, with over 80% of all cases consisting of the papillary type. It is more common in women than in men (3:1 ratio) and is more common in whites than in African-Americans. The mean age of diagnosis is 40 years old[4]. There are several case reports of thyroid cancer in patients with IBD, two of them involving UC[5,6]. Moss *et al*[7] described five cases of papillary thyroid cancer (PTC) in patients with preexisting CD. Despite these anecdotal published findings, there have been no formal studies evaluating the relationship that exists between PTC and IBD in a population cohort study.

It is uncertain whether PTC in patients with IBD is due to an association or coincidence. Knowledge of a relationship is important in order to determine the factors that link IBD and PTC. Exposure to radiation and multivitamin intake are both considered risk factors for PTC, both of which are common in patients with IBD due to diagnostic studies and malnutrition respectively[8]. However, a recent study by Peloquin *et al*[9] concluded that the radiation exposure in an IBD population was equivalent to the average annual background radiation dose from naturally occurring sources in the United States, while a smaller subset of patients had substantially higher doses. In addition, immunosuppressants such as azathioprine and 6-mercaptopurine are commonly taken by patients with IBD to induce remission and can increase the risk of malignancy, specifically lymphoproliferative disorders[10,11]. Whether these medications increase the risk of thyroid cancer is unknown.

It is already established that an inappropriate inflammatory response results in IBD. Defects in genes involving the innate immune system and activation of inflammatory cytokines such as NOD2, autophagy-related 16-like 1, and interleukin-23 lead to intestinal inflammation[12,13]. Similarly, lymphocytic infiltration is seen in PTC, suggesting that immunologic factors may contribute to neoplastic development[14]. Thus, the inflammatory modulators involved in the pathogenesis of IBD may have an effect on PTC development.

This study addressed an important question regarding the epidemiology of IBD and PTC. The specific aim of this study was to identify any relationships between the two diseases and to determine whether patients with IBD have a higher prevalence of PTC than a control population. Knowledge of an association if present has potential to impact the management of patients with IBD, which may warrant screening for thyroid cancer through regular thyroid examinations.

MATERIALS AND METHODS

Definition of the cohort

A retrospective cohort study from within the University of Pennsylvania Health System was performed using the data from the electronic health system database (EPIC Hyperspace Summer 2009 IU 6, EPIC Systems Corporation, Verona, Wisconsin, United States). Patients who were seen as outpatients in our health system from 1998 to 2009 were included in the search. Patients with CD and UC were identified based on ICD-9 codes (ICD-9 codes for CD: 555, 550.0, 555.1, 555.9; codes for UC: 556, 556.0, 556.1, 556.2, 556.3, 556.5, 556.6, 556.8, 556.9).

Inclusion criteria included the diagnosis of CD or UC and the concurrent diagnosis of thyroid cancer. The latter diagnosis was identified using the ICD-9 code for thyroid cancer: 193. The papillary type of thyroid cancer was verified using biopsy reports or the patients' medical records. Patients who met inclusion criteria were included in the final analysis and were compared to patients with inflammatory controls with diverticulitis and asthma. Controls were identified based on a search of our electronic database using ICD-9 codes (ICD-9 code

Table 1 Concomitant diagnosis of papillary thyroid cancer

Diagnosis	No. of patients diagnosed with PTC	Unadjusted OR (95%CI) compared to asthma controls	OR (95%CI) compared to asthma controls, adjusted for sex and age in 2009
CD	4	1.334 (0.485-3.672)	1.510 (0.548-4.162)
UC	9	1.544 (0.767-3.108)	1.679 (0.832-3.389)
Diverticulitis	9	1.673 (0.831-3.368)	1.338 (0.644-2.779)
Asthma	64	NA	NA

OR: Odds ratio; PTC: Papillary thyroid cancer; NA: Not available.

for diverticulitis: 562.11, 562.13; code for asthma: 493). Approval was obtained from the Institutional Review Board at the University of Pennsylvania to perform our study.

Definition of outcome

The primary outcome for this study was a diagnosis of PTC. We did not limit our outcome definition to an incident diagnosis of PTC following a diagnosis of IBD. We were interested in testing the hypothesis that patients with IBD are more likely to be diagnosed with PTC than a control population, not that IBD in and of itself predisposes patients to PTC. As such, we searched for any diagnosis of PTC at any time in the patients' records.

Statistical analysis

All analyses were performed separately for patients with CD and UC, and then as a combined IBD group. Analyses began with descriptive statistics. Continuous variables were reported as means ± SD. Categorical variables were reported as counts and percentages. Since age was approximately normally distributed, we used analysis of variance to test for differences in the mean age at diagnosis of PTC[15]. Statistical analysis was performed at the University of Pennsylvania, Philadelphia, PA using SAS software version 9.2 (SAS Institute, Cary, NC).

To estimate the association between PTC and IBD in this cross-sectional study, we calculated the odds ratio (OR) and 95%CI using logistic regression. We considered a finding to be statistically significance when $P < 0.05$.

Sample size and power calculations

A two group continuity corrected χ^2 test with a 0.050 two-sided significance level had 80% power to detect the difference between a proportion of 0.3% in the asthma group and a proportion of 0.8% in the UC group (OR of 2.41) when the sample sizes are 19 447 and 1774, respectively (a total sample size of 21 221). A two group continuity corrected χ^2 test with a 0.050 two-sided significance level had 80% power to detect the difference between a proportion of 0.3% in the asthma group and a proportion of 1.0% in the UC group (OR of 3.02) when the sample sizes are 19 447 and 912, respectively (a total sample size of 20 359).

RESULTS

Our retrospective analysis yielded a total of 2686 patients who were diagnosed with either CD or UC. A total of 912 patients with CD and 1774 patients with UC met inclusion criteria and were included in our analysis. These patients were compared to 1638 diverticulitis and 19447 asthma controls.

The overall mean age was 47.5 years (range: 18-102 years) and 66% patients were female. Using an analysis of variance model to compare the age of thyroid diagnosis between the CD, UC, asthma and diverticulitis groups, a statistically significant difference in age at PTC diagnosis was noted across all groups ($F = 6.35$, df = 3, $P = 0.0006$). Of note, the age of PTC diagnosis in CD patients was statistically significantly lower than UC, asthma, and diverticulitis patients (average PTC diagnosis age for CD: 25 years, UC 49 years, asthma: 45 years, diverticulitis: 63 years). After covarying for sex and age in 2009, the difference in age at PTC diagnosis remained statistically significant ($F = 4.13$, df = 3, $P = 0.0089$).

A total of 86 patients were diagnosed with PTC. Nine patients (0.5%) with UC were diagnosed with PTC. Patients with UC were not shown to be more likely to develop PTC (OR: 1.544, 95%CI 0.767-3.108) compared to asthma controls. Four patients (0.4%) with CD were diagnosed with PTC. Patients with CD were not shown to be more likely to develop PTC (OR: 1.334, 95%CI 0.485-3.672) compared to a control population with asthma. Nine patients (0.5%) with a history of diverticulitis were diagnosed with PTC. Patients with diverticulitis were not shown to be more likely to develop PTC (OR: 1.673, 95%CI 0.831-3.368) compared to asthma controls (Table 1).

Patients with CD or UC were not less likely to develop PTC compared to those with diverticulitis (CD OR: 0.80, 95%CI 0.25-2.60; UC OR: 0.92, 95%CI 0.37-2.33). None of the patients used immunosuppressant medications prior to the diagnosis of PTC (azathioprine, 6-mercaptopurine, and methotrexate).

DISCUSSION

The results of our present study highlights a statistically significant difference in age of diagnosis of PTC in patients with CD compared to patients with UC and the control populations studied. Patients with CD are diagnosed with PTC at a much younger age, possibly due to a unique pathogenetic mechanism of disease occurring in this subset of patients. It is established that CD results from a variety of factors, including genetic, environmental and immunological factors. An inappropriate inflammatory reaction is a key factor in the pathogenesis of CD. Similarly, PTC involves an inflammatory reaction, in particular, a lymphocytic infiltration of thyroid tissue[14]. Thus, overlapping immunologic factors/pathways may connect these two diseases together. The inflammatory modulators involved in the pathogenesis of IBD

may have an effect on PTC development or PTC may predispose patients to developing IBD.

In addition there have been specific genetic mutations described to be present in patients with CD. The initial frameshift mutation described was in the *NOD-2* gene in 2001[16]. Data from epidemiological studies, based on concordance data in family studies via linkage analysis to genomewide association studies, highlight evidence for over 30 distinct genomic loci involved in the genetic susceptibility to CD. These loci encode genes involved in a number of homeostatic mechanisms: innate pattern recognition receptors, the differentiation of Th17-lymphocytes, autophagy, maintenance of epithelial barrier integrity, and the orchestration of the secondary immune response[17] It is perceived that recognition of these loci will help to improve our understanding of the pathophysiology of CD.

Similarly, there have been several genetic mutations described in patients with PTC, the most common involving the RET/papillary thyroid carcinomas 1 (PTC1) and RET/PTC3 rearrangements which account for more than 90% of all genetic rearrangements found in PTC, and have been found in 30%-40% of adult patients with sporadic PTC in the United States, Italy, and Canada[18]. Other genetic mutations include the neurotrophic tyrosine kinase, receptor, type 1 (NTRK1) rearrangement, Ras kinases and Raf kinases[19-23]. In addition, recent literature has incited the presence of 7 gene regions to be associated with PTC[24]. These results suggest a possible role of genes involved in maintenance of genomic integrity in relation to risk of PTC.

Several studies determined significantly higher oncogenic rearrangements of RET and NTRK1 proto-oncogenes in patients with PTC that occurred primarily in children and young adults. An analysis of 92 patients with PTC determined that patients age 4-30 years had significantly higher frequency of *RET* or *NTRK1* gene compared to those 31-80 years (57% *vs* 32%, $P = 0.019$). Further, among patients ages 4-19 years, 67% of them displayed RET gene rearrangement[25]. Studies by Jhiang *et al*[26] and Soares *et al*[27] found that patients displaying RET rearrangement in their PTC had significantly lower mean age at diagnosis than those whose PTC did not present this rearrangement (32 years *vs* 50 years, $P < 0.05$ and 28 years *vs* 45 years, $P = 0.005$, respectively)[26,27]. An assessment of spontaneous PTC in 33 young patients ages 6-21 years showed that the RET/PTC1 rearrangement was common in the sporadic form of children with PTC (53%), with the RET/PTC3 mutation more commonly seen among children with PTC secondary to radiation exposure (67%-76%) as reported in prior studies[28-31].

One limitation to this study is the small number of patients who were found to have PTC. With so few patients with both diagnoses, it was difficult to elucidate a temporal relationship between the two diseases. Some patients were diagnosed with PTC followed by a CD diagnosis, and vice versa. A larger study would help establish whether there is a diagnosis pattern. The findings on prevalence do not suggest a higher prevalence of PTC in patients with IBD compared to the control populations studied. We cannot exclude the potential for a type Ⅱ error given that our study was underpowered. A study using a much larger cohort of patients would be better-powered and would more likely lead to statistically significant results and better define a lack of relationship between the two diseases.

There were several other limitations to this study. We did not test for confounding; we did not examine other thyroid conditions including Graves disease, Hashimoto's thyroiditis, goiter, and non-papillary type thyroid cancer as potential confounder variables. The other limitation of our study is that some relevant clinical information from the past medical history of patients may not have been included in the electronic database and thus may have been potentially omitted. This is a common limitation of retrospective studies.

In summary, the results of our study suggest that patients with CD who are also diagnosed with PTC are diagnosed at a much younger age than patients with UC and other controls. This is important for cancer detection in CD patients. Currently age, gender, radiation exposure, and a low iodine diet are the only known clinical risk factors for PTC; further studies are needed to establish whether patients with CD are at increased risk of developing PTC. If there is an increased risk, then more stringent methods of cancer screening may be warranted for CD patients, including initiating screening at a younger age.

COMMENTS

Background

Inflammatory bowel diseases (IBD), specifically Crohn's disease (CD) and ulcerative colitis (UC), are idiopathic chronic inflammatory disorders resulting from defects in the barrier function of the intestinal epithelium and inappropriate activation of the mucosal immune system. Early studies have suggested a relationship between thyroid abnormalities and IBD. For example, CD has been associated with Graves' disease and Hashimoto's thyroiditis. However, the association with IBD and thyroid cancer has been less critically analyzed. The aim of this study was to formerly study the age of diagnosis of papillary thyroid cancer (PTC) in IBD patients and evaluate the prevalence of PTC in IBD patients compared to a control population.

Research frontiers

This study addressed an important question regarding the epidemiology of IBD and PTC. The results suggest that CD patients are diagnosed with PTC at a much younger age, possibly due to a unique pathogenetic mechanism of disease occurs in this subset of patients.

Innovations and breakthroughs

The researchers drew a conclusion that there is a significant difference in age of diagnosis of PTC in patients with CD compared to patients with UC and the control populations studied. Patients with CD are diagnosed with PTC at a much younger age, possibly due to a unique pathogenetic mechanism of disease occurs in this subset of patients. It is established that CD results from a variety of factors, including genetic, environmental and immunological factors. An inappropriate inflammatory reaction is a key factor in the pathogenesis of CD. Similarly, PTC involves an inflammatory reaction, in particular, a lymphocytic infiltration of thyroid tissue. Thus, overlapping immunologic factors/pathways may connect these two diseases together. The inflammatory modulators involved in the pathogenesis of IBD may have an effect on PTC development or PTC may predispose patients to developing IBD.

Applications

This study answers important questions regarding the epidemiology of PTC and IBD, and also addresses the topic of cancer detection in CD patients. Currently age, gender, radiation exposure, and a low iodine diet are the only known clinical risk factors for PTC; further studies are needed to establish whether patients with CD are at increased risk of developing PTC. If there is an increased risk, then more stringent methods of cancer screening may be warranted for CD patients, including initiating screening at a younger age.

Peer review

The paper evaluated on a retrospective cohort study prevalence of PTC in IBD patients compared to a control population. This is a very interesting subject and there are many lacunae in the literature.

REFERENCES

1 **Stenson W.** Inflammatory Bowel Disease. In: Goldman L, Ausiello D, Arend W. Cecil Medicine. 23rd ed. Philadelphia: Saunders Elsevier Publications, 2008: 1042-1049

2 **Järnerot G**, Azad Khan AK, Truelove SC. The thyroid in ulverative colitis and Crohn's disease. II. Thyroid enlargement and hyperthyroidism in ulcerative colitis. *Acta Med Scand* 1975; **197**: 83-87 [PMID: 1124663]

3 **Inokuchi T**, Moriwaki Y, Takahashi S, Tsutsumi Z, KA T, Yamamoto T. Autoimmune thyroid disease (Graves' disease and hashimoto's thyroiditis) in two patients with Crohn's disease: case reports and literature review. *Intern Med* 2005; **44**: 303-306 [PMID: 15897640 DOI: 10.2169/internalmedicine.44.303]

4 **Carling T,** Udelsman R. Cancer of the Endocrine System. Thyroid Tumors. In: Devita V, Hellman S, Rosenberg S, editors. Cancer: Principles and Practice of Oncology. 7th ed. Philadephia: Lippincott Williams and Wilkins, 2005: 1663-1681

5 **Evgenikos N**, Stephen JG. Papillary thyroid carcinoma associated with ulcerative colitis. *Postgrad Med J* 1996; **72**: 621-622 [PMID: 8977948]

6 **Ginsberg GG,** Goodman ZD, Lewis JH. A 22-year-old man with thyroid cancer and cholestatic liver disease. *Semin Liver Dis* 1991; **11**: 64-71 [PMID: 2047892 DOI: 10.1055/s-2008-1040424]

7 **Moss AC**, Brennan AM, Cheifetz AS, Peppercorn MA. Thyroid cancer and Crohn's disease: association or coincidence? *Inflamm Bowel Dis* 2006; **12**: 79-80 [PMID: 16374265]

8 **Mack WJ**, Preston-Martin S, Bernstein L, Qian D. Lifestyle and other risk factors for thyroid cancer in Los Angeles County females. *Ann Epidemiol* 2002; **12**: 395-401 [PMID: 12160598 DOI: 10.1016/S1047-2797(01)00281-2]

9 **Peloquin JM**, Pardi DS, Sandborn WJ, Fletcher JG, McCollough CH, Schueler BA, Kofler JA, Enders FT, Achenbach SJ, Loftus EV. Diagnostic ionizing radiation exposure in a population-based cohort of patients with inflammatory bowel disease. *Am J Gastroenterol* 2008; **103**: 2015-2022 [PMID: 18564113 DOI: 10.1111/j.1572-0241.2008.01920.x]

10 **Kandiel A**, Fraser AG, Korelitz BI, Brensinger C, Lewis JD. Increased risk of lymphoma among inflammatory bowel disease patients treated with azathioprine and 6-mercaptopurine. *Gut* 2005; **54**: 1121-1125 [PMID: 16009685 DOI: 10.1136/gut.2004.049460]

11 **Kotlyar D,** Gisbert JP, Lewis JD, Brensinger CM, Beaugerie L, Blonski W, Hirten R, Van Domselaar M, Chaparro M, Sandilya S, Lichtenstein GR. A New Meta-Analysis of Overall Risk of Lymphoma in Patients With Inflammatory Bowel Disease on Thiopurine Therapy; Inclusion of the Eneida Population Study From Spain, and Differences Between Referral Center Studies and Population Based Studies. *Gastroenterology* 2011; **140:** S41-S42

12 **Cho JH**. The genetics and immunopathogenesis of inflammatory bowel disease. *Nat Rev Immunol* 2008; **8**: 458-466 [PMID: 18500230 DOI: 10.1038/nri2340]

13 **Rioux JD**, Xavier RJ, Taylor KD, Silverberg MS, Goyette P, Huett A, Green T, Kuballa P, Barmada MM, Datta LW, Shugart YY, Griffiths AM, Targan SR, Ippoliti AF, Bernard EJ, Mei L, Nicolae DL, Regueiro M, Schumm LP, Steinhart AH, Rotter JI, Duerr RH, Cho JH, Daly MJ, Brant SR. Genome-wide association study identifies new susceptibility loci for Crohn disease and implicates autophagy in disease pathogenesis. *Nat Genet* 2007; **39**: 596-604 [PMID: 17435756 DOI: 10.1038/ng2032]

14 **Kondo T**, Ezzat S, Asa SL. Pathogenetic mechanisms in thyroid follicular-cell neoplasia. *Nat Rev Cancer* 2006; **6**: 292-306 [PMID: 16557281 DOI: 10.1038/nrc1836]

15 **Armitage P,** Berry G. Statistical Methods in Medical Research. 2nd ed. Oxford: Blackwell Scientific Publications, 1987

16 **Ogura Y**, Bonen DK, Inohara N, Nicolae DL, Chen FF, Ramos R, Britton H, Moran T, Karaliuskas R, Duerr RH, Achkar JP, Brant SR, Bayless TM, Kirschner BS, Hanauer SB, Nuñez G, Cho JH. A frameshift mutation in NOD2 associated with susceptibility to Crohn's disease. *Nature* 2001; **411**: 603-606 [PMID: 11385577 DOI: 10.1038/35079114]

17 **Van Limbergen J**, Wilson DC, Satsangi J. The genetics of Crohn's disease. *Annu Rev Genomics Hum Genet* 2009; **10**: 89-116 [PMID: 19453248 DOI: 10.1146/annurev-genom-082908-150013]

18 **Nikiforov YE**. RET/PTC rearrangement in thyroid tumors. *Endocr Pathol* 2002; **13**: 3-16 [PMID: 12114746 DOI: 10.1385/EP]

19 **Brzeziańska E**, Pastuszak-Lewandoska D, Lewiński A. Rearrangements of NTRK1 oncogene in papillary thyroid carcinoma. *Neuro Endocrinol Lett* 2007; **28**: 221-229 [PMID: 17627253]

20 **Musholt TJ**, Musholt PB, Khaladj N, Schulz D, Scheumann GF, Klempnauer J. Prognostic significance of RET and NTRK1 rearrangements in sporadic papillary thyroid carcinoma. *Surgery* 2000; **128**: 984-993 [PMID: 11114633 DOI: 10.1067/msy.2000.110845]

21 **Pierotti MA**, Greco A. Oncogenic rearrangements of the NTRK1/NGF receptor. *Cancer Lett* 2006; **232**: 90-98 [PMID: 16242838 DOI: 10.1016/j.canlet.2005.07.043]

22 **Xing M**. BRAF mutation in thyroid cancer. *Endocr Relat Cancer* 2005; **12**: 245-262 [PMID: 15947100 DOI: 10.1677/erc.1.0978]

23 **Vasko V**, Ferrand M, Di Cristofaro J, Carayon P, Henry JF, de Micco C. Specific pattern of RAS oncogene mutations in follicular thyroid tumors. *J Clin Endocrinol Metab* 2003; **88**: 2745-2752 [PMID: 12788883 DOI: 10.1210/jc.2002-021186]

24 **Neta G**, Brenner AV, Sturgis EM, Pfeiffer RM, Hutchinson AA, Aschebrook-Kilfoy B, Yeager M, Xu L, Wheeler W, Abend M, Ron E, Tucker MA, Chanock SJ, Sigurdson AJ. Common genetic variants related to genomic integrity and risk of papillary thyroid cancer. *Carcinogenesis* 2011; **32**: 1231-1237 [PMID: 21642358 DOI: 10.1093/carcin/bgr100]

25 **Bongarzone I**, Fugazzola L, Vigneri P, Mariani L, Mondellini P, Pacini F, Basolo F, Pinchera A, Pilotti S, Pierotti MA. Age-related activation of the tyrosine kinase receptor protooncogenes RET and NTRK1 in papillary thyroid carcinoma. *J Clin Endocrinol Metab* 1996; **81**: 2006-2009 [PMID: 8626874 DOI: 10.1210/jc.81.5.2006]

26 **Jhiang SM**, Caruso DR, Gilmore E, Ishizaka Y, Tahira T, Nagao M, Chiu IM, Mazzaferri EL. Detection of the PTC/retTPC oncogene in human thyroid cancers. *Oncogene* 1992; **7**: 1331-1337 [PMID: 1620547]

27 **Soares P**, Fonseca E, Wynford-Thomas D, Sobrinho-Simões M. Sporadic ret-rearranged papillary carcinoma of the thyroid: a subset of slow growing, less aggressive thyroid neoplasms? *J Pathol* 1998; **185**: 71-78 [PMID: 9713362 DOI: 10.1002/(SICI)1096-9896(199805)185]

28 **Fenton CL**, Lukes Y, Nicholson D, Dinauer CA, Francis GL, Tuttle RM. The ret/PTC mutations are common in sporadic

papillary thyroid carcinoma of children and young adults. *J Clin Endocrinol Metab* 2000; **85**: 1170-1175 [PMID: 10720057 DOI: 10.1210/jc.85.3.1170]

29 **Fugazzola L**, Pilotti S, Pinchera A, Vorontsova TV, Mondellini P, Bongarzone I, Greco A, Astakhova L, Butti MG, Demidchik EP. Oncogenic rearrangements of the RET proto-oncogene in papillary thyroid carcinomas from children exposed to the Chernobyl nuclear accident. *Cancer Res* 1995; **55**: 5617-5620 [PMID: 7585643]

30 **Nikiforov YE**, Rowland JM, Bove KE, Monforte-Munoz H, Fagin JA. Distinct pattern of ret oncogene rearrangements in morphological variants of radiation-induced and sporadic thyroid papillary carcinomas in children. *Cancer Res* 1997; **57**: 1690-1694 [PMID: 9135009]

31 **Klugbauer S**, Lengfelder E, Demidchik EP, Rabes HM. High prevalence of RET rearrangement in thyroid tumors of children from Belarus after the Chernobyl reactor accident. *Oncogene* 1995; **11**: 2459-2467 [PMID: 8545102]

P- Reviewer Rocha R **S- Editor** Gou SX
L- Editor A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i7.1085

World J Gastroenterol 2013 February 21; 19(7): 1085-1090
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Efficacy and safety of Chlorella supplementation in adults with chronic hepatitis C virus infection

Jose Azocar, Arley Diaz

Jose Azocar, Arley Diaz, Northgate Medical Center, Springfield, MA 01103, United States
Author contributions: Azocar J designed and performed the research, collected and analyzed the data and wrote the paper; Diaz A analyzed data and revised the paper.
Supported by Northgate Medical Center, Research Program NMSC01
Correspondence to: Jose Azocar, MD, DSc, Northgate Medical Center, 1985 Main Street, Springfield, MA 01103, United States. jazocar@rcn.com
Telephone: +1-413-7339955 Fax: +1-413-7889840
Received: September 14, 2012 Revised: December 27, 2012
Accepted: January 17, 2013
Published online: February 21, 2013

Abstract

AIM: To evaluate the safety and efficacy of Chlorella in 18 patients chronically infected with hepatitis C virus (HCV) genotype 1.

METHODS: Eighteen adults with chronic infection by HCV genotype 1 received daily oral supplementation of Chlorella for 12 wk. Changes in the RNA levels of HCV, as well as those of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were evaluated following this treatment period. Paired *t* tests were conducted to compare the means of the different variables at the beginning and end of the study. Side effects and quality of life aspects were also compared between weeks 0 and 12 of the study period.

RESULTS: A majority 84.61% of the patients had a significant decrease in their ALT levels from week 0 to week 12. Evaluation of side effects showed that Chlorella was well tolerated. Quality of life assessment showed that 76.9 of the participants reported an improvement in their energy levels and 46.1% reported an improvement in their perception of general health. Although 69.23% also showed a decrease in their AST levels, this was not statistically significant. Most patients that exhibited an improvement in their ALT and AST levels also showed a tendency toward a decreased HCV viral load. The HCV RNA levels showed a decrease in 69.23% of the patients, which along with changes in AST/ALT ratios from week 0 to week 12, these results were not statistically significant.

CONCLUSION: Chlorella supplementation was well tolerated in patients with chronic HCV and associated with a significant decrease in ALT liver enzyme levels.

© 2013 Baishideng. All rights reserved.

Key words: Chlorella; Hepatitis C virus; Interferon; Aspartate and alanine aminotransferase; Ratio

Azocar J, Diaz A. Efficacy and safety of Chlorella supplementation in adults with chronic hepatitis C virus infection. *World J Gastroenterol* 2013; 19(7): 1085-1090 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i7/1085.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i7.1085

INTRODUCTION

Complementary and alternative medicines to treat chronic liver diseases, including chronic hepatitis C virus (HCV) infection, are becoming increasingly popular in North America[1-5]. Infection with HCV is global in nature, infecting approximately 160 million persons worldwide or roughly 2% of the world population, with some countries documenting a rate of 15% or more[6,7]. After an initial HCV infection, close to 70% of cases develop chronic infection that may progress to liver cirrhosis and hepatocellular carcinoma if left untreated[8]. The successful treatment of chronic HCV infection is determined by a reduced HCV-RNA viral load and improved liver function and histology. The current Food and Drug

Administration approved treatment for HCV is up to 42 wk of interferon and antiviral medications. However, there are significant costs associated with these medications and side effects which limit their use thus stressing the need for novel treatment options[9,10]. In addition, subjects that fail to respond to the initial treatment are less likely to respond to retreatment[11]. Many herbal and other natural compounds have now been used for the treatment of liver diseases, including HCV infection. Silybum marianum and Lactoferrin have been associated with a decrease in serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels[12,13]. More recently, green tea catechins have been shown to inhibit HCV attachment and transmission in human liver cells *in vitro*[14]. However, the potential benefits of herbal and other natural molecules in inhibiting the progression of HCV infection are only beginning to be understood, and more controlled studies are needed in this area.

Chlorella, a fresh water unicellular alga rich in macro- and micronutrients, has been used as a food source and nutritional supplement for centuries[15]. In animals, Chlorella has been reported to improve host resistance to viral infection and tumors[16-18]. In humans, Chlorella supplementation has been shown to enhance the antibody titer after influenza immunization[19], and to improve the outcome in several chronic diseases[20,21]. Chlorella supplementation has been also associated with an improvement in liver function in animal models[22,23]. Due to the documented benefits of Chlorella treatments in liver diseases in animals and in chronic diseases in humans, we studied the effects of Chlorella in 18 patients with chronic HCV genotype 1 infection. The plasma HCV RNA levels, hematological and chemistry results, including liver enzyme levels, and the quality of life and psychological well-being were assessed in this cohort following dietary supplementations with Chlorella-derived products.

MATERIALS AND METHODS

Population

The current study trial took place at a primary care clinic in western Massachusetts. Approval was obtained from the New England Institutional Review Board (NEIRB, Wellesley, MA, United States). The study cohort comprised 18 patients with chronic HCV infection who were either unwilling or unable to receive an interferon plus antiviral therapy. The inclusion criteria were an age of 18-65 years, evidence of chronic HCV infection by reverse transcription polymerase chain reaction (PCR), and a confirmation that the infection was due to HCV genotype 1. The exclusion criteria were any acute or chronic liver disease other than chronic HCV infection, any evidence of advanced liver disease such as a history or presence of ascites, a history of bleeding esophageal varices or encephalopathy, any known existing medical condition that could interfere with participation in the current study, co-infection with hepatitis B virus and/or human immunodeficiency virus, or a history of active alcohol or drug abuse three months prior to the beginning of the trial. Some of the patients had already been treated with interferon plus ribavirin (three years or more previously) before their participation in this study and had failed to respond.

Study design

This study examined the effects of Chlorella upon the HCV viral load, and on hematological and chemical test results, including AST and ALT liver enzyme levels, in infected patients during a 12 wk treatment period. To control for factors others than Chlorella that could have affected the results of our study, only patients with a HCV genotype 1 infection were selected for this trial. The AST/ALT ratio at week 0 and after the 12 wk of Chlorella supplementation was compared among the 13 subjects in the cohort.

We also compared the safety and efficacy of orally administered Chlorella by assessing the presence of possible side effects and their impact on quality of life. The side effects assessed included constipation, diarrhea, depression, irritability, headache/body aches, and any other significant symptoms that arose during the study period. Similarly, quality of life was assessed by evaluating changes in energy levels, general health perceptions, quality of sleep and changes in appetite. Patients were interviewed about possible side effects and impact of quality of life at baseline, and at weeks 1, 2, 4, 8 and 12. The answers were coded on a 5 level scale from 1 (much worse) to 5 (much better). Scores were then compared between baseline and weeks 1, 2, 4, 8 and 12. To assess compliance, patients were instructed to return all used and unused products at each visit.

"Sun Chlorella A™" consisted of a dry pulverized Chlorella pyrenoidosa powder plus a water soluble extract ("Wakasa Gold™"; Sun Chlorella Corp, Kyoto, Japan), which contained 82 mg/mL of Chlorella. It was administered orally to each patient as follows: three 500 mg tablets were administered twice daily on days 1-7 and then three times each day thereafter. Wakasa Gold ™ was administered at a dose of 30 mL twice a day starting on day 1. Both pulverized tablets and water soluble extracts were used in the treatment given in order to administer the greatest amount of Chlorella possible. Patients were evaluated as described above during the duration of the trial. Routine hematology, chemical tests and HCV RNA levels were done at baseline and at week 12 using a PCR-based assay conducted at a local laboratory (Life Lab; Mercy Hospital, Springfield, MA, United States).

Changes in AST and ALT liver enzyme levels and the HCV viral load in our "Chlorella treat" cohort were measured after the 12 wk study period and were compared with the same laboratory test results in a control group of 26 subjects who were also chronically infected with HCV genotype 1, but did not receive Chlorella. Subjects

Table 1 Levels of aspartate aminotransferase, alanine aminotransferase, aspartate/alanine aminotransferase ratio and hepatitis C virus viral load at weeks 0 and 12

Patient No.	AST (IU/mL)		ALT (IU/mL)		AST/ALT ratio		HCV viral load (IU/mL)		Previous
	Week 0	Week 12	Week 0	Week 12	Week 0	Week 12	Week 0	Week 12	INF Rx
1	80	63	82	68	0.975	0.926	11 420	1183	No
2	53	44	103	72	0.514	0.611	140 642	32 334	No
3	87	40	71	39	1.225	1.025	91 808	24 141	No
4	35	56	48	92	0.729	0.608	223 075	89 331	No
5	19	25	22	21	0.863	1.19	221 116	281 886	No
6	39	33	83	72	0.469	0.454	1 917 040	560 082	No
7	51	41	81	64	0.629	0.645	269 079	217 334	Yes
8	138	74	157	105	0.878	0.704	382 773	1 302 860	Yes
9	48	51	37	47	1.297	1.085	136 292	236 748	Yes
10	75	85	102	95	0.735	0.894	716 152	503 764	Yes
11	27	24	39	29	0.692	0.827	7 672 080	7 692 310	Yes
12	103	77	157	119	0.656	0.647	847 412	542 398	Yes
13	127	93	159	106	0.798	0.877	869 846	568 398	Yes

AST: Aspartate aminotransferase; ALT: Alanine aminotransferase; HCV: Hepatitis C virus; INF: Interferon.

in the control group were randomly selected, attended the same clinic and had a similar demographic profile as the Chlorella-treated group. Changes in the hematological, liver enzyme values and HCV-RNA viral load in this control group were assessed within an 11-21 wk period as part of a standard clinical evaluation. No subjects in either the study or control groups received treatment with interferon or other anti-viral drugs during the study period. This study was conducted in accordance with the Declaration of Helsinki, Good Clinical Practices and the study protocol was reviewed and approved by the NEIRB.

Statistical analysis

Data gathered during the study was used to examine statistically significant changes in the HCV RNA levels, and in the hematological and liver enzyme (AST, ALT) levels. Side effects and quality of life aspects were also compared between weeks 0 and 12 of the study period. Paired *t* tests were conducted to compare the means of the different variables at the beginning and end of the study. *P* values < 0.05 were considered statistically significant. All data was analyzed using Statistical Package for Social Sciences version 17.0.

RESULTS

Thirteen out of 18 enrolled patients completed our 12 wk study. Of the five patients who did not complete the trial, one individual discontinued treatment due to constipation on the first two days of treatment, which resolved upon treatment withdrawal. Four subjects were excluded due to poor compliance which was unrelated to side effects or changes in their general health status.

HCVRNA titer and liver enzyme analysis

Changes in the HCV RNA levels and in the AST and ALT liver enzyme levels among our study subjects are shown in Table 1. A majority of patients experienced an improvement in their liver enzyme profiles; 84.61% (11/13) had a decrease in their ALT levels from week 0 (mean ± SD, 89.30% ± 49.36%) to week 12 (mean ± SD, 71.46% ± 31.12%). Moreover, this decrease was statistically significant ($P < 0.05$). Although 69.23% of the patients (9/13) also showed a decrease in their AST levels from week 0 (mean ± SD, 67.84% ± 37.73%) to week 12 (mean ± SD, 54.30% ± 22.63%), this was not statistically significant ($P = 0.06$). The results further showed that 69.23% (9/13) of the patients had a decrease in their HCV RNA levels, although the results of the paired *t* test also showed this was not statistically significant ($P = 0.42$). Most patients that exhibited an improvement in their AST and ALT levels also showed a tendency toward a decreased HCV viral load. An exception was patient No. 8 whose viral load increased significantly (Table 1). The changes in the AST/ALT ratio between weeks 0 and 12 among the subjects in the Chlorella cohort shown in Table 1 were not statistically significant. The values obtained from additional laboratory tests, including routine hematology (complete blood count and differential) and chemical tests, showed no statistically significant changes during the study period (data not shown). No statistically significant differences in the changes of the AST and ALT liver enzymes, AST/ALT ratio and HCV viral load was observed within a 11-21 wk period, among the control group who received not Chlorella supplementation.

Side effect profile and quality of life assessment

The main side effects associated with the Chlorella treatments in our trial included constipation and diarrhea. Four of the 13 patients (30.7%) with a previous history of constipation reported their symptoms as worse or somewhat worse during the first two weeks of treatment. However, in all of these cases, the constipation symptoms were mild to moderate and resolved within the first two weeks. These patients continued in the trial for the scheduled 12 wk. Similarly, two of the 13 patients (15.3%) who completed the study complained of mild

diarrhea at week 1, but reported that these symptoms were much improved after week 2. None of the patients reported symptoms of abdominal pain, fever, depression, headache, body ache or other complaints during the 12 wk study.

Quality of life

Four variables were tested to assess changes in quality of life; 76.9 % of the patients (10/13) reported an improvement in their energy levels during the study period whereas 23.1% (3/13) reported no change in energy levels. In addition, 46.1% (6/13) of the patients reported an improvement in their perception of general health while 53.9% (7/13) reported no change in this regard. None of the patients reported issues with sleep quality or appetite during the 12 wk study period.

DISCUSSION

In our present study, most of the subject patients with chronic HCV showed a good tolerance to Chlorella oral supplementation. This was anticipated, based on previous studies which had also shown good tolerance of similar doses of Chlorella administered in pregnant, lactating[24], and elderly subjects[19], as well as in patients with diverse chronic diseases[20,21]. In addition, a significant percentage of our subjects reported health improvements in their quality of life questionnaire: 76.9% had an increase in energy levels and 46.1 % described an improvement in their general health perception.

The most significant finding from our current study was the statistically significant decrease in ALT levels, a marker of liver inflammation, among our patient cohort after 12 wk of Chlorella treatment. The cause-effect relationship between the observed significant decrease in the ALT levels and treatment with Chlorella is further suggested by the lack of significant ALT changes within the 11-21 wk period in a control group of HCV genotype 1 infected patients who had not received Chlorella. We further found that 69.23% of our patients showed a tendency toward a decrease in both their AST and HCV RNA levels after the 12 wk Chlorella treatment period, although this was not statistically significant. The AST/ALT ratio has been widely used as an indicator of liver disease; a ratio higher of 1 is used as indicator of liver fibrosis and cirrhosis[25,26]. In our cohort only four of thirteen subjects had an AST/ALT higher than 1 and there was no significant change after the Chlorella supplementation period (Table 1).

Animal studies have shown that enhanced immunocompetence provided by oral administration of Chlorella extract is mediated by augmentation of cell mediated immunity in normal and immunocompromised hosts[16-18]. More recently Kwak *et al*[27] demonstrated enhancement of natural killer cell activity and increase interferon and other cytokines production in humans after an 8 wk period of Chlorella supplementation at doses similar to the ones used in this study. We propose that the improvements in liver function tests in our population with chronic HCV infection is most likely due to the beneficial immunostimulatory effect of Chlorella supplementation. This is consistent with the finding that in our cohort most of the subjects that had been previously treated with interferon and failed to respond, were less likely to show a decrease in ALT and HCV-RNA values during Chlorella supplementation (Previous INF Rx, Table 1). The recovery from liver inflammation in all viral and non-viral cases of hepatitis is associated with a reduction in the ALT levels[28-30]. In Chronic HCV infection, early normalization of the ALT levels is predictive of the response to interferon[9,10,30]. However, whether the significant decrease in ALT values observed in our patients after 12 wk of Chlorella administration is associated with an improvement in liver histology remains to be determined.

The strength of our hypothesis; that the benefits of Chlorella supplementation are related to the well known immunoenhancement effects of Chlorella is limited by the lower number of subjects in our cohort and the limited time of supplementation (12 wk). Nevertheless, we conclude from our present findings that the benefits of Chlorella administration in the treatment of chronic HCV infection before and/or during the administration of interferon plus antiviral drugs, as well as the effects of Chlorella upon chronic infection by other HCV genotypes, warrant further study.

ACKNOWLEDGMENTS

The authors acknowledge the excellent editorial support of Camille Alvarado-Pinguelo, MPH. Sun Chlorella A™ and Wakasa Gold™ were generously provided by Sun Chlorella Corporation (Kyoto, Japan).

COMMENTS

Background

Chronic hepatitis C viral (HCV) infection are becoming increasingly popular in North America and most cases develop chronic infection that may progress to liver cirrhosis and hepatocellular carcinoma if left untreated. Chlorella supplementation has been shown to enhance the antibody titer after influenza immunization, and to improve the outcome in several chronic diseases.

Research frontiers

Animal studies have shown that enhanced immunocompetence provided by oral administration of Chlorella extract is mediated by augmentation of cell mediated immunity in normal and immunocompromised host. More recently Kwak *et al* demonstrated enhancement of natural killer cell activity and increase interferon and other cytokines production in humans after an 8 wk period of Chlorella supplementation at doses similar to the ones used in this study.

Innovations and breakthroughs

The study suggestes that the benefits of Chlorella administration in the treatment of chronic HCV infection before and/or during the administration of interferon plus antiviral drugs, as well as the effects of Chlorella upon chronic infection by other HCV genotypes, warrant further study.

Terminology

Chlorella, a fresh water unicellular alga rich in macro- and micronutrients, has been used as a food source and nutritional supplement for centurie. In animals,

Chlorella has been reported to improve host resistance to viral infection and tumors.

Peer review

The results are interesting and suggest that most of the subject patients with chronic HCV showed a good tolerance to Chlorella oral supplementation.

REFERENCES

1 **Modi AA**, Wright EC, Seeff LB. Complementary and alternative medicine (CAM) for the treatment of chronic hepatitis B and C: a review. *Antivir Ther* 2007; **12**: 285-295 [PMID: 17591018]

2 **White CP**, Hirsch G, Patel S, Adams F, Peltekian KM. Complementary and alternative medicine use by patients chronically infected with hepatitis C virus. *Can J Gastroenterol* 2007; **21**: 589-595 [PMID: 17853954]

3 **Seeff LB**, Curto TM, Szabo G, Everson GT, Bonkovsky HL, Dienstag JL, Shiffman ML, Lindsay KL, Lok AS, Di Bisceglie AM, Lee WM, Ghany MG. Herbal product use by persons enrolled in the hepatitis C Antiviral Long-Term Treatment Against Cirrhosis (HALT-C) Trial. *Hepatology* 2008; **47**: 605-612 [PMID: 18157835 DOI: 10.1002/hep.22044]

4 **Mollison L**, Totten L, Flexman J, Beaman M, Batey R. Randomized double blind placebo-controlled trial of a Chinese herbal therapy (CH100) in chronic hepatitis C. *J Gastroenterol Hepatol* 2006; **21**: 1184-1188 [PMID: 16824073 DOI: 10.1111/j.1440-1746.2006.04320.x]

5 **Batey RG**, Bensoussan A, Fan YY, Bollipo S, Hossain MA. Preliminary report of a randomized, double-blind placebo-controlled trial of a Chinese herbal medicine preparation CH-100 in the treatment of chronic hepatitis C. *J Gastroenterol Hepatol* 1998; **13**: 244-247 [PMID: 9570235 DOI: 10.1111/j.1440-1746.1998.01550.x]

6 **Shepard CW**, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. *Lancet Infect Dis* 2005; **5**: 558-567 [PMID: 16122679 DOI: 10.1016/S1473-3099(05)70216-4]

7 **Williams I**. Epidemiology of hepatitis C in the United States. *Am J Med* 1999; **107**: 2S-9S [PMID: 10653448 DOI: 10.1016/S0002-9343(99)00373-3]

8 **Seeff LB**, Hollinger FB, Alter HJ, Wright EC, Cain CM, Buskell ZJ, Ishak KG, Iber FL, Toro D, Samanta A, Koretz RL, Perrillo RP, Goodman ZD, Knodell RG, Gitnick G, Morgan TR, Schiff ER, Lasky S, Stevens C, Vlahcevic RZ, Weinshel E, Tanwandee T, Lin HJ, Barbosa L. Long-term mortality and morbidity of transfusion-associated non-A, non-B, and type C hepatitis: A National Heart, Lung, and Blood Institute collaborative study. *Hepatology* 2001; **33**: 455-463 [PMID: 11172349 DOI: 10.1053/jhep.2001.21905]

9 **Poynard T**, Marcellin P, Lee SS, Niederau C, Minuk GS, Ideo G, Bain V, Heathcote J, Zeuzem S, Trepo C, Albrecht J. Randomised trial of interferon alpha2b plus ribavirin for 48 weeks or for 24 weeks versus interferon alpha2b plus placebo for 48 weeks for treatment of chronic infection with hepatitis C virus. International Hepatitis Interventional Therapy Group (IHIT) *Lancet* 1998; **352**: 1426-1432 [PMID: 9807989]

10 **Poordad F**, McCone J, Bacon BR, Bruno S, Manns MP, Sulkowski MS, Jacobson IM, Reddy KR, Goodman ZD, Boparai N, DiNubile MJ, Sniukiene V, Brass CA, Albrecht JK, Bronowicki JP. Boceprevir for untreated chronic HCV genotype 1 infection. *N Engl J Med* 2011; **364**: 1195-1206 [PMID: 21449783 DOI: 10.1056/NEJMoa1010494]

11 **Pomilio AB**, Buschi CA, Tomes CN, Viale AA. Antimicrobial constituents of Gomphrena martiana and Gomphrena boliviana. *J Ethnopharmacol* 1992; **36**: 155-161 [PMID: 1608273 DOI: 10.1186/1471-2334-10-212]

12 **Kalantari H**, Shahshahan Z, Hejazi SM, Ghafghazi T, Sebghatolahi V. Effects of silybum marianum on patients with chronic hepatitis C. *J Res Med Sci* 2011; **16**: 287-290 [PMID: 22091246]

13 **Tanaka K**, Ikeda M, Nozaki A, Kato N, Tsuda H, Saito S, Sekihara H. Lactoferrin inhibits hepatitis C virus viremia in patients with chronic hepatitis C: a pilot study. *Jpn J Cancer Res* 1999; **90**: 367-371 [PMID: 10363572 DOI: 10.1111/j.1349-7006.1999.tb00756.x]

14 **Calland N**, Albecka A, Belouzard S, Wychowski C, Duverlie G, Descamps V, Hober D, Dubuisson J, Rouillé Y, Séron K. (-)-Epigallocatechin-3-gallate is a new inhibitor of hepatitis C virus entry. *Hepatology* 2012; **55**: 720-729 [PMID: 22105803 DOI: 10.1002/hep.24803]

15 **Kay RA**. Microalgae as food and supplement. *Crit Rev Food Sci Nutr* 1991; **30**: 555-573 [PMID: 1741951 DOI: 10.1080/10408399109527556]

16 **Miyazawa Y**, Murayama T, Ooya N, Wang LF, Tung YC, Yamaguchi N. Immunomodulation by a unicellular green algae (Chlorella pyrenoidosa) in tumor-bearing mice. *J Ethnopharmacol* 1988; **24**: 135-146 [PMID: 3253484 DOI: 10.1016/0378-8741(88)90145-6]

17 **Tanaka K**, Konishi F, Himeno K, Taniguchi K, Nomoto K. Augmentation of antitumor resistance by a strain of unicellular green algae, Chlorella vulgaris. *Cancer Immunol Immunother* 1984; **17**: 90-94 [PMID: 6565519 DOI: 10.1007/BF00200042]

18 **Hasegawa T**, Kimura Y, Hiromatsu K, Kobayashi N, Yamada A, Makino M, Okuda M, Sano T, Nomoto K, Yoshikai Y. Effect of hot water extract of Chlorella vulgaris on cytokine expression patterns in mice with murine acquired immunodeficiency syndrome after infection with Listeria monocytogenes. *Immunopharmacology* 1997; **35**: 273-282 [PMID: 9043941 DOI: 10.1016/S0162-3109(96)00150-6]

19 **Halperin SA**, Smith B, Nolan C, Shay J, Kralovec J. Safety and immunoenhancing effect of a Chlorella-derived dietary supplement in healthy adults undergoing influenza vaccination: randomized, double-blind, placebo-controlled trial. *CMAJ* 2003; **169**: 111-117 [PMID: 12874157]

20 **Merchant RE**, Andre CA. A review of recent clinical trials of the nutritional supplement Chlorella pyrenoidosa in the treatment of fibromyalgia, hypertension, and ulcerative colitis. *Altern Ther Health Med* 2001; **7**: 79-91 [PMID: 11347287]

21 **Mizoguchi T**, Takehara I, Masuzawa T, Saito T, Naoki Y. Nutrigenomic studies of effects of Chlorella on subjects with high-risk factors for lifestyle-related disease. *J Med Food* 2008; **11**: 395-404 [PMID: 18800884]

22 **Takekoshi H**, Mizoguchi T, Komasa Y, Chubachi H, Inoue Y, Imanishi H, Nakano M. Suppression of glutathione S-transferase placental form-positive foci development in rat hepatocarcinogenesis by Chlorella pyrenoidosa. *Oncol Rep* 2005; **14**: 409-414 [PMID: 16012723]

23 **Takekoshi H**, Suzuki G, Chubachi H, Nakano M. Effect of Chlorella pyrenoidosa on fecal excretion and liver accumulation of polychlorinated dibenzo-p-dioxin in mice. *Chemosphere* 2005; **59**: 297-304 [PMID: 15722102 DOI: 10.1016/j.chemosphere.2004.11.026]

24 **Nakano S**, Takekoshi H, Nakano M. Chlorella pyrenoidosa supplementation reduces the risk of anemia, proteinuria and edema in pregnant women. *Plant Foods Hum Nutr* 2010; **65**: 25-30 [PMID: 20013055 DOI: 10.1007/s11130-009-0145-9]

25 **Lackner C**, Struber G, Liegl B, Leibl S, Ofner P, Bankuti C, Bauer B, Stauber RE. Comparison and validation of simple noninvasive tests for prediction of fibrosis in chronic hepatitis C. *Hepatology* 2005; **41**: 1376-1382 [PMID: 15915455]

26 **Fouad SA**, Esmat S, Omran D, Rashid L, Kobaisi MH. Noninvasive assessment of hepatic fibrosis in Egyptian patients with chronic hepatitis C virus infection. *World J Gastroenterol* 2012; **18**: 2988-2994 [PMID: 22736923]

27 **Kwak JH**, Baek SH, Woo Y, Han JK, Kim BG, Kim OY, Lee JH. Beneficial immunostimulatory effect of short-term Chlorella supplementation: enhancement of Natural Killer cell activity and early inflammatory response (Randomized, double-blinded, placebo-controlled trial). *Nutr J* 2012; **11**: 53 [PMID: 22849818 DOI: 10.1186/1475-2891-11-53]

28 **Wedemeyer H**, Hofmann WP, Lueth S, Malinski P, Thimme R, Tacke F, Wiegand J. [ALT screening for chronic liver diseases: scrutinizing the evidence]. *Z Gastroenterol* 2010; **48**: 46-55 [PMID: 20072996]

29 **Lee MH**, Yang HI, Lu SN, Jen CL, Yeh SH, Liu CJ, Chen PJ, You SL, Wang LY, Chen WJ, Chen CJ. Hepatitis C virus seromarkers and subsequent risk of hepatocellular carcinoma: long-term predictors from a community-based cohort study. *J Clin Oncol* 2010; **28**: 4587-4593 [PMID: 20855826]

30 **Seeff LB**. Natural history of chronic hepatitis C. *Hepatology* 2002; **36**: S35-S46 [PMID: 12407575 DOI: 10.1053/jhep.2002.36806]

P- Reviewers Zhang XW, Chetty R
S- Editor Zhai HH **L- Editor** A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i7.1091

World J Gastroenterol 2013 February 21; 19(7): 1091-1097
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Efficacy of infliximab in acute severe ulcerative colitis: A single-centre experience

Stephen J Halpin, P John Hamlin, Daniel P Greer, Lisa Warren, Alexander C Ford

Stephen J Halpin, P John Hamlin, Daniel P Greer, Lisa Warren, Alexander C Ford, Leeds Gastroenterology Institute, St. James's University Hospital, Leeds, West Yorkshire LS9 7TF, United Kingdom

Alexander C Ford, Leeds Institute of Molecular Medicine, University of Leeds, Leeds, West Yorkshire LS9 7TF, United Kingdom

Author contributions: Halpin SJ, Warren L, Greer DP, Hamlin PJ and Ford AC conceived and drafted the study; Halpin SJ, Warren L and Greer DP collected all data; Halpin SJ and Ford AC analyzed and interpreted the data, and drafted the manuscript; all authors commented on drafts of the paper and approved the final draft of the manuscript.

Correspondence to: Dr. Alexander C Ford, Leeds Gastroenterology Institute, St. James's University Hospital, Room 125, 4th Floor, Bexley Wing, Beckett Street, Leeds, West Yorkshire LS9 7TF, United Kingdom. alexf12399@yahoo.com
Telephone: +44-113-2684963 Fax: +44-113-2429722

Received: November 17, 2012 Revised: December 22, 2012
Accepted: December 25, 2012
Published online: February 21, 2013

Abstract

AIM: To suggest infliximab (IFX) is effective for acute severe ulcerative colitis, from real-life clinical practice.

METHODS: All patients receiving IFX for the treatment of acute severe ulcerative colitis in a single centre were included. Data were extracted from clinical records in order to assess response to IFX therapy. The primary endpoint was colectomy-free survival, and secondary outcomes included glucocorticosteroid-free remission and safety, which was evaluated by recording deaths and adverse events. Demographic and clinical characteristics of those who underwent colectomy and those who were colectomy-free, both at discharge from their index admission, and during follow-up after an initial response to IFX were compared.

RESULTS: Forty-four patients (16 females, mean age 36 years) received IFX between May 2006 and January 2012 for acute severe ulcerative colitis. The median duration of follow-up post-first infusion was 396 d (interquartile range = 173-828 d). There were 21 (47.7%) patients with < 1 year of follow-up, 10 (22.7%) with 1 years to 2 years of follow-up, and 13 (29.5%) with > 2 years of follow-up post-first infusion of IFX. Overall, 35 (79.5%) responded to IFX, avoiding colectomy during their index admission, 29 (65.9%) were colectomy-free at last point of follow-up (median follow-up 396 d), and 25 (56.8%) were in glucocorticosteroid-free remission at end of follow-up. There was one death from post-operative sepsis, 20 d after a single IFX infusion. Colectomy rates were generally lower among those "bridging" to thiopurine. Of 18 patients "bridged" to thiopurine therapy, 17 (94.4%) were colectomy-free, and 15 (83.3%) were in glucocorticosteroid-free remission at study end. No predictors of response were identified.

CONCLUSION: IFX is effective for acute severe ulcerative colitis in real-life clinical practice. Two-thirds of patients avoided colectomy, and more than 50% were in glucocorticosteroid-free remission.

© 2013 Baishideng. All rights reserved.

Key words: Ulcerative colitis; Severe; Azathioprine; Infliximab; Remission

Halpin SJ, Hamlin PJ, Greer DP, Warren L, Ford AC. Efficacy of infliximab in acute severe ulcerative colitis: A single-centre experience. *World J Gastroenterol* 2013; 19(7): 1091-1097 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i7/1091.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i7.1091

INTRODUCTION

Ulcerative colitis (UC) is a chronic inflammatory disorder of the gastrointestinal tract of unknown etiology, with a prevalence of between 160 and 240 per 100 000 people

in Western populations[1-3]. The condition is thought to arise from dysregulation of both the innate and adaptive immune systems, leading to an abnormal inflammatory response to commensal bacteria in a genetically susceptible individual[4].

The clinical course of UC is characterized by periods of remission and relapse, with acute inflammatory exacerbations of disease activity which, when severe, are potentially life-threatening. The standard initial management of these inflammatory exacerbations includes high dose intravenous glucocorticosteroids in the first instance, but this strategy may be unsuccessful in up to 50% of patients[5-7]. Immunomodulating drugs such as azathioprine, whilst effective in maintaining remission[8], act too slowly to be of use in the acute setting. Following the publication of a randomized controlled trial (RCT) by Lichtiger *et al*[9] in 1994, ciclosporin has been used as medical rescue therapy in acute severe UC, in order to avoid colectomy in the short-term, and to act as a "bridge" to long-term thiopurine therapy[10]. Several case series have since been published[11-14], but despite response rates in the order of 50%-70%, many patients require colectomy in the longer term.

In recent years, biological therapies have emerged as a treatment option in inflammatory bowel disease (IBD). Infliximab (IFX) (Remicade®, Centocor Ortho Biotech Inc, PA, United States), a chimeric monoclonal antibody directed against human tumor necrosis factor-alpha was the first biological therapy to be approved for use in acute severe UC. The efficacy of IFX in UC has been investigated by a limited number of RCTs[15-18]. When data from all these trials were pooled in a recent meta-analysis the number needed to treat over placebo to achieve remission in one patient with moderately or severely active UC was only 4, suggesting this is a highly efficacious therapy[19].

However, only two of these trials studied the use of IFX in acute severe UC[15,18], one of which found no significant difference in response between IFX and placebo[18]. In addition, data from RCTs do not always translate into normal clinical practice. Data from small retrospective case series' suggest that as many as one-third of patients given IFX for acute severe UC still require colectomy during the acute admission[20-23], but larger datasets, with longer follow-up, may provide more accurate insight into the efficacy of IFX in this setting. We therefore report our 6-year experience of using IFX in acute severe UC.

MATERIALS AND METHODS

Participants and setting

Patients have been treated with IFX for acute severe UC since 2006 in the Leeds Gastroenterology Institute, which operates across two large teaching hospitals serving a local population of approximately 800 000, as well as receiving tertiary referrals from the surrounding area. After their first IFX infusion of 5 mg/kg in hospital, patients attend for their second and third infusions on an outpatient basis at 2 wk and 6 wk. These are administered at a dedicated biological therapy clinic by specialist IBD nurses, who maintain a prospective database detailing demographics, response to therapy, number of infusions received, and any adverse events experienced.

All patients who received at least one dose of IFX for acute severe UC in Leeds between May 2006 and January 2012 were included. Patients were identified by cross referencing our IBD database with pharmacy records, which are accurate as all IFX infusions for in-patients are prepared in the pharmacy department. The use of IFX to treat an episode of acute severe UC was defined as need for the drug during an in-patient admission with an acute inflammatory exacerbation of disease activity. Patients receiving IFX for IBD-unclassified or pouchitis were excluded.

Data collection

Inpatient medical records, computerized outpatient clinic letters, histopathology, endoscopy, and blood results were reviewed by one investigator. Data were collected onto a Microsoft Excel spreadsheet (XP professional edition; Microsoft, Redmond, WA, United States) designed prospectively. These included demographic details, date of UC diagnosis, extent of disease according to the Montreal classification[24], drug therapy at time of admission, prior or current use of thiopurine or 5-aminosalicylate (5-ASA), physiological and biochemical parameters at day 0 and day 3 (mean number of stools per day, pulse, temperature, and C-reactive protein (CRP) level in mg/L), severity of UC on day 3 of their admission, according to the Travis criteria[25], and duration of admission (in days). Total number of infusions received, any adverse events experienced (including death), requirement for colectomy, and current drug therapies were collected at discharge from the index admission, as well as at last point of follow-up.

The primary outcome of interest was colectomy-free survival, and secondary outcomes included glucocorticosteroid-free remission and safety. Patients were judged to have had an initial response to therapy if they were discharged from their index admission colectomy-free. Recording of the patient's current drug therapy at last contact allowed assessment of the achievement of glucocorticosteroid-free remission. Safety data included deaths or adverse events during the study period that were potentially related to IFX.

Statistical analysis

The proportion of individuals who were colectomy-free at discharge from their index admission, and who were in colectomy- and glucocorticosteroid- free remission at the last point of follow-up was calculated. Demographic and clinical characteristics of those who underwent colectomy and those who were colectomy-free, both at discharge from their index admission, and during follow-up, after an initial response to IFX were compared using

Table 1 Baseline characteristics and demographics of 44 patients with acute severe ulcerative colitis receiving infliximab *n* (%)

Characteristic	All patients (*n* = 44)
Age at index admission[1]	35.7 ± 15.9
Female	16 (36.4)
Extent of disease (Montreal classification)	
E1 (limited to rectum)	2 (4.5)
E2 (distal to splenic flexure)	13 (29.5)
E3 (proximal to splenic flexure)	29 (65.9)
Current or previous smoker	18 (40.9)
Prescribed oral 5-ASA on admission	24 (54.5)
Prescribed thiopurine on admission	14 (31.8)
Median disease duration, in days, prior to first IFX infusion (IQR)	409 (16.25 to 1896.5)
First presentation of UC	12 (27.3)
Mean CRP (mg/L) on day of admission[1]	90.1 ± 81.9
CRP ≤ 5 on day of admission	6 (13.6)
Mean number of stools per day on admission[1]	12.1 ± 5.8

[1]Data are presented as mean ± SD. UC: Ulcerative colitis; IQR: Interquartile range; 5-ASA: 5-aminosalicylate; CRP: C-reactive protein; IFX: Infliximab.

an independent samples *t*-test for continuous variables, and Fisher's exact test for categorical variables. Multivariate logistic regression was performed in an attempt to identify independent risk factors for colectomy during the index admission, or during follow-up, controlling for all baseline demographic and clinical characteristics. All statistical analyses were performed using StatsDirect version 2.7.2 (StatsDirect Ltd, Sale, Cheshire, England), and SPSS for Windows version 19.0 (SPSS Inc, Chicago, IL, United States).

RESULTS

Between May 2006 and January 2012, 44 patients were treated with IFX for acute severe UC. The median duration of follow-up post-first infusion was 396 d [interquartile range (IQR) 173-828 d]. There were 21 (47.7%) patients with < 1 year of follow-up, 10 (22.7%) with 1-2 years of follow-up, and 13 (29.5%) with > 2 years of follow-up post-first infusion of IFX. The mean age at presentation with acute severe UC was 35.7 years (range: 18-78 years), and mean age at diagnosis was 32.4 years (range: 13-78 years). Of the 44 patients, 16 were female (36.4%). Baseline demographic data and disease characteristics of the included patients are detailed in Table 1. All patients had abdominal X-ray performed on admission to exclude toxic megacolon, and this was repeated at the discretion of the treating physician during intravenous glucocorticosteroids. Confirmation of mucosal disease activity was obtained by flexible sigmoidoscopy.

All 44 patients met the modified Truelove and Witt criteria[26] for acute severe UC on the day of admission. Mean CRP at time of admission was 90.1 mg/L, although this was less than 5 mg/L in 6 (13.6%) patients, and mean number of stools per day on admission was 12.1. All patients received intravenous glucocorticosteroids from the time of admission for a median of 7 d prior to IFX. There were 12 (27.3%) patients for whom the index episode of acute severe UC was their first presentation with the disease. Among the other 32 patients with an existing diagnosis of UC, 24 (75.0%) were currently receiving oral 5-ASA therapy, 14 (43.8%) were currently receiving thiopurine therapy, and a further six had previously received thiopurines but were either intolerant of them, or had experienced adverse events.

Need for colectomy during index admission

Nine patients (20.5%) underwent colectomy during their index admission, at a median of 5 d after their first IFX infusion (range: 2-18 d). The remaining 35 patients were discharged after their first IFX infusion colectomy-free. Baseline demographic data and disease characteristics of patients according to colectomy status at discharge from hospital following the index admission are reported in Table 2.

Patients who underwent colectomy during the index admission were generally older (mean age 45.6 years versus 33.2 years), and a higher proportion were admitted with a first presentation of UC (55.6%), compared with those who were discharged without colectomy (20.0%), but these differences were not statistically significant (P = 0.18, and P = 0.09 respectively). Extent of disease, according to the Montreal classification, was not associated with need for colectomy on index admission. In terms of medication use, fewer patients who underwent colectomy were receiving oral 5-ASAs or thiopurines on admission to hospital, but only the latter difference was statistically significant (P = 0.04). Those who underwent colectomy had significantly higher CRP values both on admission, and at day 3, than those who avoided colectomy (P = 0.002, and P = 0.04, respectively). All nine patients who required colectomy met the Travis criteria at day 3, compared with only 15 (42.9%) of those who did not undergo surgery at the index admission (P = 0.002). Among those who were colectomy-free at discharge 31.4% received IFX at day 5 or sooner, compared with only 11.1% of those who underwent colectomy (P = 0.41). No predictors of need for colectomy during the index admission were identified by multivariate logistic regression.

Colectomy-free survival at study end

Of the 35 patients who avoided colectomy during their index admission, 33 received standard three-dose induction with IFX. At the last point of follow-up, 29 (65.9%) of 44 patients remained colectomy-free. Thus, 82.9% (29/35) of those who responded to IFX on the index admission remained colectomy-free during follow-up. Among these 35 patients, 17 (48.6%) had < 1 year of follow-up, 8 (22.9%) had 1-2 years of follow-up, and 10 (28.6%) had > 2 years of follow-up post-first infusion of IFX. Two patients in each of these groups underwent colectomy during follow-up (χ^2 for trend, P = 0.69). The median time from first IFX infusion to colectomy for the six patients who had colectomy following an initial response to IFX therapy was 278 d (IQR 136.5-401.25 d).

Table 2 Clinical characteristics and demographics of 44 patients with acute severe ulcerative colitis receiving infliximab, according to colectomy status after index admission and at last point of follow-up *n* (%)

	Colectomy status after index admission			Colectomy status at last point of follow-up		
	Colectomy during index admission (*n* = 9)	Discharged colectomy-free (*n* = 35)	*P* value[2]	Colectomy during index admission or follow-up (*n* = 15)	Colectomy-free survival (*n* = 29)	*P* value[2]
Age at index admission[1]	45.6 ± 24.7	33.2 ± 12.1	0.18	42.2 ± 21.4	32.4 ± 11.3	0.11
Male	7 (77.8)	21 (60.0)	0.45	10 (66.7)	18 (62.1)	1.0
First presentation of UC	5 (55.6)	7 (20.0)	0.09	6 (40.0)	6 (20.7)	0.28
Disease extent	E1: 0 (0)	E1: 2 (5.7)		E1: 2 (13.3)	E1: 0 (0)	
	E2: 3 (33.3)	E2: 10 (28.6)		E2: 3 (20.0)	E2: 10 (34.5)	
	E3: 6 (66.7)	E3: 23 (65.7)	0.75[3]	E3: 10 (66.7)	E3: 19 (65.5)	0.10[3]
Current or previous smoker	4 (44.4)	14 (40.0)	1.0	6 (40.0)	12 (41.4)	1.0
Prescribed oral 5-ASA on admission	3 (33.3)	21 (60.0)	0.26	8 (53.3)	16 (55.2)	1.0
Prescribed thiopurine on admission	0 (0)	14 (40.0)	0.04	3 (20.0)	11 (37.9)	0.31
CRP (mg/L): day 0[1]	163 ± 62.5	71 ± 76	0.002	111.0 ± 83.0	79.0 ± 80.6	0.23
CRP (mg/L): day[1]	96 ± 69	39 ± 41.5	0.04	65.3 ± 65.5	42.9 ± 44.3	0.25
Number of stools per day: day 0[1]	13.3 ± 5.6	11.7 ± 5.9	0.46	13.0 ± 5.7	11.6 ± 5.9	0.44
Number of stools per day: day 3[1]	8.7 ± 4.6	6.5 ± 3.3	0.21	7.7 ± 4.2	6.6 ± 3.3	0.39
Met Travis criteria at day 3	9 (100)	15 (42.9)	0.002	10 (66.7)	14 (48.3)	0.34
Received IFX on day 5 or sooner	1 (11.1)	11 (31.4)	0.41			

[1]Data are presented as mean ± SD; [2]Independent samples *t*-test for continuous data and Fisher's exact test for categorical data; [3]χ^2 for trend. UC: Ulcerative colitis; 5-ASA: 5-aminosalicylate; CRP: C-reactive protein; IFX: Infliximab.

Demographic data and clinical characteristics of those who were colectomy-free at study end and those requiring colectomy at any point during the study are reported in Table 2.

Those who were colectomy-free at end of follow-up were generally younger, more likely to have had an established diagnosis of UC prior to their index admission, more likely to be receiving thiopurines on admission, and had lower mean CRP levels at admission, and on day 3, but none of these differences were statistically significant. Again, no predictors of need for colectomy at any point during follow-up were identified by multivariate logistic regression.

There were 18 of the 35 patients who avoided colectomy during their index admission who received IFX as a "bridge" to commencement of thiopurine therapy during, or soon after, the index admission. Of these, 17 (94.4%) were colectomy-free at the end of follow-up, compared with 12 of the 17 (70.6%) who did not commence thiopurine therapy ($P = 0.09$).

Glucocorticosteroid-free remission at study end

Of the 29 individuals who were colectomy-free at the last point of follow-up, 25 (86.2%) were in glucocorticosteroid-free remission. Therefore of the original 44 patients, 56.8% were colectomy-free and in glucocorticosteroid-free remission at the end of follow-up. Of the four patients who were colectomy-free but not in glucocorticosteroid-free remission, two had experienced a relapse of disease activity at their most recent assessment, one was receiving long-term low-dose oral glucocorticosteroids for co-existent inflammatory arthritis but was in remission clinically, and the fourth patient was still tapering the dose of glucocorticosteroids following recent index admission. Of the 18 patients who were "bridged" to thiopurine therapy during, or soon after, the index admission 15 (83.3%) were in glucocorticosteroid-free remission at the end of follow-up.

Safety and tolerability of IFX

During the study period one patient died from severe sepsis in the post-operative period, 2 d post-colectomy, and 20 d after a single IFX infusion. A total of eight other patients experienced adverse events with IFX. Five of these were minor, including skin rash in two patients, flushing in one patient, elevated transaminases in one patient, and pruritus in the fifth. All of these resolved without the need for discontinuation of IFX. In the other three patients the adverse events were intolerable and led to discontinuation of the drug. These included infusion reactions in two patients, and a delayed hypersensitivity reaction in the third. Of the three patients who discontinued IFX, one underwent colectomy and ileal pouch formation 15 mo after the initial IFX infusion, one was receiving low-dose glucocorticosteroids (2.5 mg prednisolone daily) in combination with methotrexate for co-existent inflammatory arthritis, and was in clinical remission as detailed above, whilst the third was colectomy-free and in glucocorticosteroid- free remission on azathioprine at the last point of follow-up.

DISCUSSION

This study has demonstrated that IFX is an effective rescue therapy in acute severe UC. After failure of intravenous glucocorticosteroids to control the acute severe episode, 80% of patients receiving IFX avoided the need for colectomy during the index admission. Those who met the Travis criteria on day 3 and those who were not receiving thiopurine therapy on admission were more

likely to require colectomy on their index admission. Among those who responded to IFX during their index admission, 83% remained colectomy-free and, in those who were colectomy-free, glucocorticosteroid-free remission was achieved in 86%, after a median follow-up period of 396 d. Of the total cohort of patients, 57% were colectomy-free and in glucocorticosteroid-free remission at the end of follow-up. The efficacy of IFX as a "bridge" to commencing thiopurine therapy is reinforced by the finding that over 90% of those patients "bridged" to thiopurine therapy avoided subsequent colectomy. Serious adverse events, resulting in the discontinuation of IFX were rare. However, there was one post-operative death as a result of severe sepsis.

Strengths of the study include the use of our biologics database which is maintained prospectively, allowing the inclusion of data from every patient who received IFX for acute severe UC in a large tertiary referral centre. The relatively long duration of follow-up among included individuals provides valuable, real-life data on outcomes among patients with acute severe UC receiving IFX. There are some limitations of the study. We relied on data extracted from medical records and computerized outpatient clinic letters, which may not always be accurate. We did not measure improvement of disease activity using validated indices, but instead used the dichotomous outcome measures of need for colectomy and glucocorticosteroid-free remission. As the patients included in this study are from a tertiary referral centre, the data may not be generalizable to patients in other hospitals. However, the spectrum of disease is likely to be more severe in a population such as this, which may have led to an underestimate of the efficacy of IFX in this setting. Finally, although this is one of the largest retrospective single centre experiences of the use of IFX in acute severe UC reported, the absolute number of patients involved remains small, meaning that we were unable to identify any patient demographics or clinical characteristics that were independently associated with a response to IFX therapy or avoidance of colectomy during follow-up.

The results of this study are comparable with those found in an RCT conducted in Scandinavia by Järnerot *et al*[15], in which 71% of those treated with IFX for moderately severe or severe UC avoided colectomy over 90 d. Previous retrospective studies have demonstrated similar efficacy, with between 66% and 84% of other cohorts from the United Kingdom, Denmark and Canada avoiding colectomy during their index admission[20-23]. Retrospective studies comparing IFX with ciclosporin from New Zealand and Italy found that around 80% of patients treated with IFX avoided colectomy at 3 mo, compared with 37% and 72% respectively for ciclosporin[27,28]. Recent data from the United Kingdom national IBD audit suggest that, among those who failed first line treatment with intravenous glucocorticosteroids, response rates to IFX were generally higher than those with ciclosporin[29]. One multi-centre European RCT comparing IFX to ciclosporin head-to-head in this setting has been published recently[30], and another United Kingdom-based trial is ongoing[31]. The European trial recruited 115 patients with acute severe UC. There was no significant difference detected in rates of response to therapy at 7 d, failure of therapy after 98 d, or colectomy rates, leading the authors to conclude that the two treatments were equivalent, and that the choice of which of these therapies to use should be guided by physician and centre experience[30].

In the trial reported by Järnerot *et al*[15], IFX appeared to have a more marked effect in those with less severe disease activity. Our finding that surrogate measures of severity, including a higher CRP level on day 0 and day 3, and meeting the Travis criteria at day 3, were associated with higher colectomy rates during the index admission are consistent with this. In addition, over 30% of patients who avoided colectomy during their index admission received their first IFX infusion at day 5 or sooner, compared with only 11% of those who required colectomy. Although this result did not reach statistical significance it is noteworthy, and suggests that there may be a potential benefit associated with earlier use of IFX, before the acute episode has reached its full intensity. However, these results are not supported by the findings of a multi-centre Scottish study, in which colectomy rates were higher among those treated on day 5 or sooner, compared with those treated on or after day 6[20].

All patients in our study who underwent colectomy during the index admission were thiopurine-naïve. This is in contrast to both the Scandinavian and Scottish studies, in which thiopurine use prior to admission did not appear to affect need for colectomy[15,20], although the numbers of patients receiving thiopurines at the time of admission in both these studies were smaller. Those who were "bridged" to thiopurine therapy in our study, following an initial response to IFX, appeared less likely to require colectomy during subsequent follow-up, although a large French multicentre case series of IFX in UC, which included patients with both acute severe and chronic relapsing disease, found that immunomodulator use was not predictive of the need for IFX optimization, IFX failure, or colectomy[32]. However, the role of thiopurines and IFX in this setting is still evolving, with preliminary results from the UC SUCCESS trial showing superiority of combination IFX and azathioprine therapy over either therapy alone in the setting of moderate to severe UC[33].

In conclusion, this study provides further evidence for the efficacy and safety of IFX as rescue therapy in acute severe UC, in one of the largest cohorts of patients with a longer duration of follow-up than previously available from other retrospective, real-life data. Overall, 66% of patients were colectomy-free at study end, and 57% had also achieved glucocorticosteroid free-remission. Although serious adverse events were rare, the mortality rate of 2% is a reminder for clinicians of the profound effects of biological therapy on the im-

mune system, in a group of patients who are already seriously ill. The results of this study suggest that the use of IFX as a "bridge" to thiopurine therapy in patients with acute severe UC is highly effective, but even among patients who are already receiving, or are intolerant of, thiopurines the use of three-dose induction therapy with IFX may avoid the need for colectomy in a significant number.

COMMENTS

Background

Ulcerative colitis (UC) is a chronic inflammatory condition affecting the lower gastrointestinal tract. Acute exacerbations of inflammation are managed using intravenous glucocorticosteroids initially, but if these fail biological therapies can be used in an attempt to control inflammation and avoid the need for surgery. Infliximab (IFX) is a monoclonal antibody directed against tumour necrosis factor alpha.

Research frontiers

Whilst randomised controlled trials have established the efficacy of IFX in UC, there have been only two placebo controlled trials specifically in the context of acute severe UC. Since results from clinical trials are not always replicated under normal clinical conditions and over longer durations of follow-up, results from real-life experience are essential to provide further insight into the efficacy of IFX in this setting.

Innovations and breakthroughs

This single-centre review shows that, over longer follow-up periods and in real-life clinical settings, similar outcomes can be obtained to those in the original clinical trials, with two-thirds of patients avoiding the need for surgery. It also sheds light on the role of azathioprine alongside IFX therapy.

Applications

This study will inform clinicians and patients of the likely outcomes if IFX is used as rescue therapy in acute severe UC, unresponsive to steroid treatment.

Terminology

The use of IFX to treat an episode of acute severe UC was defined as need for the drug during an in-patient admission with an acute inflammatory exacerbation of disease activity.

Peer review

This is a well-done paper dealing with IFX rescue treatment of patients with UC. The authors are completely right that observations from real life situations are of much higher importance than those obtained from an artificial setting of a randomized controlled trial.

REFERENCES

1 **Kappelman MD**, Rifas-Shiman SL, Kleinman K, Ollendorf D, Bousvaros A, Grand RJ, Finkelstein JA. The prevalence and geographic distribution of Crohn's disease and ulcerative colitis in the United States. *Clin Gastroenterol Hepatol* 2007; **5**: 1424-1429 [PMID: 17904915 DOI: 10.1016/j.cgh.2007.07.012]

2 **Langholz E**, Munkholm P, Nielsen OH, Kreiner S, Binder V. Incidence and prevalence of ulcerative colitis in Copenhagen county from 1962 to 1987. *Scand J Gastroenterol* 1991; **26**: 1247-1256 [PMID: 1763295 DOI: 10.3109/00365529108998621]

3 **Loftus CG**, Loftus EV, Harmsen WS, Zinsmeister AR, Tremaine WJ, Melton LJ, Sandborn WJ. Update on the incidence and prevalence of Crohn's disease and ulcerative colitis in Olmsted County, Minnesota, 1940-2000. *Inflamm Bowel Dis* 2007; **13**: 254-261 [PMID: 17206702 DOI: 10.1002/ibd.20029]

4 **Danese S**, Fiocchi C. Ulcerative colitis. *N Engl J Med* 2011; **365**: 1713-1725 [PMID: 22047562 DOI: 10.1056/NEJMra1102942]

5 **Blomberg B**, Järnerot G. Clinical evaluation and management of acute severe colitis. *Inflamm Bowel Dis* 2000; **6**: 214-227 [PMID: 10961594 DOI: 10.1097/00054725-200008000-00008]

6 **Carbonnel F**, Gargouri D, Lémann M, Beaugerie L, Cattan S, Cosnes J, Gendre JP. Predictive factors of outcome of intensive intravenous treatment for attacks of ulcerative colitis. *Aliment Pharmacol Ther* 2000; **14**: 273-279 [PMID: 10735919 DOI: 10.1046/j.1365-2036.2000.00705.x]

7 **Ho GT**, Mowat C, Goddard CJ, Fennell JM, Shah NB, Prescott RJ, Satsangi J. Predicting the outcome of severe ulcerative colitis: development of a novel risk score to aid early selection of patients for second-line medical therapy or surgery. *Aliment Pharmacol Ther* 2004; **19**: 1079-1087 [PMID: 15142197 DOI: 10.1111/j.1365-2036.2004.01945.x]

8 **Khan KJ**, Dubinsky MC, Ford AC, Ullman TA, Talley NJ, Moayyedi P. Efficacy of immunosuppressive therapy for inflammatory bowel disease: a systematic review and meta-analysis. *Am J Gastroenterol* 2011; **106**: 630-642 [PMID: 21407186 DOI: 10.1038/ajg.2011.64]

9 **Lichtiger S**, Present DH, Kornbluth A, Gelernt I, Bauer J, Galler G, Michelassi F, Hanauer S. Cyclosporine in severe ulcerative colitis refractory to steroid therapy. *N Engl J Med* 1994; **330**: 1841-1845 [PMID: 8196726 DOI: 10.1056/NEJM199406303302601]

10 **Loftus CG**, Loftus EV, Sandborn WJ. Cyclosporin for refractory ulcerative colitis. *Gut* 2003; **52**: 172-173 [PMID: 12524395 DOI: 10.1136/gut.52.2.172]

11 **Santos J**, Baudet S, Casellas F, Guarner L, Vilaseca J, Malagelada JR. Efficacy of intravenous cyclosporine for steroid refractory attacks of ulcerative colitis. *J Clin Gastroenterol* 1995; **20**: 285-289 [PMID: 7665815 DOI: 10.1097/00004836-199506000-00005]

12 **Stack WA**, Long RG, Hawkey CJ. Short- and long-term outcome of patients treated with cyclosporin for severe acute ulcerative colitis. *Aliment Pharmacol Ther* 1998; **12**: 973-978 [PMID: 9798801 DOI: 10.1046/j.1365-2036.1998.00396.x]

13 **Carbonnel F**, Boruchowicz A, Duclos B, Soulé JC, Lerebours E, Lémann M, Belaïche J, Colombel JF, Cosnes J, Gendre JP. Intravenous cyclosporine in attacks of ulcerative colitis: short-term and long-term responses. *Dig Dis Sci* 1996; **41**: 2471-2476 [PMID: 9011460 DOI: 10.1007/BF02100145]

14 **Arts J**, D'Haens G, Zeegers M, Van Assche G, Hiele M, D'Hoore A, Penninckx F, Vermeire S, Rutgeerts P. Long-term outcome of treatment with intravenous cyclosporin in patients with severe ulcerative colitis. *Inflamm Bowel Dis* 2004; **10**: 73-78 [PMID: 15168804 DOI: 10.1097/00054725-200403000-00002]

15 **Järnerot G**, Hertervig E, Friis-Liby I, Blomquist L, Karlén P, Grännö C, Vilien M, Ström M, Danielsson A, Verbaan H, Hellström PM, Magnuson A, Curman B. Infliximab as rescue therapy in severe to moderately severe ulcerative colitis: a randomized, placebo-controlled study. *Gastroenterology* 2005; **128**: 1805-1811 [PMID: 15940615 DOI: 10.1053/j.gastro.2005.03.003]

16 **Probert CS**, Hearing SD, Schreiber S, Kühbacher T, Ghosh S, Arnott ID, Forbes A. Infliximab in moderately severe glucocorticoid resistant ulcerative colitis: a randomised controlled trial. *Gut* 2003; **52**: 998-1002 [PMID: 12801957 DOI: 10.1136/gut.52.7.998]

17 **Rutgeerts P**, Sandborn WJ, Feagan BG, Reinisch W, Olson A, Johanns J, Travers S, Rachmilewitz D, Hanauer SB, Lichtenstein GR, de Villiers WJ, Present D, Sands BE, Colombel JF. Infliximab for induction and maintenance therapy for ulcerative colitis. *N Engl J Med* 2005; **353**: 2462-2476 [PMID: 16339095 DOI: 10.1056/NEJMoa050516]

18 **Sands BE**, Tremaine WJ, Sandborn WJ, Rutgeerts PJ, Hanauer SB, Mayer L, Targan SR, Podolsky DK. Infliximab in the treatment of severe, steroid-refractory ulcerative colitis: a pilot study. *Inflamm Bowel Dis* 2001; **7**: 83-88 [PMID: 11383595 DOI: 10.1097/00054725-200105000-00001]

19 **Ford AC**, Sandborn WJ, Khan KJ, Hanauer SB, Talley NJ, Moayyedi P. Efficacy of biological therapies in inflammatory bowel disease: systematic review and meta-analysis. *Am J Gastroenterol* 2011; **106**: 644-59, quiz 660 [PMID: 21407183 DOI: 10.1038/ajg.2011.73]

20 **Lees CW**, Heys D, Ho GT, Noble CL, Shand AG, Mowat C,

Boulton-Jones R, Williams A, Church N, Satsangi J, Arnott ID. A retrospective analysis of the efficacy and safety of infliximab as rescue therapy in acute severe ulcerative colitis. *Aliment Pharmacol Ther* 2007; **26**: 411-419 [PMID: 17635376 DOI: 10.1111/j.1365-2036.2007.03383.x]

21 **Waters O,** Saunders M, Clarke M, Daneshmend T, Ahmad T. Infliximab rescue therapy for steroid refractory acute severe ulcerative colitis in the Exeter IBD cohort. *Gut* 2011; **60:** A215 [DOI:10.1136/gut.2011.239301.454]

22 **Bressler B**, Law JK, Al Nahdi Sheraisher N, Atkinson K, Byrne MF, Chung HV, Fishman M, Partovi N, Pearson D, Penner R, Enns RA. The use of infliximab for treatment of hospitalized patients with acute severe ulcerative colitis. *Can J Gastroenterol* 2008; **22**: 937-940 [PMID: 19018340]

23 **Teisner AS**, Ainsworth MA, Brynskov J. Long-term effects and colectomy rates in ulcerative colitis patients treated with infliximab: a Danish single center experience. *Scand J Gastroenterol* 2010; **45**: 1457-1463 [PMID: 20701434 DOI: 10.3109/00365521.2010.510572]

24 **Silverberg MS**, Satsangi J, Ahmad T, Arnott ID, Bernstein CN, Brant SR, Caprilli R, Colombel JF, Gasche C, Geboes K, Jewell DP, Karban A, Loftus Jr EV, Peña AS, Riddell RH, Sachar DB, Schreiber S, Steinhart AH, Targan SR, Vermeire S, Warren BF. Toward an integrated clinical, molecular and serological classification of inflammatory bowel disease: Report of a Working Party of the 2005 Montreal World Congress of Gastroenterology. *Can J Gastroenterol* 2005; **19** Suppl A: 5-36 [PMID: 16151544]

25 **Travis SP**, Farrant JM, Ricketts C, Nolan DJ, Mortensen NM, Kettlewell MG, Jewell DP. Predicting outcome in severe ulcerative colitis. *Gut* 1996; **38**: 905-910 [PMID: 8984031 DOI: 10.1136/gut.38.6.905]

26 **Truelove SC**, Witts LJ. Cortisone in ulcerative colitis; final report on a therapeutic trial. *Br Med J* 1955; **2**: 1041-1048 [PMID: 13260656 DOI: 10.1136/bmj.2.4947.1041]

27 **Dean KE**, Hikaka J, Huakau JT, Walmsley RS. Infliximab or cyclosporine for acute severe ulcerative colitis: a retrospective analysis. *J Gastroenterol Hepatol* 2012; **27**: 487-492 [PMID: 22098019 DOI: 10.1111/j.1440-1746.2011.06958.x]

28 **Mocciaro F**, Renna S, Orlando A, Rizzuto G, Sinagra E, Orlando E, Cottone M. Cyclosporine or infliximab as rescue therapy in severe refractory ulcerative colitis: early and long-term data from a retrospective observational study. *J Crohns Colitis* 2012; **6**: 681-686 [PMID: 22398101 DOI: 10.1016/j.crohns.2011.11.021]

29 **Arnott ID,** Leiper K, Down C, Lowe D, Potter J, Rhodes JM. Outcome of acute severe ulcerative colitis: data from the UK national IBD audit. *Gastroenterology* 2010; **138** (suppl I): S106 [DOI: 10.1016/S0016-5085(10)60484-2]

30 **Laharie D**, Bourreille A, Branche J, Allez M, Bouhnik Y, Filippi J, Zerbib F, Savoye G, Nachury M, Moreau J, Delchier JC, Cosnes J, Ricart E, Dewit O, Lopez-Sanroman A, Dupas JL, Carbonnel F, Bommelaer G, Coffin B, Roblin X, Van Assche G, Esteve M, Färkkilä M, Gisbert JP, Marteau P, Nahon S, de Vos M, Franchimont D, Mary JY, Colombel JF, Lémann M. Ciclosporin versus infliximab in patients with severe ulcerative colitis refractory to intravenous steroids: a parallel, open-label randomised controlled trial. *Lancet* 2012; **380**: 1909-1915 [PMID: 23063316 DOI: 10.1016/S0140-6736(12)61084-8]

31 Comparison of infliximab and ciclosporin in steroid resistant ulcerative colitis: a trial. Available from: URL: http://www.controlled-trials.com/ISRCTN22663589

32 **Oussalah A**, Evesque L, Laharie D, Roblin X, Boschetti G, Nancey S, Filippi J, Flourié B, Hebuterne X, Bigard MA, Peyrin-Biroulet L. A multicenter experience with infliximab for ulcerative colitis: outcomes and predictors of response, optimization, colectomy, and hospitalization. *Am J Gastroenterol* 2010; **105**: 2617-2625 [PMID: 20736936 DOI: 10.1038/ajg.2010.345]

33 **Panccione R,** Ghosh S, Middleton S, Marquez JR, Khalif I, Flint L, van Hoogstraten H, Zheng HZ, Danese S, Rutgeerts PJ. Infliximab, azathioprine, or infliximab plus azathioprine for treatment of moderate to severe ulcerative colitis: the UC success trial. *Gastroenterology* 2011; **140:** S134

P- Reviewer Nielsen OH **S- Editor** Wen LL
L- Editor A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i7.1098

World J Gastroenterol 2013 February 21; 19(7): 1098-1103
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Pegylated interferon alfa and ribavirin for children with chronic hepatitis C

Irit Rosen, Michal Kori, Orly Eshach Adiv, Baruch Yerushalmi, Nataly Zion, Ron Shaoul

Irit Rosen, Orly Eshach Adiv, Nataly Zion, Ron Shaoul, Pediatric Gastroenterology and Nutrition Unit, Meyer Children's Hospital, Rambam Medical Center, Bruce Rappaport Faculty of Medicine, Technion, Haifa 31096, Israel
Michal Kori, Pediatric Gastroenterology and Nutrition Unit, Kaplan Medical Center, Rehovot 76100, Israel
Baruch Yerushalmi, Pediatric Gastroenterology and Nutrition Unit, Soroka Medical Center, Ben Gurion University Faculty of Medicine, Be'er Sheva 84101, Israel
Author contributions: Shaoul R initiated the study; Rosen I and Shaoul R wrote the manuscript; Rosen I, Shaoul R, Eshach Adiv O, Kori M, Zion N, and Yerushalmi B collected patients' data and performed follow-up.
Correspondence to: Irit Rosen, MD, Pediatric Gastroenterology and Nutrition Unit, Meyer Children's Hospital, Rambam Medical Center, Bruce Rappaport Faculty of Medicine, Technion, PO Box 9697, Efron Street Bat-Galim, Haifa 31096, Israel. irita111@gmail.com
Telephone: +972-4-8542914 Fax: +972-4-8541805
Received: June 14, 2012 Revised: September 4, 2012
Accepted: October 16, 2012
Published online: February 21, 2013

Abstract

AIM: To study current treatment options for pediatric hepatitis C infection and their associated success rates.

METHODS: We retrospectively reviewed charts of thirty children who had been treated with combination therapy of pegylated interferon alfa plus ribavirin for chronic hepatitis C infection. Patients had been treated with ribavirin (15 mg/kg per day) and either pegylated interferon alfa 2a (180 mg/m^2 once weekly) or pegylated interferon alfa 2b (1.5 mg/kg once weekly). Patients' follow-up included subjective assessment of complaints, physical examination including weight and height, as well as laboratory evaluations for viral load [before treatment, at 12 wk, and 6 mo following treatment completion, as determined by sustained viral response (SVR)], complete blood count, liver enzymes, alkaline phosphatase, bilirubin, renal function tests, and thyroid function tests. For patients not achieving a two log decrease in viral load at treatment week 12, treatment was discontinued and the patient was considered a treatment non-responder.

RESULTS: Thirty children aged 3-18 years were included in the study. Twenty patients (11 males, 9 females) received pegylated interferon alfa 2b and ten patients (6 males, 4 females) received pegylated interferon alfa 2a. Twenty-three patients were infected with genotype 1, six patients were infected with genotype 3, and one patient was infected with genotype 2. Twenty patients (67%) achieved SVR. Treatment success rates were 90% with pegylated interferon alfa 2a *vs* 55% with pegylated interferon alfa 2b. Although a trend was noted for improved outcomes in the group receiving pegylated interferon alfa 2a, there were no statistically significant outcome differences between the two treatment groups (P = 0.1). Treatment success was 56.5% for patients infected with genotype 1 virus, compared to 100% for patients infected with other genotypes (P = 0.064). There was no difference in treatment response between males and females. A cut-off age of twelve years was used to dichotomize younger *vs* older participants; however, no difference in treatment response was observed between these groups. Using multivariate regression analysis, we could not determine predictors for achieving SVR from among the variables we examined (age, sex, and viral genotype). Although we noted a trend toward SVR with peginterferon alfa-2a, there was no statistical difference between the two peginterferons. A high incidence of adverse reactions to treatment was noted. Twenty-five patients (83%) suffered from at least one adverse reaction, but most experienced more than one adverse reaction. All patients except one became leukopenic (white blood cell count less than 5500 leukocytes/μL), six (20%) became anemic (hemoglobin less than 110 g/L), and one (3.3%) became thrombocytopenic (platelets less than 100 000/μL).

CONCLUSION: Combination therapy to treat hepatitis

C in children is as effective as in adults. There may be a benefit for treatment with pegylated interferon alfa 2a.

© 2013 Baishideng. All rights reserved.

Key words: Hepatitis C virus; Interferon alfa; Ribavirin; Children; Sustained viral response

Rosen I, Kori M, Eshach Adiv O, Yerushalmi B, Zion N, Shaoul R. Pegylated interferon alfa and ribavirin for children with chronic hepatitis C. *World J Gastroenterol* 2013; 19(7): 1098-1103 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i7/1098.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i7.1098

INTRODUCTION

Hepatitis C virus (HCV) infection is relatively uncommon in the pediatric population. Populations at risk of HCV infection are infants born to infected mothers who acquire the infection vertically, and children with chronic diseases who acquire the virus through infected blood products[1]. Vertical transmission is the predominant source for new pediatric HCV infections. Estimated transmission rates are 2%-5% when the mother is viremic during pregnancy[1,2]. Mothers with greater than 10^6 copies/mL of HCV RNA are more likely to transmit the infection to their infants compared to mothers with lower levels of viremia. Chronic hepatitis C (CHC) develops in 55%-80% of infected children[2,3]. The prevalence of HCV in children in developed countries ranges from 0.1% to 0.4%[4-6]. Treatment is contraindicated for patients less than three years of age because of safety concerns and to allow for spontaneous viral clearance. After age four, spontaneous viral clearance is unlikely[7]. The rate of viral clearance in children with CHC who acquired the infection vertically is 20%[8]. Most children who clear the virus do so during the first five years of follow-up.

In most cases, HCV infection in children is asymptomatic. Histological findings are minor and severe complications are uncommon. However, chronic hepatic inflammation from HCV infection can progress to advanced liver fibrosis and cirrhosis in four percent to six percent of infected children[9,10]. The risk of developing cirrhosis is approximately 20% and the risk of developing hepatocellular carcinoma is 2%-5%[11]. Hepatocellular carcinoma has been reported in adolescents with CHC[12].

The current treatment of choice for CHC in children, as in the adult population[13,14], is combination pegylated interferon alfa plus ribavirin. We have been prescribing this treatment regimen for the last seven years. We retrospectively reviewed charts of patients who had been treated for CHC with pegylated interferon alfa plus ribavirin in several Israeli Pediatric Gastroenterology Centers.

MATERIALS AND METHODS

Patient selection

Complete data were available for a total of thirty children with chronic HCV infection who were treated between 2003 and 2010. Chronic HCV infection was diagnosed by the presence of anti-HCV antibodies and HCV RNA positivity. Additional available information included: alanine aminotransferase (ALT) levels during the three mo prior to treatment, laboratory studies during follow-up, and, for some patients, liver biopsy specimens showing evidence of CHC or Actitest and Fibrotest results.

Ten patients were treated with pegylated interferon alfa 2a (180 mg/m^2 once weekly) plus ribavirin 15 mg/kg per day and 20 patients were treated with pegylated interferon alfa 2b (1.5 mg/kg once weekly) plus ribavirin (15 mg/kg per day). The decision for which peginterferon was prescribed depended on the patient's medical insurance. Length of treatment was 24 wk for patients with genotypes 2 or 3 (seven patients) and 48 wk for patients with genotype 1 (twenty-three patients). Patient follow-up included assessment of subjective complaints, physical examination with weight and height, and laboratory workup which included viral load [before treatment, at 12 wk, and 6 mo following treatment completion, as determined by sustained viral response (SVR)], complete blood count, liver enzymes, alkaline phosphatase, bilirubin, renal function tests, and thyroid function tests. In patients not achieving a two $\log_{10}$ IU/mL decrease in their viral loads at week 12, treatment was discontinued and the patient was considered a treatment non-responder.

Statistical analysis

Statistical analysis was performed using the SPSS software package version 15 (SPSS, Chicago, IL, United States).The normality of quantitative variables was tested using the Kolmogorov-Smirnov test. Because most of the quantitative variables were not normally distributed, the Mann-Whitney *U* test was used to analyze differences between SVR groups. Fisher's exact test was used to determine the relationship between SVR groups and categorical variables (gender, treatment type, genotype). Logistic regression was performed to predict relationships between SVR groups and several independent variables. A P value < 0.05 was considered statistically significant.

RESULTS

Patient characteristics

Thirty patients (13 girls and 17 boys) aged three to eighteen years were included in this study. Patients' characteristics and results are shown in Table 1. Twelve acquired HCV vertically, eleven through blood products, one by needle stick, and six patients had no identifiable source of infection.

It should be noted that several patients had underlying comorbidities: one patient had Becker Muscular Dystrophy, one patient had proctitis, one had Congenital Adrenal Hyperplasia, one had human immunodeficiency virus infection, and one had Obstructive Sleep Apnea. Twenty-three patients were infected with HCV genotype 1 (genotype 1b in twenty patients and genotype 1a in three patients). Six patients had genotype 3a, and one

Table 1 Characteristics and results for non-responders and those who achieved a sustained virologic response *n* (%)

	Non-responders (*n* = 10)	Gained SVR (*n* = 20)	*P* value
Male	6 (60)	11 (55)	1.00
Age ⩾ 12 yr	7 (70)	14 (70)	1.00
Peginterferon alfa-2a treatment	1 (10)	9 (45)	0.1
Genotype 1	10 (100)	13 (65)	0.065
WBC (cells/μL)[1]	3422.22 ± 839.47	3296.500 ± 834.57	0.39
HGB (g/L)[1]	110.99 ± 9.1	110.26 ± 10	0.07
ALT (U/L)[1]	52.33 ± 16.42	77.85 ± 41.98	0.08
AST (U/L)[1]	43.90 ± 19.31	55.65 ± 31.39	0.54
Weight (kg)[1]	48.66 ± 23.76	51.62 ± 21.96	0.66
Median viral load	747 000	332 500	0.49

[1]Data are presented mean ± SD. SVR: Sustained virologic response; WBC: White blood cells; HGB: Hemoglobin level; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase.

patient had genotype 2. Viral loads prior to treatment ranged from 134 000 to 26 200 000 copies/mL. ALT levels prior to treatment ranged from 24 to 183 U/L (mean 69.93 ± 37.64 U/L).

Liver histology ranged from mild chronic portal inflammation to moderate portal inflammation with fibrous expansion. Fibrotest scores ranged from 0 to 3 and activity ranged from 0 to 3 on the Actitest. Twenty-six patients were interferon-naïve, three patients were non-responders to previous interferon monotherapy, and one patient was a non-responder to previous pegylated interferon alfa 2a therapy (and had also been treated with pegylated interferon alfa 2b).

Treatment response

Viral load at 12 wk was undetectable in twenty-two patients, slightly positive in one patient, and positive in six patients. For one patient, viral load examination was delayed and was not tested until six months after treatment initiation, at which time it was negative (Figure 1). SVR six months following treatment completion was achieved in twenty patients, for an overall treatment success rate of 67%.

Among twenty-three patients with undetectable HCV RNA [as measured by polymerase chain reaction (PCR)] following 12 wk of treatment, 19 achieved SVR. Only one of seven patients who were positive for HCV RNA at 12 wk achieved SVR. This patient's viral load had decreased greater than two $\log_{10}$ IU/mL at 12 wk compared to baseline (Figure 1).

Forty-three percent (10/23) of those with genotype 1 did not achieve SVR; of those, four responded to treatment after twelve weeks, and six were non-responders. One patient who failed to respond was non-compliant with ribavirin, and another patient stopped ribavirin due to serious headaches and rash.

Twenty patients (eleven males, nine females) received pegylated interferon alfa 2b and ten patients (six males, four females) received pegylated interferon alfa 2a. The success rate was 90% (9/10) for those receiving pegylated interferon 2a combined with ribavirin (six patients

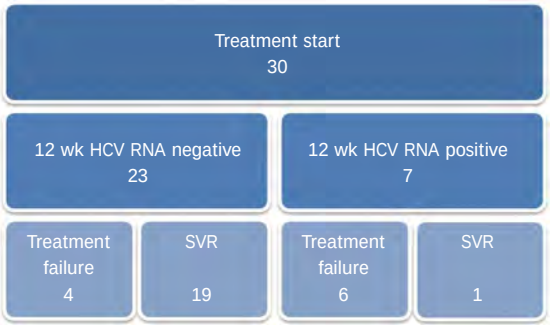


Figure 1 Hepatitis C virus RNA polymerase chain reaction results 12 wk after treatment and outcomes 6 mo after treatment completion. HCV: Hepatitis C virus; SVR: Sustained viral response.

with genotype 1b, three with genotype 1a, and one with genotype 3a). The success rate for those with genotype 1b was 83% (5/6).

Fifty-five percent (11/20) of patients receiving pegylated interferon alfa 2b and ribavirin treatment (fourteen patients with genotype 1b, five patients with genotype 3a, and one patient with genotype 2) achieved SVR. Only 36% (5/14) of patients with genotype 1b achieved SVR with this treatment. Although a trend was noted in favor of pegylated interferon alfa 2a, there were no significant differences between the two treatment groups ($P = 0.1$). The treatment success rate was 56.5% in patients infected with genotype 1 virus, compared to 100% in patients infected with non-1 genotypes ($P = 0.064$).

There was no statistical difference in response to treatment between males and females. A cut-off age of twelve years was used to compare response to treatment between younger and older patients; no statistical difference was observed. Treatment failure occurred only among those infected with genotype 1 HCV.

We could not determine predictors for achieving SVR from among the variables examined (age, sex, and viral genotype) in our multivariate regression analysis. We did observe a trend toward greater SVR when using peginterferon alfa-2a, but this difference between the two peginterferons was not statistically significant, most likely due to our small sample.

Adverse reactions

Adverse events were common, occurring in 83% of patients (25/30) and including flu-like symptoms, malaise, headaches, fever, lymphadenopathy, anorexia, weight loss, hair loss, myalgia, fat necrosis, somnolence, sleep disturbance, anemia, neutropenia, thrombocytopenia, oral aphthae and aseptic meningitis (following treatment completion). No patient experienced severe bone marrow suppression (Table 2).

Leukocyte counts before treatment ranged from 3630 to 11400 cells/μL (mean ± SD, 7540.69 ± 1950.3 cells/μL) and from 1750 to 5770 cells/μL (mean ± SD, 3335.5 ± 823 cells/μL) during treatment. The Mann-Whitney *U* test showed a significant difference between leukocyte levels before and during treatment ($P < 0.001$). Hemoglobin levels also decreased, from 116 to 169 g/L (mean ±

Table 2 Comparison of adverse reactions between those receiving peginterferon-alfa 2a and those receiving peginterferon-alfa 2b

Adverse reaction	Peginterferon-alfa 2a (n = 10)	Peginterferon-alfa 2b (n = 20)	Total
None	0	5	5
Flu-like symptoms	4	5	9
Weight loss	1	3	4
Hair loss	1	3	4
Headache	2	2	4
Aseptic meningitis	1	0	1
GI symptoms	1	4	5
Oral aphthae	1	0	1
Pruritus	1	0	1
Fat necrosis	1	1	2
Sleep disturbance	2	0	2
Lymphadenopathy	1	0	1
Absolute neutropenia	0	2	2
Anemia (HGB < 110 g/L)	2	4	6
Thrombocytopenia (platelets < 100 000 cells/μL)	1	0	1
Direct hyperbilirubinemia (mg/dL)	1	0	1
Indirect hyperbilirubinemia (mg/dL)	0	1	1

GI: Gastrointestinal; HGB: Hemoglobin level.

SD, 135.3 ± 12.8 g/L) before treatment to 95 to 139 g/L (mean ± SD, 115 ± 10.2 g/L) during treatment, as determined by t-test ($P < 0.001$).

Only one patient experienced direct hyperbilirubinemia of 15 mg/L, with a total bilirubin level of 18 mg/L. Another patient had indirect hyperbilirubinemia of 26 mg/L during treatment. All patients had normal gamma-glutamyl transferase levels and renal function throughout treatment. One patient developed hypothyroidism which subsequently resolved.

DISCUSSION

Combination therapy of pegylated interferon alfa plus ribavirin for chronic HCV infection in children was found to be as effective as in adults. Fifty-seven percent of children infected with genotype 1 achieved SVR and children infected with other HCV genotypes achieved 100% SVR. The success rates are similar to success rates reported in other pediatric studies[13,15].

Treatment strategies for CHC have evolved over the years, from interferon monotherapy to combination interferon alfa plus ribavirin[16] to combination pegylated interferon alfa plus ribavirin[14,15,17,18]. This latter combination is currently the treatment of choice for CHC in children, as in adults[13,14]. Schwarz *et al*[15] reported that combination ribavirin plus peginterferon alfa is superior to peginterferon alfa and placebo for children and adolescents with CHC. Two options exist for pegylated interferon alfa therapy: pegylated interferon alfa 2a and pegylated interferon alfa 2b[19-23]. The main treatment goal is to achieve an SVR, defined as undetectable serum HCV RNA for six months following cessation of treatment. Treatment outcomes depend on HCV genotype and viral load at the beginning of treatment[13,17,24]. The genotype is a key predictor of treatment response[24]. Wirth *et al*[14] found that patients infected with genotype 1 experienced 53% SVR, compared to patients with genotypes 2 or 3 who had 93% SVR, and patients with genotype 4 who had 80% SVR. Baseline viral load is the main response predictor for patients infected with HCV genotype 1. SVR may be more likely in patients who have lower viral loads[14]. In a systematic review, Hu *et al*[13] reported that SVR rates in children with CHC ranged from 30% to 100%, comparable to those seen among adults.

Combination treatment causes high rates of adverse reactions, with almost all children suffering from transient flu-like symptoms. Other adverse reactions are diverse. In our study, 83% of patients suffered from adverse reactions, but almost all patients remained compliant with therapy. Indeed, in most studies, the treatment is tolerated and compliance is good[24,25]. The treatment protocol has been associated with significant changes in body weight, linear growth and body composition (loss of fat mass); however, these effects seem reversible[26].

In adults, HCV RNA PCR results after twelve weeks of treatment predict treatment outcomes. Failure to respond (a less than two $\log_{10}$ drop from baseline HCV RNA levels) is associated with non-response to treatment, and the therapy should be discontinued. It is unknown whether this rule applies to pediatric patients as well[13]. However, this rule does fit with our current findings.

All treatment failures in our study occurred in patients infected with genotype 1, which was also the most frequent genotype among our patients. SVR rates depend on genotype, as in the adult population, and success rates are significantly better (greater than 90%) in patients infected with genotypes 2 and 3 compared to patients infected with genotype 1 or 4 (approximately 50%)[14].

Although both pegylated interferon alfa regimens have similar safety profiles, success rates differ. In a systematic review of twelve randomized clinical trials including 5008 patients, Awad *et al*[20] concluded that peginterferon alfa-2a is associated with higher SVR than peginterferon alfa-2b. We have also noted different success rates between the two pegylated interferon alfa products, as reported in previous studies[19,20,22,23]. With caution and consideration of our small sample size, our results show the trend by which combination treatment with pegylated interferon alfa 2a may be superior to pegylated interferon alfa 2b. Such a trend has been shown previously only in adult studies[19,20,22].

The main weakness of the study is its retrospective nature and relatively small number of patients. Nevertheless, our results are consistent with previous studies in children and adults and our study is the first to compare the two pegylated interferon products in children. Further prospective studies are highly encouraged.

COMMENTS

Background

Hepatitis C virus (HCV) is a virus that chronically infects the liver. Infection by

this virus is a known risk factor for liver disease, liver failure and its complications, and even cases of liver tumors (hepatocellular carcinoma). There are limited publications about experience in treating children, recommendations for a preferred regimen, and data on treatment success rates. In this study, thirty children with chronic hepatitis C had been treated with combination therapy of peginterferon alfa plus ribavirin (as is recommended in adults). Ten of them had been given peginterferon alfa 2a, twenty of them had been given peginterferon alfa 2b, since there is no accepted preference of either of these drugs.

Research frontiers

This paper introduces the Israeli experience in treatment of children with chronic HCV infection, including success rates and adverse reactions.

Innovations and breakthroughs

The study found that success rates in children are very similar to those reported in adults. Although no significant superiority of either of the drugs was found, (most probably due to small group size), a trend for better results was noted with peginterferon alfa 2a. Even though side effects were very common during the treatment regimen, children were found to be very compliant, and most of them completed the treatment course.

Applications

The study results suggest that success rates of treatment are similar to those noted in adults, and that treatment with combination peginterferon alfa 2a plus ribavirin may be superior.

Terminology

Peginterferon alfa: A pegylated interferon drug that is given in a subcutaneous manner once a week, as opposed to previous treatment with interferon, which was given by injections 3 times a week. Treatment success: Achieving sustained viral response means that six months after treatment completion there is no detectable HCV RNA in the blood.

Peer review

Data on HCV therapy in pediatric patients are limited, thus this paper adds important information.

REFERENCES

1 **Guido M**, Rugge M, Jara P, Hierro L, Giacchino R, Larrauri J, Zancan L, Leandro G, Marino CE, Balli F, Bagni A, Timitilli A, Bortolotti F. Chronic hepatitis C in children: the pathological and clinical spectrum. *Gastroenterology* 1998; **115**: 1525-1529 [PMID: 9834281 DOI: 10.1016/S0016-5085(98)70032-0]

2 **Chang MH**, Ni YH, Hwang LH, Lin KH, Lin HH, Chen PJ, Lee CY, Chen DS. Long term clinical and virologic outcome of primary hepatitis C virus infection in children: a prospective study. *Pediatr Infect Dis J* 1994; **13**: 769-773 [PMID: 7808843 DOI: 10.1097/00006454-199409000-00003]

3 **Vogt M**, Lang T, Frösner G, Klingler C, Sendl AF, Zeller A, Wiebecke B, Langer B, Meisner H, Hess J. Prevalence and clinical outcome of hepatitis C infection in children who underwent cardiac surgery before the implementation of blood-donor screening. *N Engl J Med* 1999; **341**: 866-870 [PMID: 10498458 DOI: 10.1056/NEJM199909163411202]

4 Hepatitis C virus infection. American Academy of Pediatrics. Committee on Infectious Diseases. *Pediatrics* 1998; **101**: 481-485 [PMID: 9499195]

5 **Ades AE**, Parker S, Walker J, Cubitt WD, Jones R. HCV prevalence in pregnant women in the UK. *Epidemiol Infect* 2000; **125**: 399-405 [PMID: 11117964 DOI: 10.1017/S0950268899004598]

6 **Gerner P**, Wirth S, Wintermeyer P, Walz A, Jenke A. Prevalence of hepatitis C virus infection in children admitted to an urban hospital. *J Infect* 2006; **52**: 305-308 [PMID: 16473408 DOI: 10.1016/j.jinf.2005.04.004]

7 **Bortolotti F**, Verucchi G, Cammà C, Cabibbo G, Zancan L, Indolfi G, Giacchino R, Marcellini M, Marazzi MG, Barbera C, Maggiore G, Vajro P, Bartolacci S, Balli F, Maccabruni A, Guido M. Long-term course of chronic hepatitis C in children: from viral clearance to end-stage liver disease. *Gastroenterology* 2008; **134**: 1900-1907 [PMID: 18439604 DOI: 10.1053/j.gastro.2008.02.082]

8 **European Paediatric Hepatitis C Virus Network.** Three broad modalities in the natural history of vertically acquired hepatitis C virus infection. *Clin Infect Dis* 2005; **41**: 45-51 [PMID: 15937762 DOI: 10.1086/430601]

9 **Goodman ZD**, Makhlouf HR, Liu L, Balistreri W, Gonzalez-Peralta RP, Haber B, Jonas MM, Mohan P, Molleston JP, Murray KF, Narkewicz MR, Rosenthal P, Smith LJ, Robuck PR, Schwarz KB. Pathology of chronic hepatitis C in children: liver biopsy findings in the Peds-C Trial. *Hepatology* 2008; **47**: 836-843 [PMID: 18167062 DOI: 10.1002/hep.22094]

10 **Guido M**, Bortolotti F, Leandro G, Jara P, Hierro L, Larrauri J, Barbera C, Giacchino R, Zancan L, Balli F, Crivellaro C, Cristina E, Pucci A, Rugge M. Fibrosis in chronic hepatitis C acquired in infancy: is it only a matter of time? *Am J Gastroenterol* 2003; **98**: 660-663 [PMID: 12650803 DOI: 10.1111/j.1572-0241.2003.07293.x]

11 **Freeman AJ**, Dore GJ, Law MG, Thorpe M, Von Overbeck J, Lloyd AR, Marinos G, Kaldor JM. Estimating progression to cirrhosis in chronic hepatitis C virus infection. *Hepatology* 2001; **34**: 809-816 [PMID: 11584380 DOI: 10.1053/jhep.2001.27831]

12 **González-Peralta RP**, Langham MR, Andres JM, Mohan P, Colombani PM, Alford MK, Schwarz KB. Hepatocellular carcinoma in 2 young adolescents with chronic hepatitis C. *J Pediatr Gastroenterol Nutr* 2009; **48**: 630-635 [PMID: 19412012 DOI: 10.1097/MPG.0b013e318170af04]

13 **Hu J**, Doucette K, Hartling L, Tjosvold L, Robinson J. Treatment of hepatitis C in children: a systematic review. *PLoS One* 2010; **5**: e11542 [PMID: 20644626 DOI: 10.1371/journal.pone.0011542]

14 **Wirth S**, Ribes-Koninckx C, Calzado MA, Bortolotti F, Zancan L, Jara P, Shelton M, Kerkar N, Galoppo M, Pedreira A, Rodriguez-Baez N, Ciocca M, Lachaux A, Lacaille F, Lang T, Kullmer U, Huber WD, Gonzalez T, Pollack H, Alonso E, Broue P, Ramakrishna J, Neigut D, Valle-Segarra AD, Hunter B, Goodman Z, Xu CR, Zheng H, Noviello S, Sniukiene V, Brass C, Albrecht JK. High sustained virologic response rates in children with chronic hepatitis C receiving peginterferon alfa-2b plus ribavirin. *J Hepatol* 2010; **52**: 501-507 [PMID: 20189674 DOI: 10.1016/j.jhep.2010.01.016]

15 **Schwarz KB**, Gonzalez-Peralta RP, Murray KF, Molleston JP, Haber BA, Jonas MM, Rosenthal P, Mohan P, Balistreri WF, Narkewicz MR, Smith L, Lobritto SJ, Rossi S, Valsamakis A, Goodman Z, Robuck PR, Barton BA. The combination of ribavirin and peginterferon is superior to peginterferon and placebo for children and adolescents with chronic hepatitis C. *Gastroenterology* 2011; **140**: 450-458.e1 [PMID: 21036173 DOI: 10.1053/j.gastro.2010.10.047]

16 **Wirth S**, Lang T, Gehring S, Gerner P. Recombinant alfa-interferon plus ribavirin therapy in children and adolescents with chronic hepatitis C. *Hepatology* 2002; **36**: 1280-1284 [PMID: 12395341 DOI: 10.1053/jhep.2002.36495]

17 **Davison SM**, Kelly DA. Management strategies for hepatitis C virus infection in children. *Paediatr Drugs* 2008; **10**: 357-365 [PMID: 18998746 DOI: 10.2165/0148581-200810060-00003]

18 **Palumbo E**. Treatment for chronic hepatitis C in children: a review. *Am J Ther* 2009; **16**: 446-450 [PMID: 19262360 DOI: 10.1097/MJT.0b013e31818a88c5]

19 **Ascione A**, De Luca M, Tartaglione MT, Lampasi F, Di Costanzo GG, Lanza AG, Picciotto FP, Marino-Marsilia G, Fontanella L, Leandro G. Peginterferon alfa-2a plus ribavirin is more effective than peginterferon alfa-2b plus ribavirin for treating chronic hepatitis C virus infection. *Gastroenterology* 2010; **138**: 116-122 [PMID: 19852964 DOI: 10.1053/j.gastro.2009.10.005]

20 **Awad T**, Thorlund K, Hauser G, Stimac D, Mabrouk M, Gluud C. Peginterferon alpha-2a is associated with higher sustained virological response than peginterferon alfa-2b in chronic hepatitis C: systematic review of randomized trials. *Hepatology* 2010; **51**: 1176-1184 [PMID: 20187106 DOI: 10.1002/hep.23504]

21 **Jansen PL**, Reesink HW. Antiviral effect of peginterferon alfa-2b and alfa-2a compared. *J Hepatol* 2006; **45**: 172-173 [PMID: 16797101 DOI: 10.1016/j.jhep.2006.06.003]
22 **Rumi MG**, Aghemo A, Prati GM, D'Ambrosio R, Donato MF, Soffredini R, Del Ninno E, Russo A, Colombo M. Randomized study of peginterferon-alpha2a plus ribavirin vs peginterferon-alpha2b plus ribavirin in chronic hepatitis C. *Gastroenterology* 2010; **138**: 108-115 [PMID: 19766645 DOI: 10.1053/j.gastro.2009.08.071]
23 **Zeuzem S**. Do differences in pegylation of interferon alfa matter? *Gastroenterology* 2010; **138**: 34-36 [PMID: 19932662 DOI: 10.1053/j.gastro.2009.11.034]
24 **Wirth S**, Kelly D, Sokal E, Socha P, Mieli-Vergani G, Dhawan A, Lacaille F, Saint Raymond A, Olivier S, Taminiau J. Guidance for clinical trials for children and adolescents with chronic hepatitis C. *J Pediatr Gastroenterol Nutr* 2011; **52**: 233-237 [PMID: 21076340 DOI: 10.1097/MPG.0b013e3181f6f09c]
25 **Wirth S**, Pieper-Boustani H, Lang T, Ballauff A, Kullmer U, Gerner P, Wintermeyer P, Jenke A. Peginterferon alfa-2b plus ribavirin treatment in children and adolescents with chronic hepatitis C. *Hepatology* 2005; **41**: 1013-1018 [PMID: 15793840 DOI: 10.1002/hep.20661]
26 **Jonas MM**, Balistreri WF, Gonzalez-Peralta RP, Haber B, Mohan P, Molleston JP, Murray KF, Narkewicz M, Rosenthal P, Schwarz KB, Smith LJ, Barton B, Shepherd JA, Robuck PR, Duggan C. Changes in body mass index and body composition in children treated with peginterferon for chronic hepatitis C in the PEDS-C trial. *Hepatology* 2009; **50:** 636a-637a

P- Reviewer Tacke F **S- Editor** Gou SX
L- Editor A **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i7.1104

World J Gastroenterol 2013 February 21; 19(7): 1104-1110
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Granulocyte-colony stimulating factor therapy improves survival in patients with hepatitis B virus-associated acute-on-chronic liver failure

Xue-Zhang Duan, Fang-Fang Liu, Jing-Jing Tong, Hao-Zhen Yang, Jing Chen, Xiao-Yan Liu, Yuan-Li Mao, Shao-Jie Xin, Jin-Hua Hu

Xue-Zhang Duan, Fang-Fang Liu, Jing-Jing Tong, Hao-Zhen Yang, Jing Chen, Xiao-Yan Liu, Shao-Jie Xin, Jin-Hua Hu, Liver Failure Treatment and Research Center, Beijing 302 Hospital, Beijing 100039, China
Yuan-Li Mao, Center for Clinical Laboratory, Beijing 302 Hospital, Beijing 100039, China
Author contributions: Duan XZ and Yang HZ analyzed the data; Duan XZ and Hu JH wrote the paper; Duan XZ and Hu JH designed the study; Liu FF and Tong JJ collected the data; Mao YL helped to measure the biochemical parameters and peripheral CD34-positive cells; Chen J, Liu XY and Xin SJ followed up the patients.
Supported by National Natural Science Foundation of China, No. 81171641; the Army Medical and Health Scientific Research Fund of China, No. 06H057
Correspondence to: Jin-Hua Hu, Associate Professor, Liver Failure Treatment and Research Center, Beijing 302 Hospital, No. 100, the 4th Ring Road,Beijing 100039, China. hjh@medmail.com.cn
Telephone: +86-10-66933434 Fax: +86-10-63879339
Received: November 5, 2012 Revised: January 8, 2013
Accepted: January 17, 2013
Published online: February 21, 2013

Abstract

AIM: To evaluate the safety and efficacy of granulocyte-colony stimulating factor (G-CSF) therapy in patients with hepatitis B virus (HBV)-associated acute-on-chronic liver failure (ACLF).

METHODS: Fifty-five patients with HBV-associated ACLF were randomized into two groups: the treatment group and the control group. Twenty-seven patients in the treatment group received G-CSF (5 μg/kg per day, six doses) treatment plus standard therapy, and 28 patients in the control group received standard therapy only. The peripheral $CD34^+$ cell count was measured consecutively by flow cytometry. Circulating white blood cell count, biochemical parameters, and other clinical data of these patients were recorded and analyzed. All patients were followed up for a period of 3 mo to evaluate the changes in liver function and survival rate.

RESULTS: The peripheral neutrophil and $CD34^+$ cell counts in the G-CSF group increased on day 3 from the onset of therapy, continued to rise on day 7, and remained elevated on day 15 compared to those of the control group. Child-Turcotte-Pugh score of patients in the treatment group was improved on day 30 from the onset of G-CSF therapy, compared to that in the controls (P = 0.041). Model for End-Stage of Liver Disease score of patients in the treatment group was improved on day 7 (P = 0.004) and remained high on day 30 from the onset of G-CSF therapy (P < 0.001) compared to that in controls. After 3 mo of follow-up observation, the survival rate in the treatment group (48.1%) was significantly higher than that in the control group (21.4%) (P = 0.0181).

CONCLUSION: G-CSF therapy promoted $CD34^+$ cell mobilization in patients with HBV-associated ACLF, and improved the liver function and the survival rate of these patients.

© 2013 Baishideng. All rights reserved.

Key words: Acute-on-chronic liver failure; Granulocyte-colony stimulating factor; Hepatitis B virus

Duan XZ, Liu FF, Tong JJ, Yang HZ, Chen J, Liu XY, Mao YL, Xin SJ, Hu JH. Granulocyte-colony stimulating factor therapy improves survival in patients with hepatitis B virus-associated acute-on-chronic liver failure. *World J Gastroenterol* 2013; 19(7): 1104-1110 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i7/1104.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i7.1104

INTRODUCTION

Hepatitis B virus (HBV) infection is one of the major public health problems. It is estimated that over 350 million individuals are chronically infected with HBV worldwide[1]. Some of these patients develop severe liver diseases such as acute-on-chronic liver failure (ACLF), liver cirrhosis, and hepato-cellular carcinoma[2]. The mortality rate of HBV-associated ACLF varies between 70%-80%[3,4]. Liver transplantation is the only effective treatment currently available, but its application is limited by the shortage of donors and the high cost.

In order to overcome these problems, alternative approaches have been proposed, such as an artificial liver support system[5], liver cell transplantation[6] and stem cell transplantations[7]. In particular, the great potential of stem cells to differentiate into multiple cell lineages raises the exciting hypothesis that these cells can be used in tissue repair and tissue-specific cell regeneration, when tissue-resided stem cells are not sufficient for the regeneration of a failing organ.

During liver regeneration, bone marrow-derived hematopoietic stem cells (HSC) may mobilize to the liver and, together with hepatocytes and intrahepatic stem cells, contribute to the proliferation of liver cells[8]. Hepatocytes carrying a Y chromosome were detected in the livers of female recipients who had received bone marrow transplantation from male donors[9]. Granulocyte-colony stimulating factor (G-CSF) can be used to mobilize stem cells to the periphery in patients with advanced liver disease, or to promote adequate numbers of cells for further transplantation. The safety and efficacy of this protocol has been investigated[10]. In experimental animal models, G-CSF ameliorated liver injury and improved the survival rate in rats with D-galactosamine-induced acute liver failure[11,12]. When administered to patients with severe liver cirrhosis, G-CSF boosted the numbers of peripheral leukocytes and $CD34^+$ bone marrow-derived HSCs[13]. Therefore, G-CSF therapy may be beneficial for liver regeneration in patients with different kinds of liver injuries.

Our objective in this clinical study was to evaluate the effects of G-CSF therapy on the proliferation of peripheral $CD34^+$ cells and on liver function in patients with HBV-associated ACLF. The parameters of liver function in these patients were consecutively measured. Child-Turcotte-Pugh (CTP) score, Model for End Stage of Liver Disease (MELD) score and survival rate of these patients were evaluated during a 3-mo follow-up study.

MATERIALS AND METHODS

Patients

A total of 55 patients with HBV-associated ACLF were recruited to our center from June 2009 to May 2011. HBV-associated ACLF, defined by the Asian Pacific Association for the Study of the Liver Working Party[2], is an acute hepatic insult manifested as jaundice (serum bilirubin $\geqslant$ 5 mg/dL) and coagulopathy [international normalized ratio (INR) $\geqslant$ 1.5 or prothrombin activity < 40%], with complications of ascites and/or encephalopathy within 4 wk in patients previously diagnosed or undiagnosed with chronic liver disease. Other inclusion criteria included: (1) the presence of hepatitis B surface antigen in the serum for at least 6 mo; (2) the evidence of active viral replication as indicated by detectable HBV DNA in the serum ($\geqslant 1 \times 10^4$ copies/mL); (3) flares of hepatitis, marked by increased serum alanine aminotransferase (ALT) level to more than 5-fold of the upper limit of normal value; and (4) age between 18 to 65 years.

The exclusion criteria included the following: (1) super-infection or co-infection with hepatitis A, C, D, E, Epstein-Barr virus, cytomegalovirus, or human immunodeficiency virus; (2) a previous course of any antiviral, immuno-modulator or cytotoxic/immunosuppressive therapy for chronic hepatitis within the prior 12 mo; (3) evidence of decompensated liver disease prior to the enrollment; (4) hepato-cellular carcinoma diagnosed by computed tomography or magnetic resonance imaging; (5) co-existence of any other serious medical illnesses or other liver diseases such as autoimmune hepatitis, alcoholic liver disease, drug-induced liver injury or Wilson's disease; (6) any concurrent evidence of sepsis; (7) malignant jaundice induced by obstructive jaundice and hemolytic jaundice; and (8) prolonged prothrombin time due to blood system disease.

Groups

This was a randomized, controlled, and double-blinded study. The sample size was determined as follows: Based on the hypothesis that G-CSF therapy can improve survival rate by 10% in the treatment group compared to the control group, with a power of 95% and an alpha error of 5%, the number of patients should be 25 in each group. A randomization number code was generated for each patient. Based on this code, each patient was assigned to receive G-CSF therapy plus standard medical therapy (G-CSF group), or standard medical therapy alone (control group). Both the patients and the investigators were blinded to the treatment regimen. The patients in the G-CSF treatment group received G-CSF (SL Pharm, Beijing, China) subcutaneously at the dosage of 5 μg/kg per day for six consecutive days, and were monitored with daily physical examination and laboratory tests. All patients received entecavir (0.5 mg/d, Squibb Pharmaceuticals Ltd., Shang Hai, China) and standard therapy (including reduced glutathione, glycyrrhizin, ademetionine, polyene phosphatidylcholine, alprostadil, and human serum albumin) on the day of admission. The white blood cell (WBC) counts were assessed twice per week in the first two weeks. In addition, abdominal ultrasound examination was performed on days 1 and 7 to evaluate the diameters of spleen and the hepatic portal vein.

Quantification of peripheral $CD34^+$ cells

$CD34^+$ cells in the peripheral venous blood were measured consecutively twice per week in the first two weeks

Table 1 Characteristics of patients with hepatitis B virus-associated acute-on-chronic liver failure at the time of admission

Parameters	G-CSF group	Control group	*P* value
Gender (male %)	22 (81.5)	22 (78.6)	0.755
Age (yr)	43.5 (29-63)	45.9 (22-65)	0.332
WBC (10^9/L)	5.79 ± 1.81	6.61 ± 1.71	0.443
Neutrophil (10^9/L)	3.53 ± 1.46	3.82 ± 1.17	0.114
Platelets (10^9/L)	182 (147-215)	174 (149-175)	0.680
ALT (U/L)	276 (197-801)	252 (189-1239)	0.430
AST (U/L)	246 (195-788)	251 (187-980)	0.544
Total bilirubin (μmol/L)	336 (181-519)	320.0 (174.5-519.8)	0.605
Cr (μmol/L)	83.8 ± 16.9	85.4 ± 53.87	0.475
INR	2.11 ± 0.28	2.34 ± 0.34	0.606
ALB (g/L)	29.11 ± 4.05	28.75 ± 4.63	0.596
HBV DNA (log_{10})	5.11 ± 1.37	5.55 ± 1.59	0.280
CTP score	12.17 ± 1.47	12.25 ± 1.29	0.349
MELD score	25.11 ± 3.30	26.30 ± 4.12	0.588

Values are shown as mean ± SD. WBC: White blood cell; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; Cr: Creatine; INR: International normalized ratio; ALB: Albumin; CTP: Child-Turcotte-Pugh; MELD: Model for end stage of liver disease; HBV: Hepatitis B virus; G-CSF: Granulocyte-colony stimulating factor.

in all patients. Briefly, small aliquots of peripheral blood were incubated with PE-conjugated anti-CD34 monoclonal-antibody (BD Company, United States) for 30 min on ice. Erythrocytes were lysed with ammonium chloride for 10 min at room temperature. Cells were washed with phosphate-buffered saline, and kept on ice till flow cytometric analysis.

Follow-up scheduling

All patients had daily follow-ups and physical examinations in the first month, and then at least weekly for the next 2 mo. During the 3-mo follow-up period, patients were monitored for the following parameters: the levels of serum bilirubin and albumin, prothrombin time and concentration, INR, the levels of ALT and aspartate aminotransferase, the levels of blood urea and serum creatinine, complete blood analysis, estimation of the degree of ascites, CTP score, MELD score, and hepatic encephalopathy. The survival rates were evaluated over a period of 3-mo.

Sera from patients were collected and direct polymerase chain reaction (PCR) sequencing was used to screen for HBV reverse transcriptase (RT) domain if serum HBV DNA tests were positive after patients received entecavir therapy. The *HBV* gene fragment (nt 54-1278) encompassing the complete *RT* gene (nt 130-1161) was amplified by nested PCR. The primers and reaction conditions have been described previously[14]. Substitutions at positions rt180, rt184, rt202, rt204 and rt250 were taken as resistance mutations for analysis.

Ethics

The protocol of our study is in compliance with the ethical guidelines of the 1975 Declaration of Helsinki, and was approved by the Clinical Research Ethics Committee of the Beijing 302 Hospital. All patients or their relatives in the G-CSF treatment group provided written informed consent prior to the enrollment.

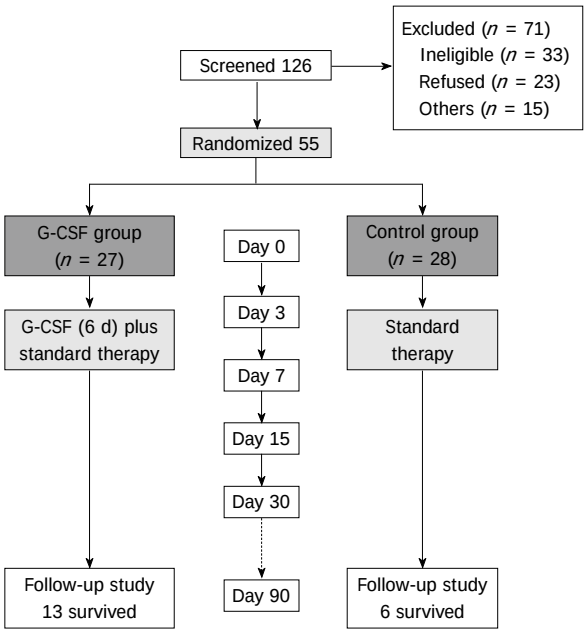


Figure 1 Study design and flow algorithm of patient selection. G-CSF: Granulocyte-colony stimulating factor.

Statistical analysis

Data were compiled using Excel XP and processed using Statistical Package for Science and Society (SPSS) version 12.0 (SPSS Inc., Chicago, IL). All quantitative variables were presented as mean ± SD. All qualitative data were described as frequency or percentage. Comparisons between groups for qualitative data were carried out using χ^2 test, Fischer's exact test, or McNemar test when appropriate. Independent sample *t* test and paired sample *t* test were used for quantitative variables with normal distribution, whereas non-parametric Mann-Whitney test and Wilcoxon signed-rank test were used for quantitative variables with non-normal distribution. In all tests, $P < 0.05$ were considered as statistically significant.

RESULTS

Baseline conditions

Of the 126 patients screened, 71 were excluded and 55 patients enrolled in our study. Among them, 27 were randomized to receive G-CSF therapy, and the other 28 were included as controls (Figure 1).

The two groups showed no statistical differences in gender, age, the baseline values of peripheral WBC, platelets, and other parameters (Table 1). All patients had a history of chronic hepatitis B, and had not received anti-viral therapy prior to the enrollment.

Peripheral neutrophil counts and CD34-positive cell counts

The baseline value of the circulating neutrophil count was compared between the G-CSF and the control groups

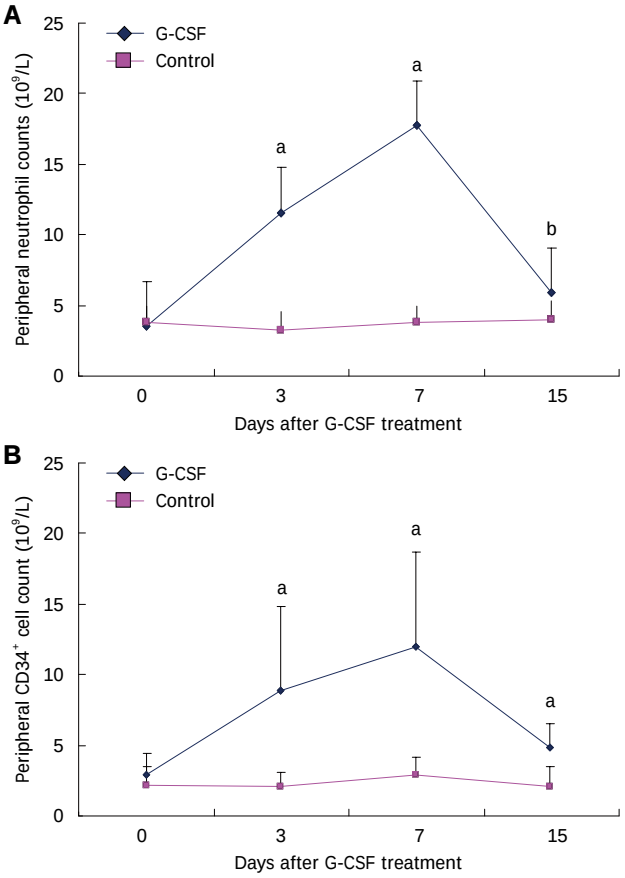


Figure 2 Kinetics of peripheral neutrophil count (A) and CD34$^+$ cell count (B) in patients treated with granulocyte-colony stimulating factor and controls. $^aP < 0.05$, $^bP < 0.01$ *vs* controls. G-CSF: Granulocyte-colony stimulating factor.

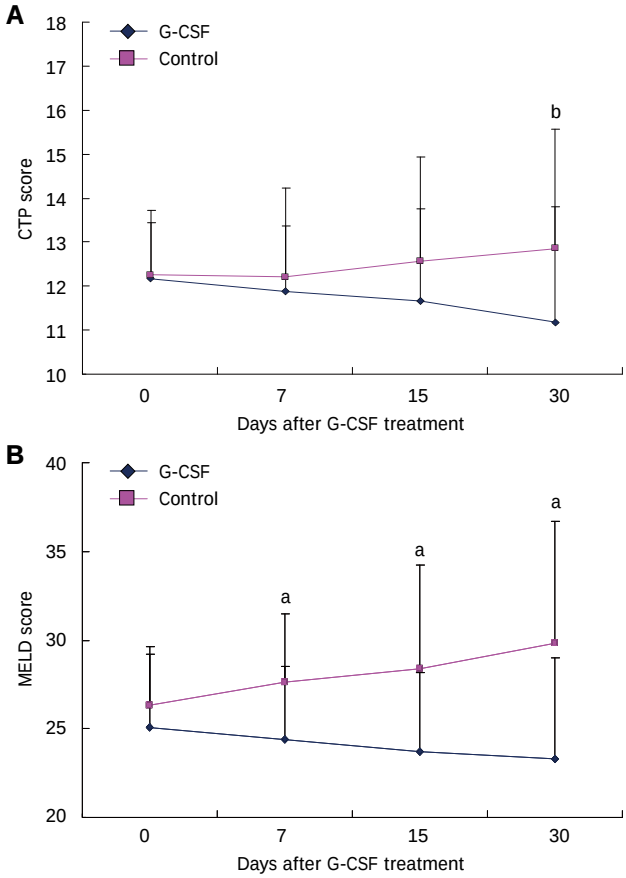


Figure 3 Changes in Child-Turcotte-Pugh score (A) and Model for End stage of Liver Disease score (B) in patients treated with granulocyte-colony stimulating factor and control groups. $^aP < 0.05$, $^bP < 0.01$ *vs* controls. G-CSF: Granulocyte-colony stimulating factor; CTP: Child-Turcotte-Pugh; MELD; Model for End Stage of Liver Disease.

(Table 1). No statistical difference was found between the baselines of the two groups ($P = 0.443$). However, on day 3 from the onset of G-CSF therapy, the peripheral neutrophil count increased to (11.59 ± 6.40) × 10^9/L, significantly higher than that of the control group (3.29 ± 1.25) × 10^9/L ($P < 0.001$). It continued to rise on day 7 to (17.76 ± 10.07) × 10^9/L in the G-CSF group, significantly higher than (3.82 ± 1.17) × 10^9/L in the control group ($P < 0.001$). On day 15, the neutrophil count decreased to (5.88 ± 3.69) × 10^9/L in the G-CSF group, but was still higher than (4.02 ± 1.33) × 10^9/L in the control group ($P = 0.032$) (Figure 2A).

Prior to G-CSF therapy, circulating CD34$^+$ cell counts were comparable in the two groups - (2.97 ± 1.52) × 10^6/L in the G-CSF group and (2.23 ± 1.29) × 10^6/L in the control group ($P = 0.085$). On day 3 of treatment, circulating CD34$^+$ cell counts in the G-CSF group increased to (8.96 ± 5.97) × 10^6/L, compared to (2.09 ± 1.02) × 10^6/L in the control group ($P < 0.001$). On day 7, the circulating CD34$^+$ cell counts continued to rise to (12.05 ± 6.70) × 10^6/L, compared to (2.97 ± 1.22) × 10^6/L in the control ($P < 0.001$). On day 15, the CD34$^+$ cell counts decreased to (4.92 ± 1.63) × 10^6/L in the G-CSF treatment group, still significantly higher than that in the control group (2.11 ± 1.39) ($P < 0.001$) (Figure 2B). On day 30, the CD34$^+$ cell counts were nearly at the same level between the two groups.

In the course of the G-CSF therapy, all patients demonstrated good tolerance. Some patients were affected by minor side effects, such as fever (8 cases), headache (5 cases), and nausea (4 cases), but all the side effects resolved within 5 d after the withdrawal of G-CSF treatment.

Changes of liver function after G-CSF therapy

Prior to the G-CSF therapy, the CTP score was 12.17 ± 1.47 in the G-CSF group, and 12.25 ± 1.29 in the control group ($P = 0.841$). The CTP score decreased gradually after the G-CSF therapy, and reached 11.17 ± 2.76 on day 30 of treatment in the G-CSF group, compared to 12.86 ± 2.63 in the control group ($P = 0.041$) (Figure 3A).

The MELD score demonstrated an early decrease in the G-CSF group (Figure 3B). On day 7 of the therapy, the MELD score decreased to 24.4 ± 3.9 in the G-CSF group, compared to 27.6 ± 4.1 in the control group ($P = 0.004$). On day 15, the MELD score was 23.7 ± 5.8 in the G-CSF group, compared to 28.4 ± 4.5 in the control group ($P < 0.001$). This trend continued during the 30-d observation period (23.3 ± 6.9 *vs* 29.8 ± 5.7) ($P < 0.001$).

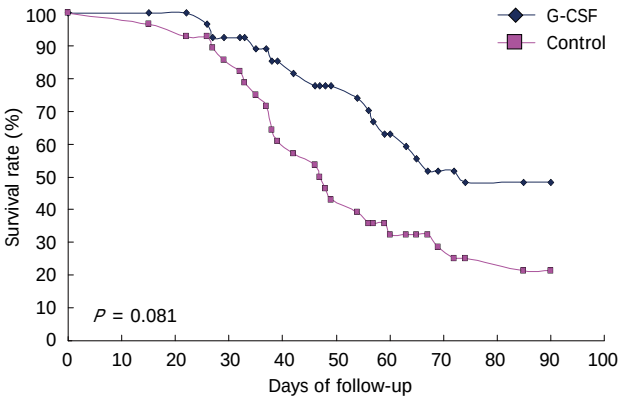


Figure 4 Survival rate of patients treated with granulocyte-colony stimulating factor and in controls. G-CSF: Granulocyte-colony stimulating factor.

Levels of serum HBV DNA and drug-resistant HBV variant monitoring

All patients enrolled in our study received entecavir antiviral treatment throughout the course of the study. After 3 mo of treatment, all the surviving patients demonstrated effective antiviral results. Of the 19 surviving patients, 16 (11 from G-CSF group, and 5 from control group) were detected negative for serum HBV DNA (below 10^2 copies/mL), and 3 (2 from G-CSF group and 1 from control group) were positive, but the concentrations of HBV DNA were all below 10^3 copies/mL. Serum HBV mutations were determined by direct sequencing methods and an entecavir-resistant mutation was not detected in these patients. No serious adverse effects of entecavir were observed in all patients.

Survival rate

The patients' survival rates were evaluated at the end of 3 mo (Figure 4). In the G-CSF group, 13 of 27 patients survived, but only 6 of 28 patients survived in the control group (χ^2 value 5.584, $P = 0.0181$). All the patients had complications of ascites and electrolyte disturbances. In the treatment group, 4 patients died of encephalopathy, 5 of gastric intestinal bleeding, 2 of hepato-renal syndrome (HRS), and 3 of sepsis. In the control group, 6 died of encephalopathy, 3 of gastric intestinal bleeding, 6 died from HRS, and 7 of sepsis. More patients in the control group died of sepsis and HRS compared to those in the G-CSF group (χ^2 value 4.863, $P = 0.027$).

DISCUSSION

In China, HBV-associated ACLF accounts for 85%-95% of all liver failure. Approximately 60% of the patients die from complications due to the lack of early liver transplantation[15,16]. Increasing evidence indicates that stem cells contribute to hepatic regeneration, which is essential for the restoration of liver function and thus the survival of the patients. Stem cells have been shown to induce the repairing process after acute liver injuries[10,12]. Therefore, G-CSF therapy may be a promising approach in the clinical treatment of patients with ACLF.

Some previous findings on the efficacy of G-CSF in patients with liver failure remain controversial. Di Campli *et al*[17] investigated the safety and efficacy of G-CSF in patients with ACLF. They found an increased number of $CD34^+$ cells in the peripheral blood, down-regulated expression of C-X-C chemokine receptor type 4, very late activation of antigen 4 and vascular endothelial growth factor receptor in the G-CSF treatment group, but they did not show the effect of G-CSF on the survival rates of these patients. Similarly, Spahr *et al*[18] investigated the efficacy of G-CSF in patients with alcoholic steatohepatitis, and observed an elevated peripheral $CD34^+$ cell count and proliferating hemopoietic cells in the liver tissues, although they failed to demonstrate the improvement of liver function. Recently Garg *et al*[19] also investigated the efficacy of G-CSF in ACLF patients with promising results. Sixteen of the 23 patients in the G-CSF group survived for 60 d, compared to merely 7 of the 24 in the control group. The discrepancies between the results by different groups may be attributable to the background of the enrolled patients. For example, Di Campli enrolled patients with alcohol liver failure, whereas Garg recruited patients with various types of liver diseases, including alcoholic-related liver diseases and HBV-associated ACLF.

In our current study we selected patients with HBV-associated ACLF for investigation. This is the first report to apply G-CSF therapy to patients with chronic HBV infection. All the patients were positive for serum HBV DNA and received entecavir antiviral treatment after enrollment. The two groups showed no statistically significant differences in gender, WBC count, CTP score and MELD score prior to the onset of the G-CSF therapy.

We observed that G-CSF therapy increased the peripheral neutrophil count and $CD34^+$ cell count in patients with HBV-associated ACLF. In addition, the G-CSF treatment group demonstrated improved liver function compared to the control group, as demonstrated by the CTP and MELD scores. Our finding on survival rate was in consistency with that of Garg *et al*[19].

It is plausible to speculate that $CD34^+$ HSC migrated from the bone marrow to the liver, and contributed to the regeneration of the liver. Future studies, such as liver biopsy or autopsy, may provide evidence for the mobilization of the stem cells. In these patients, MELD score improved sooner than CTP score after G-CSF therapy, but the mechanism is yet to be elucidated.

Currently, there are two classical pathways for stem cells to travel from the bone marrow to the liver. The first is G-CSF mobilization. Secondly, $CD34^+$ cells can be isolated from bone marrow, purified with magnetic columns, and then re-injected into the liver through the hepatic artery or the portal vein[13,20]. However, patients with ACLF often have coagulation disorders; therefore, it is risky to apply the aforementioned complicated procedures. It is a reasonable argument that the approach of G-CSF administration is probably easier and less risky to implement than the separation, purification, and autologous transplantation of bone marrow-derived HSC cells to ACLF

patients. Furthermore, it has been reported that contamination by red blood cells during $CD34^+$ cell isolation can impair the efficacy of autologous HSC therapy[21].

Antiviral therapy has been deemed as an important therapy for patients with HBV-associated ACLF[2,22]. Previously, Huang *et al*[16] reported that antiviral therapy decreased the mortality rate in patients with HBV-associated ACLF, but the patients recruited to their study had lower ALT levels and higher prothrombin activity (over 30%), which may explain the different results between these two studies. In our study, the mortality rate in the control group was higher (78.6%) than those reported in the literature. This may be attributable to several factors. Firstly, the patients in our study had larger MELD scores, of 25 to 29, indicating more serious conditions. Secondly, most patients enrolled in our study had middle-stage (PA between 20%-30%) or late-stage ACLF (PA below 20%) according to the standards of the Chinese classification system[23], which may contribute to an increased mortality rate. Thirdly, antiviral therapy can suppress HBV replication; however, there may not be enough time for HBV suppression and liver function recovery to occur due to the short life expectancy in ACLF patients. A similar phenomenon was also observed by other researchers[24]. We also consider the possibility of HBV viral breakthrough in these patients. Serum HBV DNA mutations were determined by a direct sequencing method when serum HBV DNA was positive in patients, but we did not find any entecavir-resistant mutations. Although we did not find poor compliance in these patients, we could not exclude the possibility. In our study, all the patients received antiviral therapy to help mitigate additional hepatic insult and even liver failure caused by the re-activation of HBV.

Patients treated with G-CSF not only demonstrated a significantly better 90 d survival rate, CTP and MELD scores, but were also less likely to develop HRS and sepsis compared to the controls. This could be explained by increased numbers of neutrophils in these patients. Neutrophil dysfunction has been shown to cause sepsis and further the development of HRS in patients with ACLF[25]. We did not find a correlation between $CD34^+$ cell number and liver function improvement, which means cell numbers, micro-environment of liver tissue and other possible factors may work together to improve liver function. That mechanism needs to be further investigated.

In conclusion, this randomized, controlled study clearly demonstrated the clinical safety and efficacy of G-CSF therapy in patients with HBV-associated ACLF. The convenience of administration makes G-CSF therapy readily implementable in large-scale, multi-center clinical sites, and further supports its benefits in restoring hepatic function and improving survival rate.

COMMENTS

Background

Hepatitis B virus-associated acute-on-chronic liver failure (HBV-ACLF) is associated with a high mortality. Liver transplantation could significantly improve the survival rate, but is limited by many factors, especially donor shortages. To overcome these problems, alternative approaches have been proposed. Granulocyte-colony stimulating factor (G-CSF) can be used to mobilize stem cells from bone marrow to the periphery, and then to the liver, in patients with advanced liver disease, and promote liver regeneration.

Research frontiers

In experimental animal models, G-CSF ameliorated liver injury and improved survival rate in rats with D-galactosamine-induced acute liver failure. When administered to patients with severe liver cirrhosis, G-CSF boosted the numbers of peripheral leukocytes and $CD34^+$ bone marrow-derived hematopoietic stem cells. Therefore, G-CSF therapy may be beneficial for liver regeneration in patients with different kinds of liver injuries. The safety and efficacy of a G-CSF protocol has been investigated but the efficacy is still controversial in patients with liver diseases.

Innovations and breakthroughs

In the current study, the authors selected patients with HBV-associated ACLF for investigation. This is the first randomized trial to apply G-CSF therapy to patients with chronic HBV infection. The authors observed that G-CSF therapy increased peripheral neutrophil count and $CD34^+$ cell count in patients with HBV-associated ACLF. In addition, the G-CSF treatment group demonstrated improved liver function and survival rate compared to the control group.

Applications

HBV-associated ACLF patients treated with G-CSF not only demonstrated a significantly better 90 d survival rate, and improved CTP and MELD scores, but were also less likely to develop HRS and sepsis compared to the controls. The convenience of administration makes G-CSF therapy readily implementable in large-scale, multi-center clinical sites to further support its benefits in restoring hepatic function and improving survival rate.

Terminology

HBV-associated ACLF, defined by the Asian Pacific Association for the Study of the Liver Working Party, is an acute hepatic insult manifested as jaundice (serum bilirubin $\geqslant$ 5 mg/dL) and coagulopathy (international normalized ratio $\geqslant$ 1.5 or prothrombin activity < 40%), with complications of ascites and/or encephalopathy within 4 wk in patients previously diagnosed or undiagnosed with chronic liver disease.

Peer review

This is an interesting study in patients with decompensated hepatitis B patients.

REFERENCES

1 **Tujios SR**, Lee WM. New advances in chronic hepatitis B. *Curr Opin Gastroenterol* 2012; **28**: 193-197 [PMID: 22450894 DOI: 10.1097/MOG.0b013e32835297ef]

2 **Sarin SK**, Kumar A, Almeida JA, Chawla YK, Fan ST, Garg H, de Silva HJ, Hamid SS, Jalan R, Komolmit P, Lau GK, Liu Q, Madan K, Mohamed R, Ning Q, Rahman S, Rastogi A, Riordan SM, Sakhuja P, Samuel D, Shah S, Sharma BC, Sharma P, Takikawa Y, Thapa BR, Wai CT, Yuen MF. Acute-on-chronic liver failure: consensus recommendations of the Asian Pacific Association for the Study of the Liver (APASL). *Hepatol Int* 2009; **3:** 269-282 [DOI: 10.1007/s12072-008-9106-x]

3 **Chan HL**, Tsang SW, Hui Y, Leung NW, Chan FK, Sung JJ. The role of lamivudine and predictors of mortality in severe flare-up of chronic hepatitis B with jaundice. *J Viral Hepat* 2002; **9**: 424-428 [PMID: 12431204]

4 **Tsubota A**, Arase Y, Suzuki Y, Suzuki F, Sezaki H, Hosaka T, Akuta N, Someya T, Kobayashi M, Saitoh S, Ikeda K, Kumada H. Lamivudine monotherapy for spontaneous severe acute exacerbation of chronic hepatitis B. *J Gastroenterol Hepatol* 2005; **20**: 426-432 [PMID: 15740488]

5 **Laleman W**, Wilmer A, Evenepoel P, Elst IV, Zeegers M, Zaman Z, Verslype C, Fevery J, Nevens F. Effect of the molecular adsorbent recirculating system and Prometheus devices on systemic haemodynamics and vasoactive agents in patients with acute-on-chronic alcoholic liver failure. *Crit Care* 2006; **10**: R108 [PMID: 16859530]

6 **Fitzpatrick E**, Mitry RR, Dhawan A. Human hepatocyte transplantation: state of the art. *J Intern Med* 2009; **266**: 339-357 [PMID: 19765179 DOI: 10.1111/j.1365-2796.2009.02152.x]

7 **Souza BS**, Nogueira RC, de Oliveira SA, de Freitas LA, Lyra LG, Ribeiro dos Santos R, Lyra AC, Soares MB. Current status of stem cell therapy for liver diseases. *Cell Transplant* 2009; **18**: 1261-1279 [PMID: 19660179 DOI: 10.3727/096368909X470522]

8 **Vassilopoulos G**, Wang PR, Russell DW. Transplanted bone marrow regenerates liver by cell fusion. *Nature* 2003; **422**: 901-904 [PMID: 12665833]

9 **Theise ND**, Nimmakayalu M, Gardner R, Illei PB, Morgan G, Teperman L, Henegariu O, Krause DS. Liver from bone marrow in humans. *Hepatology* 2000; **32**: 11-16 [PMID: 10869283]

10 **Yannaki E**, Anagnostopoulos A, Kapetanos D, Xagorari A, Iordanidis F, Batsis I, Kaloyannidis P, Athanasiou E, Dourvas G, Kitis G, Fassas A. Lasting amelioration in the clinical course of decompensated alcoholic cirrhosis with boost infusions of mobilized peripheral blood stem cells. *Exp Hematol* 2006; **34**: 1583-1587 [PMID: 17046578]

11 **Theocharis SE**, Papadimitriou LJ, Retsou ZP, Margeli AP, Ninos SS, Papadimitriou JD. Granulocyte-colony stimulating factor administration ameliorates liver regeneration in animal model of fulminant hepatic failure and encephalopathy. *Dig Dis Sci* 2003; **48**: 1797-1803 [PMID: 14561004]

12 **Yannaki E**, Athanasiou E, Xagorari A, Constantinou V, Batsis I, Kaloyannidis P, Proya E, Anagnostopoulos A, Fassas A. G-CSF-primed hematopoietic stem cells or G-CSF per se accelerate recovery and improve survival after liver injury, predominantly by promoting endogenous repair programs. *Exp Hematol* 2005; **33**: 108-119 [PMID: 15661404]

13 **Salama H**, Zekri AR, Zern M, Bahnassy A, Loutfy S, Shalaby S, Vigen C, Burke W, Mostafa M, Medhat E, Alfi O, Huttinger E. Autologous hematopoietic stem cell transplantation in 48 patients with end-stage chronic liver diseases. *Cell Transplant* 2010; **19**: 1475-1486 [PMID: 20587151 DOI: 10.3727/096368910X514314]

14 **Liu Y**, Zhong Y, Zou Z, Xu Z, Li B, Ren X, Bai S, Wang L, Li X, Dai J, Wang Y, Mao P, Xu D. Features and clinical implications of hepatitis B virus genotypes and mutations in basal core promoter/precore region in 507 Chinese patients with acute and chronic hepatitis B. *J Clin Virol* 2010; **47**: 243-247 [PMID: 20080060 DOI: 10.1016/j.jcv.2009.12.013]

15 **Yang WB**, Chen EQ, Bi HX, Bai L, Chen XB, Feng P, Tang H. Different models in predicting the short-term prognosis of patients with hepatitis B virus-related acute-on-chronic liver failure. *Ann Hepatol* 2012; **11**: 311-319 [PMID: 22481448]

16 **Huang K**, Hu JH, Wang HF, He WP, Chen J, Duan XZ, Zhang AM, Liu XY. Survival and prognostic factors in hepatitis B virus-related acute-on-chronic liver failure. *World J Gastroenterol* 2011; **17**: 3448-3452 [PMID: 21876637 DOI: 10.3748/wjg.v17.i29.3448]

17 **Di Campli C**, Zocco MA, Saulnier N, Grieco A, Rapaccini G, Addolorato G, Rumi C, Santoliquido A, Leone G, Gasbarrini G, Gasbarrini A. Safety and efficacy profile of G-CSF therapy in patients with acute on chronic liver failure. *Dig Liver Dis* 2007; **39**: 1071-1076 [PMID: 17964871]

18 **Spahr L**, Lambert JF, Rubbia-Brandt L, Chalandon Y, Frossard JL, Giostra E, Hadengue A. Granulocyte-colony stimulating factor induces proliferation of hepatic progenitors in alcoholic steatohepatitis: a randomized trial. *Hepatology* 2008; **48**: 221-229 [PMID: 18537187 DOI: 10.1002/hep.22317]

19 **Garg V**, Garg H, Khan A, Trehanpati N, Kumar A, Sharma BC, Sakhuja P, Sarin SK. Granulocyte colony-stimulating factor mobilizes CD34(+) cells and improves survival of patients with acute-on-chronic liver failure. *Gastroenterology* 2012; **142**: 505-512.e1 [PMID: 22119930 DOI: 10.1053/j.gastro.2011.11.027]

20 **Khan AA**, Parveen N, Mahaboob VS, Rajendraprasad A, Ravindraprakash HR, Venkateswarlu J, Rao P, Pande G, Narusu ML, Khaja MN, Pramila R, Habeeb A, Habibullah CM. Management of hyperbilirubinemia in biliary atresia by hepatic progenitor cell transplantation through hepatic artery: a case report. *Transplant Proc* 2008; **40**: 1153-1155 [PMID: 18555137 DOI: 10.1016/j.transproceed.2008.03.110]

21 **Assmus B**, Tonn T, Seeger FH, Yoon CH, Leistner D, Klotsche J, Schächinger V, Seifried E, Zeiher AM, Dimmeler S. Red blood cell contamination of the final cell product impairs the efficacy of autologous bone marrow mononuclear cell therapy. *J Am Coll Cardiol* 2010; **55**: 1385-1394 [PMID: 20338501 DOI: 10.1016/j.jacc.2009.10.059]

22 **Garg H**, Sarin SK, Kumar M, Garg V, Sharma BC, Kumar A. Tenofovir improves the outcome in patients with spontaneous reactivation of hepatitis B presenting as acute-on-chronic liver failure. *Hepatology* 2011; **53**: 774-780 [PMID: 21294143 DOI: 10.1002/hep.24109]

23 **Liver Failure and Artificial Liver Group,** Chinese Society of Infectious Diseases, Chinese Medical Association; Severe Liver Diseases and Artificial Liver Group, Chinese Society of Hepatology, Chinese Medical Association. [Diagnostic and treatment guidelines for liver failure]. *Zhonghua Ganzangbing Zazhi* 2006; **14**: 643-646 [PMID: 16995974]

24 **Cui YL**, Yan F, Wang YB, Song XQ, Liu L, Lei XZ, Zheng MH, Tang H, Feng P. Nucleoside analogue can improve the long-term prognosis of patients with hepatitis B virus infection-associated acute on chronic liver failure. *Dig Dis Sci* 2010; **55**: 2373-2380 [PMID: 20512414 DOI: 10.1007/s10620-010-1257-7]

25 **Leber B**, Spindelboeck W, Stadlbauer V. Infectious complications of acute and chronic liver disease. *Semin Respir Crit Care Med* 2012; **33**: 80-95 [PMID: 22447263 DOI: 10.1055/s-0032-1301737]

P- Reviewer Spahr L **S- Editor** Song XX
L- Editor Logan S **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i7.1111

World J Gastroenterol 2013 February 21; 19(7): 1111-1118
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Risk factors for hepatic decompensation in patients with primary biliary cirrhosis

Tian-Yan Shi, Li-Na Zhang, Hua Chen, Li Wang, Min Shen, Xuan Zhang, Feng-Chun Zhang

Tian-Yan Shi, Li-Na Zhang, Hua Chen, Li Wang, Min Shen, Xuan Zhang, Feng-Chun Zhang, Department of Rheumatology, Key Laboratory of Rheumatology and Clinical Immunology, Peking Union Medical College Hospital, Chinese Academy of Medical Science, Beijing 100032, China

Author contributions: Zhang FC designed the research plan; Chen H, Wang L, Shen M, Zhang X, and Zhang FC recruited patients; Shi TY and Zhang LN analyzed data; Shi TY wrote the paper.

Supported by National Science Technology Pillar Program in the 11th Five-Year Plan, No. 2008BAI59B03; and Research Special Fund for the Public Welfare Industry of Health, No. 201202004

Correspondence to: Feng-Chun Zhang, MD, Department of Rheumatology, Key Laboratory of Rheumatology and Clinical Immunology, Peking Union Medical College Hospital, Chinese Academy of Medical Science, No. 41 Da Mu Cang, Western District, Beijing 100032, China. zhangsamfch@hotmail.com
Telephone: +86-10-88068795 Fax: +86-10-88068794

Received: October 29, 2012 Revised: January 2, 2013
Accepted: January 11, 2013
Published online: February 21, 2013

Abstract

AIM: To examine the clinical features and analyze prognostic factors in a prospective study of primary biliary cirrhosis (PBC) patients.

METHODS: From 1995 to 2010, PBC patients without hepatic decompensation seen at the Peking Union Medical College Hospital were enrolled. Clinical signs and manifestations (pruritus, persistent fatigue, jaundice and pain in the right hypochondrium), laboratory parameters (auto-antibodies for autoimmune hepatic disease, biliary and hepatic enzymes, immunoglobulin, bilirubin, and albumin) and imaging findings were recorded at entry and at specific time points during follow-up. Cox regression and Kaplan-Meier analyses, respectively, assessed the risk factors for hepatic decompensation and survival.

RESULTS: Two hundred and sixty-two PBC patients were enrolled with a median follow-up of 75.2 mo (range, 21-201 mo). The 240 patients were aged 51.5 ± 10.2 years at diagnosis and 91.6% were female. Two hundred and forty-five (93.5%) were seropositive for anti-mitochondrial antibodies. At presentation, 170 patients (64.9%) were symptomatic, while 96 patients (36.6%) had extra-hepatic autoimmune disease. During the follow-up period, 62 (23.7%) patients developed hepatic decompensation of whom four underwent liver transplantation and 17 died. The cumulative survival rate and median survival time were 83.9% and 181.7 mo, respectively. Cox regression analysis revealed that an incomplete ursodeoxycholic acid (UDCA) response or inconsistent treatment [$P < 0.001$; hazard risk (HR) 95%CI = 2.423-7.541], anti-centromere antibodies (ACA) positivity ($P < 0.001$; HR 95%CI = 2.516-7.137), alanine aminotransferase ratio (AAR) elevations ($P < 0.001$; HR 95%CI = 1.357-2.678), and histological advanced liver disease ($P = 0.006$; HR 95%CI = 1.481-10.847) were predictors of hepatic decompensation. The clinical features and survival of PBC in China are consistent with those described in Western countries.

CONCLUSION: Incomplete UDCA response or inconsistent treatment, ACA positivity, AAR elevations, and advanced histological stage are predictors of decompensation.

© 2013 Baishideng. All rights reserved.

Key words: Primary biliary cirrhosis; Risk factor; Hepatic decompensation; Survival; Ursodeoxycholic acid response; Anti-centromere antibodies; Histological stage

Shi TY, Zhang LN, Chen H, Wang L, Shen M, Zhang X, Zhang FC. Risk factors for hepatic decompensation in patients with primary biliary cirrhosis. *World J Gastroenterol* 2013; 19(7): 1111-1118 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i7/1111.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i7.1111

INTRODUCTION

Primary biliary cirrhosis (PBC) is a chronic cholestatic autoimmune liver disease that causes lymphocytic portal hepatitis and bile duct destruction, leading to cirrhosis and liver failure[1]. The annual incidence rates range between 0.7 and 49 cases per million persons, while prevalence rates range between 6.7 and 402 cases per million[2,3]. Currently, treatment with ursodeoxycholic acid (UDCA) at a dose of 13-15 mg/kg/d is the recommended first-line therapy.

According to clinical observations, the progression and prognosis of PBC vary significantly among patients, including a relatively slow progression in some patients, while others have a more rapid progression to hepatic failure[4,5]. Therefore, knowledge of the factors affecting disease progression and prognosis would be of great value in clinical management.

Several prognostic models have been developed for PBC. The most important is the Mayo score model, which has been widely used to predict the survival of PBC patients, and assess the timing of liver transplantation[6,7]. However, some patients are asymptomatic at the time of diagnosis, which limits the application of the Mayo model; and this model was not originally developed to assess disease progression in the early stages of PBC[8]. In terms of treatment, UDCA has been reported to delay histological progression and improve long-term prognosis[9]. Previous studies have shown that anti-gp210 and anti-centromere antibodies (ACA) were associated with severe disease course and poor prognosis. Bilirubin, prothrombin time, hypoalbuminemia and serum immunoglobulin (Ig) G have also been reported as prognostic markers[10-12]. However, these studies were mainly retrospective and had small sample sizes.

We aimed to assess the clinical features and risk factors for hepatic decompensation in a large prospective cohort study in China.

MATERIALS AND METHODS

Study population and data collection

Patients with PBC seen at the Peking Union Medical College Hospital (PUMCH) from 1995 to 2010 were recruited. The ethics committee at PUMCH approved the study, and written informed consent was obtained from all participants prior to enrollment. The diagnosis of PBC was made if at least two of the following three criteria were fulfilled: (1) elevated serum alkaline phosphatase (ALP) (at least 1.5 times the upper limit of normal); (2) the presence of anti-mitochondrial antibodies (AMAs) in serum; and (3) representative histological manifestations of portal area inflammation and bile duct injury. Early PBC was defined by seropositivity for AMA, and the presence of histological manifestations, but normal liver function[4]. The liver histology was graded according to the Ludwig classification[13]. Patients with liver decompensation, autoimmune hepatitis (AIH), viral hepatitis and other causes of liver damage were excluded. Patients with PBC/AIH overlap syndrome were identified according to the criteria proposed by Chazouillères *et al*[14]. In the current study, serum antinuclear antibody (ANA), AMA, anti-smooth muscle antibody, anti-liver kidney microsomal antibody, and anti-parietal cell antibody were tested. In patients who were ANA positive, the level of alanine aminotransferase (ALT), aspartate aminotransferase (AST), IgG and autoantibodies for AIH were assessed to exclude PBC/AIH. The extra-hepatic autoimmune diseases were diagnosed according to the generally accepted criteria.

The following information was recorded: (1) demographic features, clinical signs and manifestations of liver disease including pruritus, persistent fatigue, jaundice and pain in the right hypochondrium; (2) laboratory parameters, including ANA, AMA, ACA, ALT, AST, ALP, γ-glutamyl-transferase (GGT), total bilirubin (TBil), direct bilirubin (DBil), albumin (ALB), IgG, IgA and IgM; and (3) imaging findings: liver ultrasound or CT demonstrating morphologic changes of liver and spleen, and portal vein blood flow. UDCA at the dose of 13-15 mg/kg per day was prescribed for all patients.

Follow-up

Patients were followed up in the outpatient clinic, and data were collected at 3-mo intervals during the first year, 6-mo intervals or yearly thereafter. During each visit, the clinical signs, symptoms and laboratory parameters (AST, ALT, ALP, GGT, ALB, TBil, DBil, IgG, IgA, IgM, white blood cell, hemoglobin and platelet count) were assessed. Liver ultrasound was performed annually. Gastroscopy was carried out if necessary. If the patients had symptoms of hepatic decompensation (ascites, esophageal varices, variceal bleeding, hypersplenism, coagulant function abnormality, hypoproteinemia, and hepatic encephalopathy), they were permitted to visit doctors at any time or to go to emergency departments. After treatment for one year, patients whose ALP levels decreased by more than 40% of baseline values or returned to normal were defined as UDCA responders[15].

Outcome evaluation

Two clinical outcomes were studied: hepatic decompensation, and liver-related death. Hepatic decompensation was defined as the occurrence of severe functional damage of liver and one or more complications of liver cirrhosis[16]. We investigated the following features of decompensation: hypersplenism, ascites, esophageal varices (variceal bleeding), encephalopathy, hypoproteinemia, coagulant function abnormality, and spontaneous bacterial peritonitis. Figure 1 shows a diagram of the study design.

Statistical analysis

Data were expressed as median (range) or mean ± SD. Analysis was performed using SPSS 17.0 statistical software (Chicago, IL, United States). A two-sided P-value < 0.05 was considered statistically significant. Categorical variables were compared by the χ^2 test while continuous

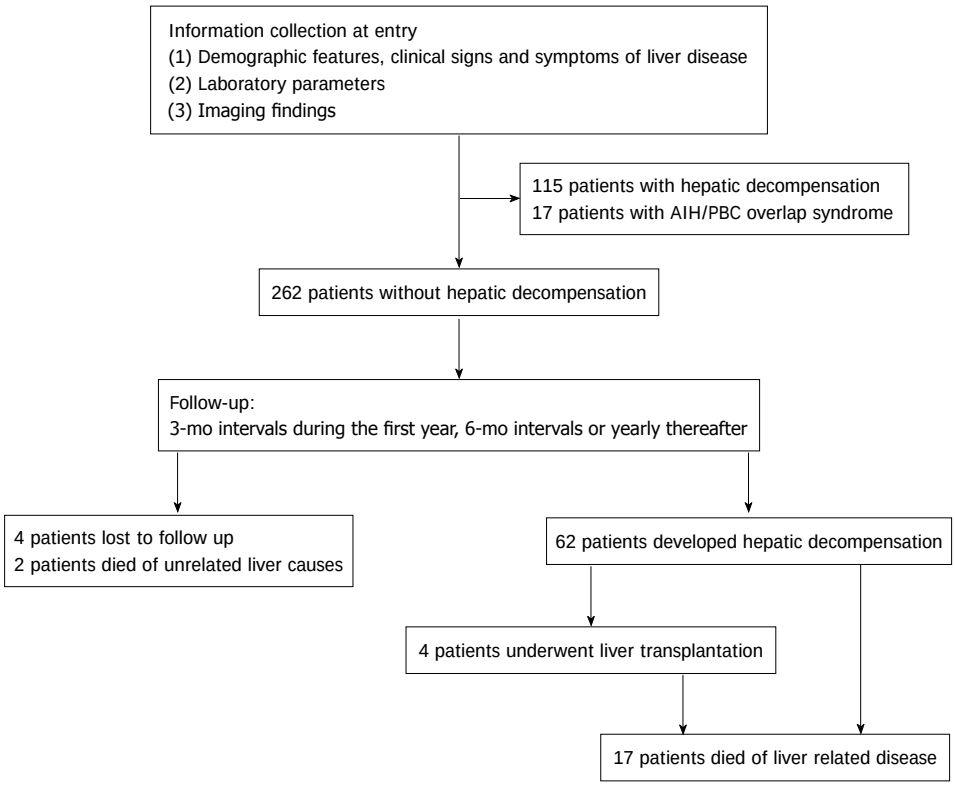


Figure 1 Diagram showing the study enrollment.

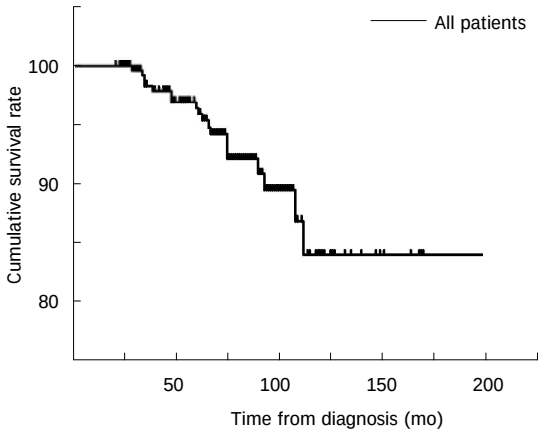


Figure 2 Survival curve of all patients in this cohort.

variables were compared by the Student's *t*-test or Mann-Whitney *U* test. Variables that were significant in univariate analysis were submitted to Cox regression analysis to predict the independent risk factors. Hazard risk (HR) and 95%CI were presented. Kaplan-Meier analysis was used to assess survival. Patients who were lost to follow-up or died of causes unrelated to liver failure were censored.

RESULTS

Clinical profiles of the study group at the entry

In this study, 262 patients with a mean age of 51.5 ± 10.2 years, including 240 (91.6%) women, were enrolled. The median duration of follow-up was 75.2 mo (range, 21-201 mo). The demographic and clinical features are shown in Tables 1 and 2.

On entry into the study, 170 (64.9%) patients presented with manifestations of liver disease, and 96 (36.6%) patients had extra-hepatic autoimmune disease. In addition, we enrolled 19 (7.3%) patients with early PBC. Two hundred and forty-five (93.5%) patients were seropositive for AMA. ANA and ACA were detected in 181 (69.1%) and 73 (27.9%) patients, respectively. Among 110 patients who underwent needle liver biopsies, 97 (88.2%) were at stages Ⅰ-Ⅱ, while 13 (11.8%) were at stages Ⅲ-Ⅳ. One hundred and ninety (72.5%) patients received UDCA regularly and responded, while 46 patients (17.6%) took UDCA consistently, but failed to respond. Of the latter, eight patients terminated treatment on their own. Others (9.9%) did not take UDCA consistently, as prescribed.

Incidence of outcomes and survival

During the study period, 62 (23.7%) patients developed adverse events, and the median duration from diagnosis to hepatic decompensation was 57.4 mo (range, 7-151 mo). Among these patients, four (1.5%) patients underwent liver transplantation at the end of follow-up or before death, and 17 (6.5%) patients died of liver failure. The median duration from diagnosis to death was 63.6 mo (range, 29-112 mo). According to Kaplan-Meier analysis, the cumulative survival rate and estimated median survival time were 83.9% and 181.7 mo, respectively (Figure 2 and Table 3).

Table 1 Demographic and clinical laboratory characteristics of primary biliary cirrhosis patients at the time of entry (*n* = 262) *n* (%)

Age (yr), mean ± SD	51.5 ± 10.2
Gender (female/male), *n*	240/22
The symptomatic	170 (64.9)
Jaundice	78 (29.8)
Pruritus	69 (26.3)
Persistent fatigue	63 (24.0)
Pain in the right hypochondrium	40 (15.3)
UDCA responders	190 (72.5)
Early PBC	19 (7.3)
Extra-hepatic autoimmune diseases	96 (36.6)
Sjögren's syndrome	54 (20.6)
Autoimmune thyroid diseases	25 (9.5)
CREST/systemic sclerosis	8 (3.1)/3 (1.1)
Polymyositis/dermatomyositis	8 (3.1)/1 (0.4)
Systemic lupus erythematosus	4 (1.5)
Psoriasis	4 (1.5)
Rheumatoid arthritis	3 (1.1)
Takayasu's arteritis	2 (0.8)
Histological stages	110 (42.0)
Ⅰ - Ⅱ	97 (88.2)
Ⅲ-Ⅳ	13 (11.8)

UDCA: Ursodeoxycholic acid; PBC: Primary biliary cirrhosis; PBC: Primary biliary cirrhosis; CREST: Comprehensive overview covers symptoms, causes, treatment of this autoimmune disorder.

Risk factors for hepatic decompensation

Possible risk factors were first identified by univariate analysis. Subsequently, all variables, except histological stage, that showed statistical differences in univariate analysis were introduced into the Cox regression model. Incomplete UDCA response or inconsistent UDCA treatment ($P < 0.001$; HR 95%CI = 2.423-7.541), ACA positivity ($P < 0.001$; HR 95%CI = 2.516-7.137), alanine aminotransferase ratio (AAR) elevations ($P < 0.001$; HR 95%CI = 1.357-2.678), TBil ($P = 0.004$; HR 95%CI = 1.002-1.009), and ALP ($P = 0.008$; HR 95%CI = 1.000-1.002) showed statistical significance in the model (Figure 3 and Tables 4 and 5). Although histological stage was not introduced into the model because of limited numbers, Cox regression analysis indicated that it was also a prognostic factor ($P = 0.006$; HR 95%CI = 1.481-10.847; Figure 3).

DISCUSSION

PBC is a chronic autoimmune liver disease that has the potential to progress to cirrhosis and, eventually, hepatic failure. Although it is less common in East Asia[17], there is a rising frequency attributable to a more widespread awareness of this disease among physicians[18]. In this study, with a follow-up for 17 years, we identified the clinical profiles and risk factors for hepatic decompensation of PBC patients. To the best of our knowledge, this is the first prospective study with such a large number of patients in China.

In the current cohort, the demographic features and autoantibody profiles were consistent with those of previous studies[19]. Although PBC is an organ-specific disease, association with extra-hepatic autoimmune diseases is an important characteristic. The prevalence of such co-existing diseases was high (36.6%), the most common of which were Sjögren's syndrome (SS) (20.6%) and autoimmune thyroid diseases (9.5%). These findings were similar to the results obtained by Marasini *et al*[20] in Italy and Silveira *et al*[21] in the United States.

Table 2 Laboratory characteristics of primary biliary cirrhosis patients at the time of entry (*n* = 262)

ANA	181(69.1)
AMA	245 (93.5)
ACA	73 (27.9)
ALT (IU/L)	91.0 (9-598)
AST (IU/L)	86.2 (15-428)
AST/ALT	1.10 (0.22-4.85)
ALP (IU/L)	348.5 (21-1486)
GGT (IU/L)	428.2 (9-2614)
ALB (g/L)	40.7 (25-69)
TBil (μmol/L)	28.05 (4.90-334.40)
DBil (μmol/L)	14.39 (0.34-237.40)
IgG (g/L)	16.81 (5.12-56.60)
IgA (g/L)	3.15 (0.19-8.84)
IgM (g/L)	4.66 (0.41-22.30)

Continuous data were expressed as *n* (%) or median (range). ANA: Anti-nuclear antibody; AMA: Anti-mitochondrial antibody; ACA: Anti-centromere antibody; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT: γ-glutamyl-transferase; ALB: Albumin; TBil: Total bilirubin; DBil: Direct bilirubin; Ig: Immunoglobulin.

Clinically, PBC patients can progress from an asymptomatic stage to a symptomatic stage because of liver damage[22]. In the current cohort, 64.9% patients had hepatic-related symptoms at diagnosis, which is higher than that reported by Prince *et al*[23], but lower than the results by Su *et al*[11]. In the current study, the most common manifestation was jaundice, which was different from other reports in which the most common manifestation was fatigue[22,24,25]. The median survival time for asymptomatic patients has been reported to be longer than that for symptomatic patients[26]. In addition, other studies indicated that asymptomatic patients had shorter life spans than people in the general population[26,27]. However, several studies have indicated that asymptomatic patients did not have a better prognosis[23,28].

In the current study, at the end of follow-up, sixty-tow (23.7%) patients developed hepatic decompensation. Among them, all had portal hypertension, and 17 died of liver failure. Only four patients underwent transplantation because of the enormous financial cost for patients, and limited medical resources. However, the survival rate of our patients was similar to those presented in other recent studies[29-31].

Although there has been controversy about efficacy, UDCA is currently accepted as the first-line drug for PBC. Several studies have shown that UDCA not only ameliorates laboratory indices, but also delays histological progression, and improves survival without transplantation[9,15,32-35], especially for patients at early histological stages[33]. In the current study, 72.5% patients took

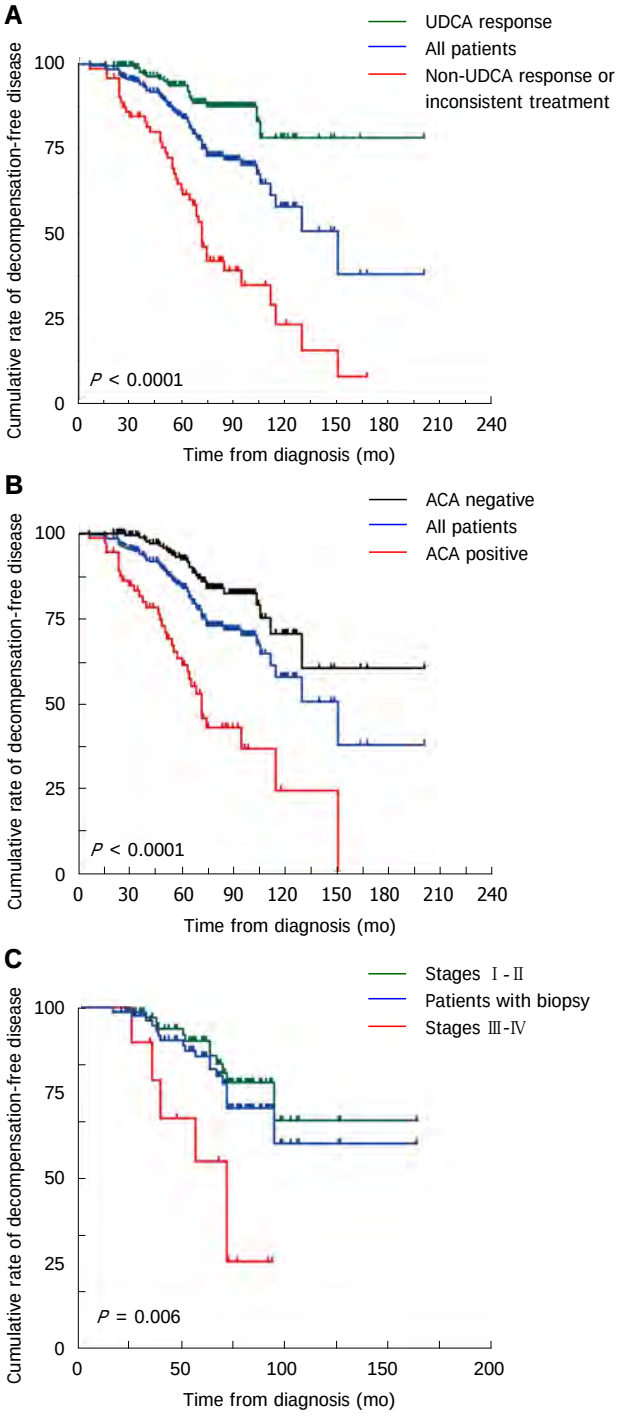


Figure 3 Cumulative rates of decompensation-free disease in primary biliary cirrhosis patients stratified by ursodeoxycholic acid treatment, anti-centromere antibody, and histological stage, respectively. A: Ursodeoxycholic acid (UDCA); B: Anti-centromere antibody (ACA); C: Histological stage.

UDCA as prescribed and responded according to the Barcelona criteria; their prognoses were significantly better than those of non-responders, which is consistent with results of a previous study[9]. Therefore, the data suggest that all PBC patients should take UDCA regularly, but novel therapeutic options are needed for patients who do not respond to UDCA.

ACA had been observed in patients with various rheumatic disorders, including CREST syndrome (comprehensive overview covers symptoms, causes, treatment of this autoimmune disorder)[36], SS[37], Raynaud's phenomenon[38], interstitial pneumonia in systemic lupus erythematosus[39], SS overlap syndrome[40] and 15%-30% PBC patients[41-44]. In the current study, ACA was detected in 27.9% patients and 56.5% of decompensated patients. It was found to be an independent prognostic factor for patients. Previous cross-sectional studies have also indicated that ACA was associated with the development of liver failure[45] and progression to portal hypertension in PBC[10]. However, Rigamonti *et al*[46] found that PBC patients with or without ACA had the same rate of liver-related death, while Parveen *et al*[47] reported that ACA was not correlated with either laboratory or histological manifestations.

Table 3 Complications of chronic liver disease in the primary biliary cirrhosis cohort (*n* = 262) *n* (%)

Hepatic decompensation	62 (23.7)
Portal hypertension	62 (23.7)
Hypersplenism	44 (16.8)
Esophageal varices	47 (17.9)
Variceal bleeding	35 (13.4)
Ascites	43 (16.4)
Encephalopathy	19 (7.3)
Coagulant function abnormality	12 (4.6)
Hypoproteinemia	35 (13.4)
Spontaneous bacterial peritonitis	3 (1.1)
Liver transplantation	4 (1.5)
Death-liver related	17 (6.5)[1]
Censor	6 (2.3)
Lost to follow-up	4 (1.5)
Death from causes unrelated to liver failure	2 (0.8)

[1]One patient who underwent liver transplantation died.

Elevation of AAR may be explained by a reduction in AST clearance, and by mitochondrial injury in severe liver disease[48]. A previous study showed that AAR exhibited modest correlations to Mayo and Child scores for evaluating the severity of PBC, and it was higher in histological stages Ⅲ-Ⅳ than Ⅰ-Ⅱ disease. Patients with an AAR of 1 or less had better prognoses than their counterparts[49]. These results are consistent with the current data.

The beneficial value of liver biopsy in patients with PBC is controversial because the pattern of cirrhosis in PBC is irregular, and sampling error may lead to misinterpretation[50]. However, several studies have indicated a relationship between histology and clinical profile. AST and bilirubin were positively related to portal fibrosis for the AMA-positive patients[51]. Prognosis was found to be correlated with the histological stages of hepatic fibrosis, cholestasis and periportal cell necrosis[52]. Cirrhosis was found to have developed within four years in 31% and 50% of patients who were initially at stage Ⅰ and stage Ⅱ, respectively[53]. Cox analysis demonstrated that histological stage was a predictor of liver decompensation.

Although TBil and ALP were included in our model, the HR approached one. Therefore, they had a relatively low correlation with survival compared with other fac-

Table 4 Univariate analysis of possible risk factors for hepatic decompensation in primary biliary cirrhosis

	Decompensation free (*n* = 196)	Decompensation (*n* = 62)	*P* value
Age at diagnosis (yr)	50.2	55.6	< 0.001
Gender (female/male)	178/18	59/3	0.275
Symptoms	60.20%	80.65%	0.003
UDCA treatment	85.20%	30.65%	< 0.001
ANA	64.80%	80.65%	0.019
AMA	92.86%	95.16%	0.731
ACA	19.39%	56.45%	< 0.001
ALT (IU/L)	89.6	96.6	0.109
AST (IU/L)	80.6	103.2	< 0.001
AST/ALT	1.05	1.27	0.013
ALP (IU/L)	311.6	454.1	< 0.001
GGT (IU/L)	395.4	524.3	0.001
ALB (g/L)	41.5	38.4	< 0.001
TBil (μmol/L)	20.92	48.84	< 0.001
DBil (μmol/L)	9.27	29.06	< 0.001
IgG (g/L)	16.61	17.43	0.386
IgA (g/L)	2.99	3.62	0.004
IgM (g/L)	4.61	4.83	0.285
Histological stage[1] (Ⅰ-Ⅱ)	92.22%	66.67%	0.008
Early PBC	9.70%	0.00%	0.023
Extra-hepatic autoimmune disease	37.24%	37.10%	0.983

[1]*n* = 108. ANA: Anti-nuclear antibody; AMA: Anti-mitochondrial antibody; ACA: Anti-centromere antibody; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; ALP: Alkaline phosphatase; GGT: γ-glutamyl-transferase; ALB: Albumin; TBil: Total bilirubin; DBil: Direct bilirubin; UDCA: Ursodeoxycholic acid; Ig: Immunoglobulin.

tors, and their value for this purpose still needs to be confirmed by other, large long-term studies.

There are some limitations of the current study: (1) only patients without decompensation were enrolled, which resulted in the expected better survival rates compared to studies that included patients with decompensation; and (2) this was a single center study with a relatively short follow-up. Consequently, these risk factors still need to be confirmed further.

In summary, the clinical features and survival of patients with PBC in China are consistent with those described in Western countries. Incomplete UDCA response or inconsistent treatment, ACA positivity, higher AAR, and advanced histological stage are risk factors for hepatic decompensation.

ACKNOWLEDGMENTS

We thank Xu-Hua Shi and Li-Xia Gao for the collection of data and patient information.

COMMENTS

Background

Primary biliary cirrhosis (PBC) is a chronic cholestatic autoimmune liver disease with a rising frequency, attributable to a widespread awareness from physicians. The progression and prognosis of PBC vary significantly among patients. However, the clinical features and risk factors for hepatic decompensation have not yet been well-documented in a large prospective cohort study in China.

Table 5 Cox regression analysis of risk factors for hepatic decompensation in primary biliary cirrhosis (*n* = 262)

	HR	HR 95%CI	*P* value
Poor UDCA response or inconsistent treatment	4.275	2.423-7.541	< 0.001
ACA positivity	4.237	2.516-7.137	< 0.001
AAR	1.906	1.357-2.678	< 0.001
TBil	1.005	1.002-1.009	0.004
ALP	1.001	1.000-1.002	0.008

ACA: Anti-centromere antibody; ALP: Alkaline phosphatase; TBil: Total bilirubin; UDCA: Ursodeoxycholic acid; AAR: Alanine aminotransferase ratio; HR: Hazard risk.

Research frontiers

In the current study, the authors identified the clinical profiles and risk factors for hepatic decompensation of PBC patients. In the authors' view, this is the first prospective study with such a large number of patients in China.

Innovations and breakthroughs

Recent reports have highlighted the importance of ursodeoxycholic acid (UDCA) treatment for improving prognosis in patients with PBC. This is the first prospective cohort study showing that incomplete UDCA response or inconsistent treatment, anti-centromere antibodies positivity, higher alanine aminotransferase ratio, and advanced histological stage are risk factors for hepatic decompensation.

Applications

Knowledge of the factors for hepatic decompensation in PBC patients would be of great value in clinical management. Novel treatments are needed for patients with poor prognostic factors.

Terminology

PBC is an autoimmune liver disease characterized by the presence of anti-mitochondrial antibodies, and destruction of intrahepatic bile ducts, which can ultimately lead to cirrhosis and hepatic failure. Early UDCA treatment, excellent biochemical response to UDCA, and histological stages are predictors of survival.

Peer review

The authors have documented the clinical profiles and analyzed risk factors for hepatic decompensation in patients with PBC. It is a relatively large prospective study and gives important information on the prognosis of PBC patients.

REFERENCES

1 **Kaplan MM**, Gershwin ME. Primary biliary cirrhosis. *N Engl J Med* 2005; **353**: 1261-1273 [PMID: 16177252 DOI: 10.1056/NEJMra043898]
2 **Prince MI**, James OF. The epidemiology of primary biliary cirrhosis. *Clin Liver Dis* 2003; **7**: 795-819 [PMID: 14594132]
3 **Lazaridis KN**, Talwalkar JA. Clinical epidemiology of primary biliary cirrhosis: incidence, prevalence, and impact of therapy. *J Clin Gastroenterol* 2007; **41**: 494-500 [PMID: 17450033 DOI: 10.1097/01.mcg.0000225653.07932.8f]
4 **Ishibashi H**, Komori A, Shimoda S, Ambrosini YM, Gershwin ME, Nakamura M. Risk factors and prediction of long-term outcome in primary biliary cirrhosis. *Intern Med* 2011; **50**: 1-10 [PMID: 21212566]
5 **Poupon R**. Primary biliary cirrhosis: a 2010 update. *J Hepatol* 2010; **52**: 745-758 [PMID: 20347176]
6 **Dickson ER**, Grambsch PM, Fleming TR, Fisher LD, Langworthy A. Prognosis in primary biliary cirrhosis: model for decision making. *Hepatology* 1989; **10**: 1-7 [PMID: 2737595]
7 **Grambsch PM**, Dickson ER, Wiesner RH, Langworthy A. Application of the Mayo primary biliary cirrhosis survival model to Mayo liver transplant patients. *Mayo Clin Proc* 1989; **64**: 699-704 [PMID: 2664365]

8 **Mayo MJ**, Parkes J, Adams-Huet B, Combes B, Mills AS, Markin RS, Rubin R, Wheeler D, Contos M, West AB, Saldana S, Getachew Y, Butsch R, Luketic V, Peters M, Di Bisceglie A, Bass N, Lake J, Boyer T, Martinez E, Boyer J, Garcia-Tsao G, Barnes D, Rosenberg WM. Prediction of clinical outcomes in primary biliary cirrhosis by serum enhanced liver fibrosis assay. *Hepatology* 2008; **48**: 1549-1557 [PMID: 18846542 DOI: 10.1002/hep.22517]

9 **Kuiper EM**, Hansen BE, de Vries RA, den Ouden-Muller JW, van Ditzhuijsen TJ, Haagsma EB, Houben MH, Witteman BJ, van Erpecum KJ, van Buuren HR. Improved prognosis of patients with primary biliary cirrhosis that have a biochemical response to ursodeoxycholic acid. *Gastroenterology* 2009; **136**: 1281-1287 [PMID: 19208346 DOI: 10.1053/j.gastro.2009.01.003]

10 **Gao L**, Tian X, Liu B, Zhang F. The value of antinuclear antibodies in primary biliary cirrhosis. *Clin Exp Med* 2008; **8**: 9-15 [PMID: 18385935]

11 **Su CW**, Hung HH, Huo TI, Huang YH, Li CP, Lin HC, Lee PC, Lee SD, Wu JC. Natural history and prognostic factors of primary biliary cirrhosis in Taiwan: a follow-up study up to 18 years. *Liver Int* 2008; **28**: 1305-1313 [PMID: 18346129 DOI: 10.1111/j.1478-3231.2008.01715.x]

12 **Zhao DT**, Liao HY, Liu YM, Zhao Y, Feng X, Yan HP. Prognostic factors and survival analysis of antimitochondrial antibody-positive primary biliary cirrhosis in Chinese patients. *Dig Dis Sci* 2011; **56**: 2750-2757 [PMID: 21409375 DOI: 10.1007/s10620-011-1661-7]

13 **Ludwig J**, Dickson ER, McDonald GS. Staging of chronic nonsuppurative destructive cholangitis (syndrome of primary biliary cirrhosis). *Virchows Arch A Pathol Anat Histol* 1978; **379**: 103-112 [PMID: 150690]

14 **Chazouillères O**, Wendum D, Serfaty L, Montembault S, Rosmorduc O, Poupon R. Primary biliary cirrhosis-autoimmune hepatitis overlap syndrome: clinical features and response to therapy. *Hepatology* 1998; **28**: 296-301 [PMID: 9695990 DOI: 10.1002/hep.510280203]

15 **Parés A**, Caballería L, Rodés J. Excellent long-term survival in patients with primary biliary cirrhosis and biochemical response to ursodeoxycholic Acid. *Gastroenterology* 2006; **130**: 715-720 [PMID: 16530513 DOI: 10.1053/j.gastro.2005.12.029]

16 **Murray-Lyon IM**, Pugh RN, Nunnerley HB, Laws JW, Dawson JL, Williams R. Treatment of bleeding oesophageal varices by infusion of vasopressin into the superior mesenteric artery. *Gut* 1973; **14**: 59-63 [PMID: 4540294]

17 **Farrell GC**. Primary biliary cirrhosis in Asians: less common than in Europeans, but just as depressing. *J Gastroenterol Hepatol* 2008; **23**: 508-511 [PMID: 18397481 DOI: 10.1111/j.1440-1746.2008.05379.x]

18 **Liu H**, Liu Y, Wang L, Xu D, Lin B, Zhong R, Gong S, Podda M, Invernizzi P. Prevalence of primary biliary cirrhosis in adults referring hospital for annual health check-up in Southern China. *BMC Gastroenterol* 2010; **10**: 100 [PMID: 20815889 DOI: 10.1186/1471-230X-10-100]

19 **Hohenester S**, Oude-Elferink RP, Beuers U. Primary biliary cirrhosis. *Semin Immunopathol* 2009; **31**: 283-307 [PMID: 19603170 DOI: 10.1007/s00281-009-0164-5]

20 **Marasini B,** Gagetta M, Rossi V, Ferrari P. Rheumatic disorders and primary biliary cirrhosis: an appraisal of 170 Italian patients. *Ann Rheum Dis* 2001; **60:** 1046-1049 [PMID: 11602476]

21 **Silveira MG**, Mendes FD, Diehl NN, Enders FT, Lindor KD. Thyroid dysfunction in primary biliary cirrhosis, primary sclerosing cholangitis and non-alcoholic fatty liver disease. *Liver Int* 2009; **29**: 1094-1100 [PMID: 19291181 DOI: 10.1111/j.1478-3231.2009.02003.x]

22 **Prince M**, Chetwynd A, Newman W, Metcalf JV, James OF. Survival and symptom progression in a geographically based cohort of patients with primary biliary cirrhosis: follow-up for up to 28 years. *Gastroenterology* 2002; **123**: 1044-1051 [PMID: 12360466]

23 **Prince MI**, Chetwynd A, Craig WL, Metcalf JV, James OF. Asymptomatic primary biliary cirrhosis: clinical features, prognosis, and symptom progression in a large population based cohort. *Gut* 2004; **53**: 865-870 [PMID: 15138215]

24 **Selmi C**, Bowlus CL, Gershwin ME, Coppel RL. Primary biliary cirrhosis. *Lancet* 2011; **377**: 1600-1609 [PMID: 21529926]

25 **Milkiewicz P**, Heathcote EJ. Fatigue in chronic cholestasis. *Gut* 2004; **53**: 475-477 [PMID: 15016736]

26 **Mahl TC**, Shockcor W, Boyer JL. Primary biliary cirrhosis: survival of a large cohort of symptomatic and asymptomatic patients followed for 24 years. *J Hepatol* 1994; **20**: 707-713 [PMID: 7930469]

27 **Springer J**, Cauch-Dudek K, O'Rourke K, Wanless IR, Heathcote EJ. Asymptomatic primary biliary cirrhosis: a study of its natural history and prognosis. *Am J Gastroenterol* 1999; **94**: 47-53 [PMID: 9934730 DOI: 10.1111/j.1572-0241.1999.00770.x]

28 **Floreani A**, Caroli D, Variola A, Rizzotto ER, Antoniazzi S, Chiaramonte M, Cazzagon N, Brombin C, Salmaso L, Baldo V. A 35-year follow-up of a large cohort of patients with primary biliary cirrhosis seen at a single centre. *Liver Int* 2011; **31**: 361-368 [PMID: 21059170 DOI: 10.1111/j.1478-3231.2010.02366.x]

29 **Baldursdottir TR**, Bergmann OM, Jonasson JG, Ludviksson BR, Axelsson TA, Björnsson ES. The epidemiology and natural history of primary biliary cirrhosis: a nationwide population-based study. *Eur J Gastroenterol Hepatol* 2012; **24**: 824-830 [PMID: 22562114 DOI: 10.1097/MEG.0b013e328353753d]

30 **Ngu JH**, Gearry RB, Frampton CM, Stedman CA. Mortality and the risk of malignancy in autoimmune liver diseases: a population-based study in Canterbury, New Zealand. *Hepatology* 2012; **55**: 522-529 [PMID: 21994151 DOI: 10.1002/hep.24743]

31 **Myers RP**, Shaheen AA, Fong A, Burak KW, Wan A, Swain MG, Hilsden RJ, Sutherland L, Quan H. Epidemiology and natural history of primary biliary cirrhosis in a Canadian health region: a population-based study. *Hepatology* 2009; **50**: 1884-1892 [PMID: 19821525 DOI: 10.1002/hep.23210]

32 **Angulo P**, Batts KP, Therneau TM, Jorgensen RA, Dickson ER, Lindor KD. Long-term ursodeoxycholic acid delays histological progression in primary biliary cirrhosis. *Hepatology* 1999; **29**: 644-647 [PMID: 10051462 DOI: 10.1002/hep.510290301]

33 **Corpechot C**, Carrat F, Bahr A, Chrétien Y, Poupon RE, Poupon R. The effect of ursodeoxycholic acid therapy on the natural course of primary biliary cirrhosis. *Gastroenterology* 2005; **128**: 297-303 [PMID: 15685541]

34 **Poupon RE**, Lindor KD, Parés A, Chazouillères O, Poupon R, Heathcote EJ. Combined analysis of the effect of treatment with ursodeoxycholic acid on histologic progression in primary biliary cirrhosis. *J Hepatol* 2003; **39**: 12-16 [PMID: 12821038]

35 **Corpechot C**, Abenavoli L, Rabahi N, Chrétien Y, Andréani T, Johanet C, Chazouillères O, Poupon R. Biochemical response to ursodeoxycholic acid and long-term prognosis in primary biliary cirrhosis. *Hepatology* 2008; **48**: 871-877 [PMID: 18752324 DOI: 10.1002/hep.22428]

36 **Miyawaki S**, Asanuma H, Nishiyama S, Yoshinaga Y. Clinical and serological heterogeneity in patients with anticentromere antibodies. *J Rheumatol* 2005; **32**: 1488-1494 [PMID: 16078324]

37 **Salliot C**, Gottenberg JE, Bengoufa D, Desmoulins F, Miceli-Richard C, Mariette X. Anticentromere antibodies identify patients with Sjögren's syndrome and autoimmune overlap syndrome. *J Rheumatol* 2007; **34**: 2253-2258 [PMID: 17937465]

38 **Hossny E**, Hady HA, Mabrouk R. Anti-centromere antibodies as a marker of Raynaud's phenomenon in pediatric rheumatologic diseases. *Pediatr Allergy Immunol* 2000; **11**: 250-255 [PMID: 11110581]

39 **Takada K**, Suzuki K, Matsumoto M, Okada M, Nakanishi T,

Horikoshi H, Higuchi T, Ohsuzu F. Clinical characteristics of patients with both anti-U1RNP and anti-centromere antibodies. *Scand J Rheumatol* 2008; **37**: 360-364 [PMID: 18686191 DOI: 10.1080/03009740802116190]
40 **Pakozdi A**, Nihtyanova S, Moinzadeh P, Ong VH, Black CM, Denton CP. Clinical and serological hallmarks of systemic sclerosis overlap syndromes. *J Rheumatol* 2011; **38**: 2406-2409 [PMID: 21844148 DOI: 10.3899/jrheum.101248]
41 **Agmon-Levin N**, Shapira Y, Selmi C, Barzilai O, Ram M, Szyper-Kravitz M, Sella S, Katz BS, Youinou P, Renaudineau Y, Larida B, Invernizzi P, Gershwin ME, Shoenfeld Y. A comprehensive evaluation of serum autoantibodies in primary biliary cirrhosis. *J Autoimmun* 2010; **34**: 55-58 [PMID: 19897339 DOI: 10.1016/j.jaut.2009.08.009]
42 **Muratori P**, Muratori L, Ferrari R, Cassani F, Bianchi G, Lenzi M, Rodrigo L, Linares A, Fuentes D, Bianchi FB. Characterization and clinical impact of antinuclear antibodies in primary biliary cirrhosis. *Am J Gastroenterol* 2003; **98**: 431-437 [PMID: 12591064]
43 **Granito A**, Muratori P, Muratori L, Pappas G, Cassani F, Worthington J, Ferri S, Quarneti C, Cipriano V, de Molo C, Lenzi M, Chapman RW, Bianchi FB. Antibodies to SS-A/Ro-52kD and centromere in autoimmune liver disease: a clue to diagnosis and prognosis of primary biliary cirrhosis. *Aliment Pharmacol Ther* 2007; **26**: 831-838 [PMID: 17767467 DOI: 10.1111/j.1365-2036.2007.03433.x]
44 **Nakamura M**, Kondo H, Mori T, Komori A, Matsuyama M, Ito M, Takii Y, Koyabu M, Yokoyama T, Migita K, Daikoku M, Abiru S, Yatsuhashi H, Takezaki E, Masaki N, Sugi K, Honda K, Adachi H, Nishi H, Watanabe Y, Nakamura Y, Shimada M, Komatsu T, Saito A, Saoshiro T, Harada H, Sodeyama T, Hayashi S, Masumoto A, Sando T, Yamamoto T, Sakai H, Kobayashi M, Muro T, Koga M, Shums Z, Norman GL, Ishibashi H. Anti-gp210 and anti-centromere antibodies are different risk factors for the progression of primary biliary cirrhosis. *Hepatology* 2007; **45**: 118-127 [PMID: 17187436 DOI: 10.1002/hep.21472]
45 **Yang WH**, Yu JH, Nakajima A, Neuberg D, Lindor K, Bloch DB. Do antinuclear antibodies in primary biliary cirrhosis patients identify increased risk for liver failure? *Clin Gastroenterol Hepatol* 2004; **2**: 1116-1122 [PMID: 15625657]
46 **Rigamonti C,** Shand LM, Feudjo M, Bunn CC, Black CM, Denton CP, Burroughs AK. Clinical features and prognosis of primary biliary cirrhosis associated with systemic sclerosis. *Gut* 2006; **55:** 388-394 [PMID: 16150855 DOI: 10.1136/gut.2005.075002]
47 **Parveen S,** Morshed SA, Nishioka M. High prevalence of antibodies to recombinant CENP-B in primary biliary cirrhosis: nuclear immunofluorescence patterns and ELISA reactivities. *J Gastroenterol Hepatol* 1995; **10:** 438-445 [PMID: 8527711]
48 **Okuda M**, Li K, Beard MR, Showalter LA, Scholle F, Lemon SM, Weinman SA. Mitochondrial injury, oxidative stress, and antioxidant gene expression are induced by hepatitis C virus core protein. *Gastroenterology* 2002; **122**: 366-375 [PMID: 11832451]
49 **Su CW**, Chan CC, Hung HH, Huo TI, Huang YH, Li CP, Lin HC, Tsay SH, Lee PC, Lee SD, Wu JC. Predictive value of aspartate aminotransferase to alanine aminotransferase ratio for hepatic fibrosis and clinical adverse outcomes in patients with primary biliary cirrhosis. *J Clin Gastroenterol* 2009; **43**: 876-883 [PMID: 19247208 DOI: 10.1097/MCG.0b013e31818980ac]
50 **Garrido MC**, Hubscher SG. Accuracy of staging in primary biliary cirrhosis. *J Clin Pathol* 1996; **49**: 556-559 [PMID: 8813953]
51 **Drebber U**, Mueller JJ, Klein E, Kasper HU, Schulze F, Schardt K, Quasdorff M, Schulte S, Odenthal M, Dienes HP. Liver biopsy in primary biliary cirrhosis: clinicopathological data and stage. *Pathol Int* 2009; **59**: 546-554 [PMID: 19627538 DOI: 10.1111/j.1440-1827.2009.02405.x]
52 **Roll J**, Boyer JL, Barry D, Klatskin G. The prognostic importance of clinical and histologic features in asymptomatic and symptomatic primary biliary cirrhosis. *N Engl J Med* 1983; **308**: 1-7 [PMID: 6847917 DOI: 10.1056/NEJM198301063080101]
53 **Locke GR**, Therneau TM, Ludwig J, Dickson ER, Lindor KD. Time course of histological progression in primary biliary cirrhosis. *Hepatology* 1996; **23**: 52-56 [PMID: 8550048 DOI: 10.1002/hep.510230108]

P- Reviewers Caboclo JLF, Beltran MA, Shimizu Y, Weekitt K
S- Editor Gou SX **L- Editor** Stewart G **E- Editor** Xiong L



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i7.1119

World J Gastroenterol 2013 February 21; 19(7): 1119-1123
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

A temporary self-expanding metallic stent for malignant colorectal obstruction

Xiao-Li Ding, Yong-Dong Li, Rui-Min Yang, Fen-Bao Li, Ming-Qiu Zhang

Xiao-Li Ding, Yong-Dong Li, Rui-Min Yang, Fen-Bao Li, Ming-Qiu Zhang, Department of Imaging and Interventional Center, The First Affiliated Hospital of Xinxiang Medical College, Weihui 453100, Henan Province, China
Yong-Dong Li, Institute of Diagnostic and Interventional Radiology, The Sixth Affiliated People's Hospital, Shanghai Jiao Tong University, Shanghai 200233, China
Author contributions: Yang RM, Li FB and Zhang MQ performed the majority of experiments; Li YD and Yang RM provided vital reagents and analytical tools and were also involved in revising the manuscript; Yang RM collected all the human materials and provided financial support for this work; Ding CL designed the study and wrote the manuscript.
Correspondence to: Dr. Rui-Min Yang, MD, Professor, Department of Imaging and Interventional Center, The First Affiliated Hospital of Xinxiang Medical College, No. 88, Jian Kang Road, Weihui 453100, Henan Province, China. y_rmin@163.com
Telephone: +86-373-4402942 Fax: +86-373-4402573
Received: December 6, 2012 Revised: January 17, 2013
Accepted: January 23, 2013
Published online: February 21, 2013

Abstract

AIM: To investigate the clinical safety and efficacy of a temporary self-expanding metallic stent (SEMS) for malignant colorectal obstruction.

METHODS: From September 2007 to June 2012, 33 patients with malignant colorectal obstruction were treated with a temporary SEMS. The stent had a tubular configuration with a retrieval lasso attached inside the proximal end of the stent to facilitate its removal. The SEMS was removed one week after placement. Clinical examination, abdominal X-ray and a contrast study were prospectively performed and both initial and follow-up data before and at 1 d, 1 wk, and 1 mo, 3 mo, 6 mo and 12 mo after stent placement were obtained. Data collected on the technical and clinical success of the procedures, complications, need for re-insertion and survival were analyzed.

RESULTS: Stent placement and removal were technically successful in all patients with no procedure-related complications. Post-procedural complications included stent migration (n = 2) and anal pain (n = 2). Clinical success was achieved in 31 (93.9%) of 33 patients with resolution of bowel obstruction within 3 d of stent removal. Eleven of the 33 patients died 73.81 ± 23.66 d (range 42-121 d) after removal of the stent without colonic re-obstruction. Clinical success was achieved in another 8 patients without symptoms of obstruction during the follow-up period. Reinsertion of the stent was performed in the remaining 12 patients with re-obstruction after 84.33 ± 51.80 d of follow-up. The mean and median periods of relief of obstructive symptoms were 97.25 ± 9.56 d and 105 ± 17.43 d, respectively, using Kaplan-Meier analysis.

CONCLUSION: Temporary SEMS is a safe and effective approach in patients with malignant colorectal obstruction due to low complication rates and good medium-term outcomes.

© 2013 Baishideng. All rights reserved.

Key words: Self-expanding metallic stents; Colorectum; Malignant obstruction; Complications

Ding XL, Li YD, Yang RM, Li FB, Zhang MQ. A temporary self-expanding metallic stent for malignant colorectal obstruction. *World J Gastroenterol* 2013; 19(7): 1119-1123 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i7/1119.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i7.1119

INTRODUCTION

Over the last decade, colorectal stenting has been reported to be an effective method of relieving colonic obstruction in palliative treatment[1-5] or as a pre-operative bridge to facilitate one-stage surgical resection of primary colonic tumors[6-10]. Overall technical and clinical

success has been reported in 80%-100% of treated patients[11-15]. However, metallic stent placement has been plagued by tumor ingrowth (3%-46%) following bare stent placement[16-19], perforation (4%)[12,13,15,20], and stent migration (30%-50%)[10,21-24] following covered stent placement.

To overcome these problems, a temporary expandable colorectal stent was devised for use in patients with unresectable malignant colorectal obstruction. Our hypothesis was that the duration of placement of the self-expanding colorectal stent could be reduced to prevent perforation and stent migration. The purpose of this study was to evaluate the clinical safety and efficacy of a temporary self-expandable colorectal stent for the treatment of unresectable malignant colorectal obstruction, with a focus on preventing colonic perforation and stent migration.

MATERIALS AND METHODS

Study design

This pilot study was approved by the Institutional Review Board, and informed consent was obtained from all the patients. From September 2007 to June 2012, consecutive patients with malignant colorectal obstruction were treated with a temporary self-expanding metallic stent (Micro-Tech, Nanjing, China) in our department.

Diagnoses were established by reviewing patient histories, computed tomography (CT) imaging, colon studies and pathologic results. Patients were eligible for this procedure if the following criteria were met: (1) documented malignancy; (2) colorectal obstruction as defined by symptoms resulting in difficulty in defecation; (3) expandable metallic stent placement; and (4) life expectancy of more than 6 mo. Exclusion criteria were: (1) nonsymptomatic patients with malignant colorectal obstruction; (2) clinical evidence of perforation or peritonitis combined with multiple small-bowel obstructions; (3) extension of rectal cancer to the anal sphincter; and (4) right-sided acute obstruction (due to difficult access).

Stent

The colon stent (Micro-Tech) is woven from a single thread of highly elastic nitinol wire 0.16 mm in diameter. The stent had a tubular configuration with an elliptic structure at the proximal and distal ends. The body section was 25-30 mm in diameter when fully expanded and 70-100 mm in length. The elliptic structure at the proximal and distal ends was 5 mm wider in diameter than the body section and 10 mm in length. To facilitate removal of the stent, a retrieval lasso was attached inside the proximal end of the stent to allow removal after placement. For implantation under fluoroscopic guidance, the stent was delivered in a compressed form inside an introducer sheath with a diameter of 16 F.

Stent placement technique

The procedure was performed by one senior interventional radiologist (Yang RM), who had 18 years of experience in interventional radiology, under fluoroscopic guidance. Neither analgesia nor sedation was administered during the procedure.

Patients were initially placed in the left lateral decubitus position. Rotating the patient into the supine position allowed for a better anatomic view under fluoroscopy. The site of obstruction was established both endoscopically by direct vision and with water soluble contrast (Ultravist 300, Schering, Guangzhou, China) administered *via* a catheter passed through the endoscope and into the stricture. The procedure we used has been described elsewhere[1,6,7,10,25]. After the anal sphincter was lubricated, the distal ends of the tumors were identified by endoscopy. Then, a 0.035-inch guide wire (Radiofocus M; Terumo, Tokyo, Japan) with a 5-Fr catheter (Torcon NB; Cook, Bloomington, United States) was advanced and passed through the area of the stricture. The stricture location, length and morphology of the colon were identified by the injection of 30% diluted nonionic contrast medium (Ultravist) *via* the catheter. To avoid perforation, balloon dilation was not generally performed. After exchanging the guide wire for a 0.035-inch super-stiff guide wire (Boston Scientific/Medi-Tech, Watertown, United States), a 16-Fr delivery system (Micro-Tech) was passed over the stiff guide wire until the proximal and distal edges of the prosthesis bridged the stricture under fluoroscopic control, and the stent was then deployed by pulling back the introducer sheath. Finally, contrast medium (Ultravist) was injected through the catheter to assess correct placement and expansion of the stent.

Upon completion of the procedure, patients were transferred to the ward for observation. Once obstructive symptoms had remitted about one week after stent placement, the stent was grasped by the retrieval lasso and gently pulled out. A contrast study was performed to evaluate the patency to rule out possible concomitant lesions in the proximal and distal colon.

Postoperative outcome evaluation

Before SEMS placement, a routine workup, including CT of the abdomen and chest, as well as calculation of Karnofsky performance status, was conducted. An abdominal radiograph was taken during hospitalization 1-3 d after placement to confirm the correct deployment and expansion. After successful removal of the stent, patients were monitored in the outpatient clinic until either death, surgery was performed, patient was lost to follow-up or a complication developed. Clinical examination, abdominal X-ray and a contrast study were performed by two of our authors, who gathered both initial and follow-up data before and at 1 d, 1 wk, and 1 mo, 3 mo, 6 mo and 12 mo after stent placement. In cases where clinical exams could not be performed, data were obtained by means of telephone calls to the patient or the closest relative and by reviewing medical records. CT examination or an endoscopic procedure was performed if there was persistence or reappearance of symptoms such as abdominal pain, constipation, or rectal bleeding. Technical and clinical success of the procedure, occur-

rence and timing of complications, and the need for surgical intervention were analyzed.

Patients were considered to have incurable cancer when curative resection of metastatic disease was impossible due to extensive liver metastases or extrahepatic disease. Technical success was defined as successful placement of the SEMS, with correct deployment, positioning at the level of the stenosis, and removal of the stent determined with radiologic procedures. Clinical success was defined as complete colonic decompression and relief of obstructive symptoms as judged by clinical symptoms and radiographic observations, without intervention or device-related complications within 72 h after SEMS removal. Death was considered to be related to SEMS complications if the patient died within 7 d of insertion or removal. Major complications were events leading to surgery or reintervention or requiring admission to the intensive care unit. Perforation, stent obstruction, and migration were considered to be major complications. Mild complications were events leading to rehospitalization or prolonged hospital stay without fulfilling the major complications criteria.

Statistical analysis

Descriptive data are expressed as the mean ± SD. Categorical data are reported as numbers and percentages. Time to considered end points (occurrence of complications, surgical intervention, or death) was determined. Kaplan-Meier analysis of relief of obstructive symptoms was performed to calculate the cumulative rate of clinical success, such as sustained relief of obstruction and lack of complications. All statistical analyses were performed using the SPSS package, version 13.0 (SPSS, Chicago, Illinois, United States).

RESULTS

Patients

A total of 38 patients were included in this study initially. Five of these were excluded due to patients being lost to follow-up, thus, a total of 33 eligible patients were included (19 men, 14 women; mean age 61.55 ± 14.59 years, range 30-85 years). Stenoses were located in: the transverse colon (n = 3); left colon (n = 7); sigmoid colon (n = 12); rectum (n = 11). The mean distance of the lesion from the anus was 19.23 cm (range 5-71 cm), and the mean lesion length was 5.97 ± 2.07 cm (range 4-12 cm). The obstructions were complete (with no passage of contrast medium during contrast studies before or during stent placement) in 8 patients and incomplete in the remaining 25 patients.

Technical and initial clinical results

Stent placement in the target colon stricture was technically successful in all patients without procedure-related complications. Initially, all patients required the placement of one stent to cover the length of the obstruction. Complete expansion of the placed stent occurred within 2 d of stent placement. No patient underwent balloon dilation, either before or after stent placement. The mean procedure time was 42 min (range 30-120 min). Removal of the stents was successful in all patients.

In patients with technically successful removal of the stent, clinical success was achieved in 31 of the 33 patients within 72 h of removal, with a success rate of 93.9%. Two patients who did not achieve clinical success after removal of the stents due to paralytic ileus or extension of the tumor were retreated with the stent.

Complications

No perforations occurred following placement or removal of the stents. Stent migration occurred in 2 patients (distal partial migration in two patients), and none of these patients required a second stent placement due to improvement of the obstruction at the time of stent removal. Two patients who had a stent placed in the rectum complained of moderate rectal pain within 2 d of stent placement without requiring analgesics.

Follow-up results

Eleven of the 33 patients died 73.81 ± 23.66 d (range 42-121 d) after removal of the stent without colonic re-obstruction due to diffuse metastatic cancer, cachexia, or myocardial infarction. Clinical success was achieved in 8 patients without symptoms of obstruction. Stent reinsertion was performed in the remaining 12 patients with re-obstruction after a mean follow-up period of 84.33 ± 51.80 d (range 27-201 d). The mean and median periods of relief of obstructive symptoms were 97.25 ± 9.56 d (95%CI: 79, 116) and 105 ± 17.43 d (95%CI: 70, 139), respectively, using Kaplan-Meier analysis.

DISCUSSION

The present study was designed to test the hypothesis that a temporary self-expanding metallic colorectal stent could reduce the risk of colonic perforation and stent migration. In this uncontrolled prospective study of 33 patients with unresectable malignant colorectal obstruction, temporary self-expanding metallic colorectal stent placement was technically successful in all patients with a clinical success rate of 93.9%. There was no procedure-related mortality or perforations in this study, and all complications were managed without surgical intervention. These results suggest that a temporary self-expanding metallic colorectal stent can be considered a viable and effective treatment for patients with unresectable malignant colorectal obstruction. To date, this is the first report to describe the treatment of unresectable malignant colorectal obstruction with a temporary self-expanding metallic colorectal stent.

Colonic or rectal stent placement is associated with some complications, including stent migration, perforations, rectal bleeding, fecal impaction, abdominal pain, and tenesmus, of which stent migration and perforation are the most serious complications. In systematic reviews, migration was reported to occur in approximately 10%-12% of patients[15,20], and is usually detected on

follow-up radiographs within 1 wk of insertion. A comparatively small diameter and limited flexibility may have contributed to the more frequent occurrence of stent migration in earlier studies[26]. Although, Repici *et al*[1] and Song *et al*[6] have reported a lower migration rate (2% and 3%, respectively) with the use of the newly designed colorectal stents - WallFlex stent and dual stent (with a large diameter and flared ends) in the treatment of malignant colorectal obstruction, however, these stents may result in another serious complication - perforation[6]. Song *et al*[6] reported the lowest migration rate (0%) in the bridge-to-surgery group, but they also yielded the highest complication rate - colon perforation (22%) in 11 of the 50 patients in the same group after successful placement of the dual stent.

Perforation is found in 3.7%-4.0% of patients with a colorectal SEMS[15,20], which is lower than stent migration, but it is the most serious complication which may threaten the life of patients. Balloon dilatation of a stricture to obtain access can result in excessive manipulation of the wire through the colonic wall and anatomical sites with a comparatively high perforation risk. It is probable that stent design and long-term placement may play an important role in colonic perforation. Large diameter stents with flared ends and the stent eroding through the colonic wall during colonic peristalsis may directly result in perforation.

The ideal colorectal stent should have adequate radial expansile force and smooth edges. Enough radial expansile force is spontaneously and evenly generated, and the final diameter is reached over the course of 2-5 d, so that dilation of the stricture is gentle, as well as effective[27]. Thus, predilation is not generally necessary, and the potential for migration is reduced. A colorectal stent with smooth edges, obviating sharp hooks in the stent design, can maximally reduce the risk of perforation. The stent made by Micro-Tech has a relatively large profile and an elliptic structure to prevent stent migration, and the elliptic structure has a shrunken edge at both sides to minimize the risk of perforation and satisfies the requirements for prevention of perforation.

The significant improvement in perforation rate and stent migration during the follow-up period in the present study was predominantly attributable to the stent design and its temporary use, and demonstrated that the stent can be safely used in malignant colorectal obstruction. Our 93.9% clinical success rate in the relief of colonic obstruction following stent removal was in line with that of other researchers who reported 80%-100% relief of colonic obstruction in patients with malignant colorectal obstruction treated with SEMSs[5,9,14,15,20], and demonstrated the efficacy of the temporary stent for patients with malignant colorectal obstruction.

Our results have important clinical implications. The use of a temporary self-expanding metallic colorectal stent rather than a long-term self-expanding metallic colorectal stent in patients with unresectable malignant colorectal obstruction will substantially decrease the risk of perforation and stent migration. The findings from this study may encourage more studies on temporary self-expanding metallic colorectal stents in the palliative treatment of patients with unresectable malignant colorectal obstruction.

Our study has some limitations. The number of patients treated was relatively small with a short lifespan, and death due to rapid progression of the disease may have masked both the benefits and risks of the procedure. Secondly, we did not include a control group, therefore, future randomized trials are needed to compare the temporary self-expanding metallic colorectal stent with the long-term self-expanding metallic colorectal stent in terms of efficacy, risk of complications, and recurrent obstruction, with particular attention to stent migration, tumoral and nontumoral tissue overgrowth and perforation.

In conclusion, our preliminary study demonstrated that the temporary SEMS was a safe and effective approach for colon decompression in patients with colorectal malignant obstruction, with a low rate of complications and good medium-term outcomes. Reinsertion of the stent can be performed in patients with re-obstruction. Although the initial results are promising, longer follow-up and expanded clinical trials are needed.

COMMENTS

Background

Colorectal stenting has been reported to be an effective method of relieving colonic obstruction, however, metallic stent placement has been plagued by tumor ingrowth, perforation and migration.

Research frontiers

A temporary self-expanding metallic stent (SEMS) has been widely used for the treatment of patients with malignant esophageal stenosis, however, the use of this type of stent in patients with malignant colorectal obstruction has rarely been reported. Authors investigated the clinical safety and efficacy of a temporary self-expandable colorectal stent for malignant colorectal obstruction.

Innovations and breakthroughs

A temporary SEMS was specially devised for the management of patients with malignant colorectal obstruction associated with colonic cancer. All procedures were performed under fluoroscopic control. This is the first study to report the use of a temporary SEMS for malignant colorectal obstruction.

Applications

By determining the efficacy and safety of a temporary SEMS, this may provide a new therapeutic intervention for the treatment of patients with malignant colorectal obstruction.

Terminology

Malignant colorectal obstruction is a common presentation of colorectal cancer, accounting for about 15%-20% of initial presentations in patients with colorectal malignancy.

Peer review

The authors present a prospective study of temporary SEMS for malignant colorectal obstruction, with the aim of investigating the clinical safety and efficacy of the approach. The results reveal that the clinical success was achieved in 31 (93.9%) of 33 patients with resolution of bowel obstruction within 3 d after stent removal, as well as lower complication rates. In addition, the mean and median periods of relief of obstructive symptom were 97.25 ± 9.56 d and 105 ± 17.43 d, respectively. The results are interesting. A temporary SEMS may be a good attempt to seek the new clinical therapy for unresectable colorectal cancer patients and has a potential clinical significance.

REFERENCES

1 **Repici A**, De Caro G, Luigiano C, Fabbri C, Pagano N,

Preatoni P, Danese S, Fuccio L, Consolo P, Malesci A, D'Imperio N, Cennamo V. WallFlex colonic stent placement for management of malignant colonic obstruction: a prospective study at two centers. *Gastrointest Endosc* 2008; **67**: 77-84 [PMID: 18155427 DOI: 10.1016/j.gie.2007.08.019]

2 **Shin SJ**, Kim TI, Kim BC, Lee YC, Song SY, Kim WH. Clinical application of self-expandable metallic stent for treatment of colorectal obstruction caused by extrinsic invasive tumors. *Dis Colon Rectum* 2008; **51**: 578-583 [PMID: 18259816 DOI: 10.1007/s10350-008-9207-6.]

3 **Fregonese D**, Naspetti R, Ferrer S, Gallego J, Costamagna G, Dumas R, Campaioli M, Morante AL, Mambrini P, Meisner S, Repici A, Andreo L, Masci E, Mingo A, Barcenilla J, Petruzziello L. Ultraflex precision colonic stent placement as a bridge to surgery in patients with malignant colon obstruction. *Gastrointest Endosc* 2008; **67**: 68-73 [PMID: 18028916 DOI: 10.1016/j.gie.2007.03.1042]

4 **Meisner S**, Hensler M, Knop FK, West F, Wille-Jørgensen P. Self-expanding metal stents for colonic obstruction: experiences from 104 procedures in a single center. *Dis Colon Rectum* 2004; **47**: 444-450 [PMID: 14994110]

5 **Ronnekleiv-Kelly SM**, Kennedy GD. Management of stage IV rectal cancer: palliative options. *World J Gastroenterol* 2011; **17**: 835-847 [PMID: 21412493 DOI: 10.3748/wjg.v17.i7.835]

6 **Song HY**, Kim JH, Shin JH, Kim HC, Yu CS, Kim JC, Kang SG, Yoon CJ, Lee JY, Koo JH, Lee KH, Kim JK, Kim DH, Shin TB, Jung GS, Han YM. A dual-design expandable colorectal stent for malignant colorectal obstruction: results of a multicenter study. *Endoscopy* 2007; **39**: 448-454 [PMID: 17516352 DOI: 10.1055/s-2007-966270]

7 **Fregonese D**, Naspetti R, Ferrer S, Gallego J, Costamagna G, Dumas R, Campaioli M, Morante AL, Mambrini P, Meisner S, Repici A, Andreo L, Masci E, Mingo A, Barcenilla J, Petruzziello L. Ultraflex precision colonic stent placement as a bridge to surgery in patients with malignant colon obstruction. *Gastrointest Endosc* 2008; **67**: 68-73 [PMID: 18028916 DOI: 10.1016/j.gie.2007.05.022]

8 **Fischer A**, Schrag HJ, Goos M, Obermaier R, Hopt UT, Baier PK. Transanal endoscopic tube decompression of acute colonic obstruction: experience with 51 cases. *Surg Endosc* 2008; **22**: 683-688 [PMID: 17623242 DOI: 10.1007/s00464-007-9461-z]

9 **Li YD**, Cheng YS, Li MH, Fan YB, Chen NW, Wang Y, Zhao JG. Management of acute malignant colorectal obstruction with a novel self-expanding metallic stent as a bridge to surgery. *Eur J Radiol* 2010; **73**: 566-571 [PMID: 19167177 DOI: 10.1016/j.ejrad.2008.12.004]

10 **Camúñez F**, Echenagusia A, Simó G, Turégano F, Vázquez J, Barreiro-Meiro I. Malignant colorectal obstruction treated by means of self-expanding metallic stents: effectiveness before surgery and in palliation. *Radiology* 2000; **216**: 492-497 [PMID: 10924576]

11 **Fiori E**, Lamazza A, Schillaci A, Femia S, Demasi E, Decesare A, Sterpetti AV. Palliative management for patients with subacute obstruction and stage IV unresectable rectosigmoid cancer: colostomy versus endoscopic stenting: final results of a prospective randomized trial. *Am J Surg* 2012; **204**: 321-326 [PMID: 22575396 DOI: 10.1016/j.amjsurg.2011.11.013]

12 **Baron TH**. Colonic stenting: technique, technology, and outcomes for malignant and benign disease. *Gastrointest Endosc Clin N Am* 2005; **15**: 757-771 [PMID: 16278137 DOI: 10.1016/j.giec.2005.08.005]

13 **Repici A**, Pagano N, Hervoso CM, Danese S, Nicita R, Preatoni P, Malesci A. Metal stents for malignant colorectal obstruction. *Minim Invasive Ther Allied Technol* 2006; **15**: 331-338 [PMID: 17190657 DOI: 10.1080/13645700601037954]

14 **Dionigi G**, Villa F, Rovera F, Boni L, Carrafiello G, Annoni M, Castano P, Bianchi V, Mangini M, Recaldini C, Laganà D, Bacuzzi A, Dionigi R. Colonic stenting for malignant disease: review of literature. *Surg Oncol* 2007; **16** Suppl 1: S153-S155 [PMID: 18023172 DOI: 10.1016/j.suronc.2007.10.017]

15 **Khot UP**, Lang AW, Murali K, Parker MC. Systematic review of the efficacy and safety of colorectal stents. *Br J Surg* 2002; **89**: 1096-1102 [PMID: 12190673 DOI: 10.1046/j.1365-2168.2002.02148.x]

16 **Lee KM**, Shin SJ, Hwang JC, Cheong JY, Yoo BM, Lee KJ, Hahm KB, Kim JH, Cho SW. Comparison of uncovered stent with covered stent for treatment of malignant colorectal obstruction. *Gastrointest Endosc* 2007; **66**: 931-936 [PMID: 17767930 DOI: 10.1016/j.gie.2007.02.064]

17 **Athreya S**, Moss J, Urquhart G, Edwards R, Downie A, Poon FW. Colorectal stenting for colonic obstruction: the indications, complications, effectiveness and outcome--5 year review. *Eur J Radiol* 2006; **60**: 91-94 [PMID: 16806783 DOI: 10.1016/j.ejrad.2006.05.017]

18 **Breitenstein S**, Rickenbacher A, Berdajs D, Puhan M, Clavien PA, Demartines N. Systematic evaluation of surgical strategies for acute malignant left-sided colonic obstruction. *Br J Surg* 2007; **94**: 1451-1460 [PMID: 17968980 DOI: 10.1002/bjs.6007]

19 **Baron TH**. Expandable metal stents for the treatment of cancerous obstruction of the gastrointestinal tract. *N Engl J Med* 2001; **344**: 1681-1687 [PMID: 11386268]

20 **Sebastian S**, Johnston S, Geoghegan T, Torreggiani W, Buckley M. Pooled analysis of the efficacy and safety of self-expanding metal stenting in malignant colorectal obstruction. *Am J Gastroenterol* 2004; **99**: 2051-2057 [PMID: 15447772 DOI: 10.1111/j.1572-0241.2004.40017.x]

21 **Small AJ**, Coelho-Prabhu N, Baron TH. Endoscopic placement of self-expandable metal stents for malignant colonic obstruction: long-term outcomes and complication factors. *Gastrointest Endosc* 2010; **71**: 560-572 [PMID: 20189515 DOI: 10.1016/j.gie.2009.10.012]

22 **Choo IW**, Do YS, Suh SW, Chun HK, Choo SW, Park HS, Kang SK, Kim SK. Malignant colorectal obstruction: treatment with a flexible covered stent. *Radiology* 1998; **206**: 415-421 [PMID: 9457194]

23 **Jung GS**, Song HY, Kang SG, Huh JD, Park SJ, Koo JY, Cho YD. Malignant gastroduodenal obstructions: treatment by means of a covered expandable metallic stent-initial experience. *Radiology* 2000; **216**: 758-763 [PMID: 10966707]

24 **Kang SG**, Jung GS, Cho SG, Kim JG, Oh JH, Song HY, Kim ES. The efficacy of metallic stent placement in the treatment of colorectal obstruction. *Korean J Radiol* 2002; **3**: 79-86 [PMID: 12087197 DOI: 10.3348/kjr.2002.3.2.79]

25 **Fan YB**, Cheng YS, Chen NW, Xu HM, Yang Z, Wang Y, Huang YY, Zheng Q. Clinical application of self-expanding metallic stent in the management of acute left-sided colorectal malignant obstruction. *World J Gastroenterol* 2006; **12**: 755-759 [PMID: 16521189]

26 **Soonawalla Z**, Thakur K, Boorman P, Macfarlane P, Sathananthan N, Parker M. Use of self-expanding metallic stents in the management of obstruction of the sigmoid colon. *AJR Am J Roentgenol* 1998; **171**: 633-636 [PMID: 9725287]

27 **Akle CA**. Endoprostheses for colonic strictures. *Br J Surg* 1998; **85**: 310-314 [PMID: 9529481 DOI: 10.1046/j.1365-2168.1998.00713.x]

P- Reviewer Cui G **S- Editor** Wen LL
L- Editor A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i7.1124

World J Gastroenterol 2013 February 21; 19(7): 1124-1134
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

META-ANALYSIS

Roux-en-Y versus Billroth Ⅰ reconstruction after distal gastrectomy for gastric cancer: A meta-analysis

Jun-Jie Xiong, Kiran Altaf, Muhammad A Javed, Quentin M Nunes, Wei Huang, Gang Mai, Chun-Lu Tan, Rajarshi Mukherjee, Robert Sutton, Wei-Ming Hu, Xu-Bao Liu

Jun-Jie Xiong, Gang Mai, Chun-Lu Tan, Wei-Ming Hu, Xu-Bao Liu, Department of Hepato-Biliary-Pancreatic Surgery, West China Hospital, Sichuan University, Chengdu 610041, Sichuan Province, China

Kiran Altaf, Muhammad A Javed, Quentin M Nunes, Wei Huang, Rajarshi Mukherjee, Robert Sutton, NIHR Liverpool Pancreas Biomedical Research Unit, Royal Liverpool University Hospital, University of Liverpool, Liverpool L69 3GA, Merseyside County, United Kingdom

Author contributions: Xiong JJ and Altaf K contributed equally to this work; Liu XB, Hu WM and Sutton R designed the research, corrected and approved the manuscript; Xiong JJ, Altaf K and Tan CL developed the literature search and carried out statistical analyses of the studies; Javed MA, Huang W, Mai G and Mukherjee R performed data extraction; Xiong JJ, Altaf K and Nunes QM wrote the manuscript; All authors read and approved the final manuscript.

Correspondence to: Xu-Bao Liu, MD, PhD, Professor, Department of Hepato-Biliary-Pancreatic Surgery, West China Hospital, Sichuan University, Guoxue Road 37, Chengdu 610041, Sichuan Province, China. liuxb2011@126.com
Telephone: +86-28-85422474 Fax: +86-28-85422872
Received: December 19, 2012 Revised: January 11, 2013
Accepted: January 23, 2013
Published online: February 21, 2013

Abstract

AIM: To conduct a meta-analysis to compare Roux-en-Y (R-Y) gastrojejunostomy with gastroduodenal Billroth Ⅰ (B-Ⅰ) anastomosis after distal gastrectomy (DG) for gastric cancer.

METHODS: A literature search was performed to identify studies comparing R-Y with B-Ⅰ after DG for gastric cancer from January 1990 to November 2012 in Medline, Embase, Science Citation Index Expanded and the Cochrane Central Register of Controlled Trials in The Cochrane Library. Pooled odds ratios (OR) or weighted mean differences (WMD) with 95%CI were calculated using either fixed or random effects model. Operative outcomes such as operation time, intraoperative blood loss and postoperative outcomes such as anastomotic leakage and stricture, bile reflux, remnant gastritis, reflux esophagitis, dumping symptoms, delayed gastric emptying and hospital stay were the main outcomes assessed. Meta-analyses were performed using RevMan 5.0 software (Cochrane library).

RESULTS: Four randomized controlled trials (RCTs) and 9 non-randomized observational clinical studies (OCS) involving 478 and 1402 patients respectively were included. Meta-analysis of RCTs revealed that R-Y reconstruction was associated with a reduced bile reflux (OR 0.04, 95%CI: 0.01, 0.14; P < 0.00 001) and remnant gastritis (OR 0.43, 95%CI: 0.28, 0.66; P = 0.0001), however needing a longer operation time (WMD 40.02, 95%CI: 13.93, 66.11; P = 0.003). Meta-analysis of OCS also revealed R-Y reconstruction had a lower incidence of bile reflux (OR 0.21, 95%CI: 0.08, 0.54; P = 0.001), remnant gastritis (OR 0.18, 95%CI: 0.11, 0.29; P < 0.00 001) and reflux esophagitis (OR 0.48, 95%CI: 0.26, 0.89; P = 0.02). However, this reconstruction method was found to be associated with a longer operation time (WMD 31.30, 95%CI: 12.99, 49.60; P = 0.0008).

CONCLUSION: This systematic review point towards some clinical advantages that are rendered by R-Y compared to B-Ⅰ reconstruction post DG. However there is a need for further adequately powered, well-designed RCTs comparing the same.

© 2013 Baishideng. All rights reserved.

Key words: Gastric cancer; Distal gastrectomy; Roux-en-Y; Billroth Ⅰ; Reconstruction; Meta-analysis

Xiong JJ, Altaf K, Javed MA, Nunes QM, Huang W, Mai G, Tan CL, Mukherjee R, Sutton R, Hu WM, Liu XB. Roux-en-Y versus Billroth Ⅰ reconstruction after distal gastrectomy for gastric cancer: A meta-analysis. *World J Gastroenterol*

2013; 19(7): 1124-1134 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i7/1124.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i7.1124

INTRODUCTION

Gastric cancer is one of the most common cancers worldwide with approximately 989 600 new cases and 738 000 deaths per year, accounting for about 8 percent of new cancers[1]. Surgical resection is still the only option for providing definitive treatment of this malignant disease[2]. Due to the early diagnosis of gastric cancer, there has been a significant improvement in the long-term survival of patients undergoing surgery within the past decade[3]. Surgeons have therefore focused on improving the patients' postoperative quality of life by modifying the surgical technique and the type of reconstruction performed after distal gastrectomy (DG)[4]. The three mainly used reconstruction techniques after DG are: (1) gastroduodenal anastomosis (Billroth-Ⅰ, B-Ⅰ); (2) gastrojejunal anastomosis (Billroth-Ⅱ, B-Ⅱ); and (3) Roux-en-Y (R-Y) gastrojejunostomy. Although both B-Ⅰ and R-Y anastomoses are recognized as standard reconstruction procedures after DG[5], it is yet to be established, which of these is the better of the two. The B-Ⅰ reconstruction has been commonly performed, because of its technical simplicity, with only one anastomotic site and maintaining physiological intestinal continuity[5,6]. However, gastroesophageal and duodenogastric reflux are well documented in patients who undergo this type of reconstruction following DG[7], and severe gastritis, esophagitis and gastric cancer can subsequently occur[8-11]. The aforementioned complications seriously affect postoperative quality of life of patients undergoing DG[12].

For several decades, R-Y reconstruction has been the preferred method to prevent reflux gastritis, esophagitis and decrease probability of gastric cancer recurrence[9,10,13,14]. However, the choice of surgical reconstruction is often based on personal preferences of surgeons, *e.g.*, majority of surgeons in the East favor a B-Ⅰ reconstruction, while R-Y is the procedure of choice in the West[9,15]. Although a few studies have directly compared B-Ⅰ and R-Y techniques, these studies have failed to reach a consensus and establish which method is the best choice after DG for gastric carcinoma. Thus it is difficult to choose a particular type of reconstruction, based on the current evidence base. We therefore sought to compare the perioperative outcomes and postoperative complications of patients undergoing R-Y and B-Ⅰ reconstruction after DG for gastric cancer by undertaking a meta-analysis of published data.

MATERIALS AND METHODS

Literature search

A comprehensive literature search of Medline, Embase, Science Citation Index Expanded and the Cochrane Central Register of Controlled Trials in the Cochrane Library between January 1990 and November 2012 was carried out for comparing R-Y and B-Ⅰ reconstructions after DG for gastric cancer. Medical subject headings as well as keywords "Roux-en-Y"; "Billroth-Ⅰ"; "reconstruction"; "distal gastrectomy"; "gastric cancer" and "stomach cancer" were used. All abstract supplements from published literature were searched manually. Relevant papers were also identified from the reference lists of previous papers, including those obtained through the search of abstracts and recent international meetings. Randomized controlled trials (RCTs) and non-randomized observational clinical studies (OCS) with full-text descriptions were included. Final inclusion of articles was determined by consensus; when this failed, a third author adjudicated. The results of the search strategy are shown in Figure 1.

Inclusion criteria and exclusion criteria

Two authors identified and screened the search findings for potentially eligible studies. Inclusion criteria were: (1) English language articles published in peer-reviewed journals; (2) Human trials of patients with gastric cancer undergoing DG as the main procedure; (3) Studies with at least one of the outcomes mentioned; and (4) When similar studies were reported by the same institution or author, either the better quality study or the more recent publication was included. Following studies were excluded: (1) Abstracts, letters, editorials, expert opinions, reviews and case reports; (2) Studies without available data; (3) Studies without control group; and (4) Studies including patients with benign disease.

Outcomes of interest

Perioperative outcomes and postoperative complications were evaluated. Operation time, intraoperative blood loss and hospital stay were the main perioperative outcomes to be assessed. Postoperative complications included anastomotic leakage and stricture, bile reflux, remnant gastritis, reflux esophagitis, dumping symptoms and delayed gastric emptying.

Data extraction and quality assessment

Data were extracted by two independent observers using standardized forms. RCTs were qualitatively analyzed using Jadad scoring system[16]. Non-randomized OCS were similarly evaluated using Newcastle-Ottawa scoring system[17]. The quality assessment was also carried out by two independent observers and is displayed in Tables 1 and 2. Quantitative data extracted from the selected studies including: population characteristics (study year, country, design, gender, mean age) and outcome parameters (operation time, intraoperative blood loss, hospital stay, anastomotic leakage and stricture, bile reflux, remnant gastritis, reflux esophagitis, dumping symptoms and delayed gastric emptying.

Statistical analysis

Meta-analyses were performed by using Review Man-

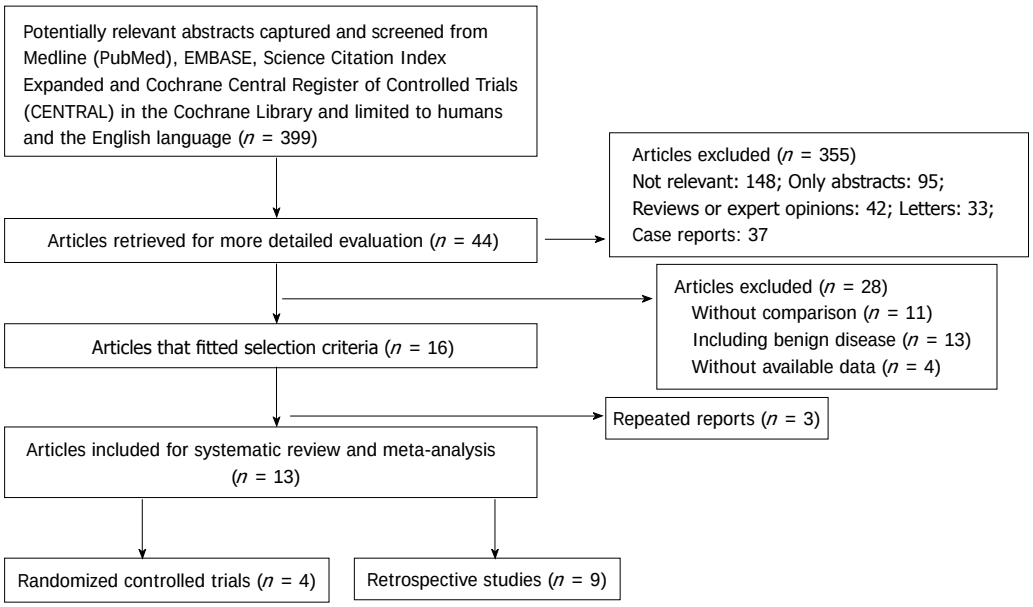


Figure 1 Flow diagram depicting the process of identification and inclusion of selected studies.

Table 1 Quality assessment of the included randomized controlled studies based on the Jadad scoring system

Ref.	Randomized	Appropriate randomization	Appropriately double blinded	Description of withdrawals	Total Jadad score
Ishikawa *et al*[20]	Yes	Yes	No	Yes	3
Lee *et al*[26]	Yes	Yes	No	Yes	3
Imamura *et al*[27] or Hirao *et al*[31]	Yes	Yes	No	Yes	3

Table 2 Newcastle-Ottawa scoring system for nonrandomized comparative studies

Ref.	Selection star	Comparability star[1]	Outcome star	Total star
Osugi *et al*[9]	3	2	3	8
Shinoto *et al*[10]	3	2	3	8
Nunobe *et al*[13]	4	2	3	9
Fukuhara *et al*[14]	3	2	3	8
Kojima *et al*[21]	3	2	3	8
Namikawa *et al*[22]	3	2	2	7
Tanaka *et al*[23]	3	2	3	8
Kumagai *et al*[24]	3	2	1	6
Kim *et al*[25]	3	2	2	7

[1]Factors considered: Age, gender; American Society of Anesthesiologists grading and tumor stage.

ager Version 5.0 software (The Cochrane Collaboration, Oxford, United Kingdom). For categorical variables, treatment effects were expressed as odds ratio (OR) with corresponding 95%CIs. For continuous variables, treatment effects were expressed as weighted mean difference (WMD) with corresponding 95%CI. Meta-analyses were performed using fixed- or random-effects model, depending on the absence or presence of significant heterogeneity. Heterogeneity was evaluated using the χ^2 test, with a $P < 0.1$ was considered significant; I^2 values were used for the evaluation of statistical heterogeneity[18]. If the test rejected the assumption of homogeneity of studies, then the random effects analysis was performed[19]. Sensitivity analyses were also performed by removing individual studies from the data set and analyzing the effect on the overall results to identify sources of significant heterogeneity. Funnel plots were constructed to evaluate potential publication bias.

RESULTS

Study characteristics

There were 399 papers relevant to the search terms. Sixteen studies[9,10,13,14,20-31] met the inclusion criteria. Four studies had previously been reported by the same institution[27-29,31]. Three studies were excluded[27-29], however, one study had some outcomes which we can include[31]. Finally, four RCTs[20,26,27,31], and 9 OCS[9,10,13,14,21-25] with 478 and 1402 patients respectively were included. All these studies have been carried out in Japan and Korea. The number of patients in the included studies ranged from 43 to 424. Characteristics of studies included in the meta-analysis are presented in Table 3.

Meta-analysis results

Included RCTs and OCS were analyzed separately to determine outcome measures in the study groups. All the results are summarized in Table 4.

RCTs comparison: To date, 4 RCTs have been under-

Table 3 Characteristics of included studies in the meta-analysis

Ref.	Country	Design	Group	Patients (*n*)	Male/female (*n*)	Mean age (yr)
Osugi *et al*[9]	Japan	Retro	R-Y	18	13/5	60.2
			B-Ⅰ	25	12/13	64.7
Shinoto *et al*[10]	Japan	Retro	R-Y	20	NR	63 ± 12
			B-Ⅰ	43	NR	63 ± 9
Nunobe *et al*[13]	Japan	Retro	R-Y	182	117/65	58.8
			B-Ⅰ	203	127/76	58.7
Fukuhara *et al*[14]	Japan	Retro	R-Y	29	23/6	56.1
			B-Ⅰ	41	19/22	66.0
Ishikawa *et al*[20]	Japan	RCT	R-Y	24	17/7	64 (43-80)
			B-Ⅰ	26	19/7	61 (34-84)
Kojima *et al*[21]	Japan	Retro	R-Y	68	43/25	62.8 ± 12.2
			B-Ⅰ	65	48/17	62.0 ± 8.9
Namikawa *et al*[22]	Japan	Retro	R-Y	38	22/16	71 (41-80)
			B-Ⅰ	47	25/22	72 (33-86)
Tanaka *et al*[23]	Japan	Retro	R-Y	51	34/17	65.2
			B-Ⅰ	50	34/16	66.2
Kumagai *et al*[24]	Japan	Retro	R-Y	95	74/21	62.7 (42-81)
			B-Ⅰ	329	197/132	63.5 (29-90)
Kim *et al*[25]	South Korea	Retro	R-Y	26	21/5	⩾ 60 (9)
			B-Ⅰ	72	54/18	⩾ 60 (41)
Lee *et al*[26]	South Korea	RCT	R-Y	47	28/19	58.5 ± 10.7
			B-Ⅰ	49	31/18	60.0 ± 11.6
Imamura *et al*[27] or Hirao *et al*[31]	Japan	RCT	R-Y	169	115/54	63.9 ± 10.5
			B-Ⅰ	163	105/58	64.4 ± 9.3

Retro: Retrospective observational study; RCT: Randomized controlled trial; R-Y: Roux-en-Y; B-Ⅰ: Billroth-Ⅰ.

Table 4 Meta-analysis of outcomes of interest

Outcome of interest	Studies	Patients (*n*)	OR/WMD	95%CI	*P* value
RCT					
Operation time	3	478	40.02	13.93, 66.11	0.003
Intraoperative blood loss	3	478	26.99	-9.35, 63.33	0.15
Hospital stay	3	478	2.96	-0.00, 5.93	0.05
Anastomotic leakage	3	478	0.56	0.12, 2.66	0.47
Anastomotic stricture	3	478	1.79	0.52, 6.13	0.36
Bile reflux	2	145	0.04	0.01, 0.14	< 0.00 001
Reflux esophagitis	3	458	0.49	0.20, 1.23	0.13
Remnant gastritis	2	363	0.43	0.28, 0.66	0.000
Delayed gastric emptying	2	363	2.31	0.12	44.41
OCS					
Operation time	4	718	31.3	12.99, 49.60	0.001
Intraoperative blood loss	3	620	26.9	-46.54, 100.34	0.47
Hospital stay	2	522	1.40	-0.17, 2.97	0.08
Anastomotic leakage	5	813	1.26	0.40, 3.97	0.70
Anastomotic stricture	3	658	0.94	0.31, 2.89	0.91
Bile reflux	6	757	0.21	0.08, 0.54	0.001
Reflux esophagitis	5	719	0.48	0.26, 0.89	0.02
Remnant gastritis	6	784	0.18	0.11, 0.29	< 0.00 001
Dumping symptoms	4	347	0.59	0.32, 1.12	0.11
Delayed gastric emptying	4	701	0.95	0.24, 3.74	0.94

RCT: Randomized controlled trials; OCS: Non-randomized observational clinical studies; OR: Odds ratio; WMD: Weighted mean differences.

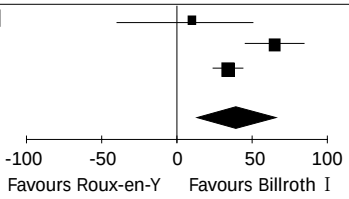
taken to compare R-Y with B-Ⅰ reconstruction[20,26,27,31]. However, two studies[27,31] have same study populations. All studies had a clear description of the sample size calculation and were found to be of high quality according to Jadad scoring system. The detailed results of meta-analysis are given in Figure 2.

Meta-analysis revealed that R-Y reconstruction was associated with a significant reduction in the incidence of bile reflux (OR 0.04, 95%CI: 0.01, 0.14; $P < 0.00001$) and remnant gastritis (OR 0.43, 95%CI: 0.28, 0.66; $P = 0.0001$). No significant differences were observed between the groups in terms of intraoperative blood loss (OR 26.99, 95%CI: -9.35, 63.33; $P = 0.15$), hospital stay (OR 2.96, 95%CI: -0.00, 5.93; $P = 0.05$), anastomotic leakage (OR 0.56, 95%CI: 0.12, 2.66; $P = 0.47$), stricture (OR 1.79, 95%CI: 0.52, 6.13; $P = 0.92$), reflux esopha-

A

Study or Subgroup	Roux-en-Y Mean	SD	Total	Billroth I Mean	SD	Total	Weight	Mean Difference IV, Random, 95%CI
Ishikawa *et al*[20]	260	68	24	250	79	26	21.5%	10.00 [-30.77, 50.77]
Lee *et al*[26]	228.4	52.2	47	163.4	45.1	49	36.1%	65.00 [45.45, 84.55]
Imamura *et al*[27]	214	44	169	180	48	163	42.4%	34.00 [24.09, 43.91]
Total (95%CI)			240			238	100.0%	40.02 [13.93, 66.11]

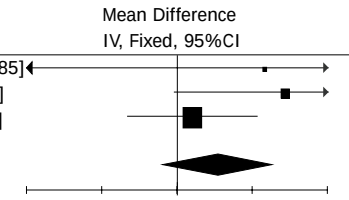
Heterogeneity: τ^2 = 391.92; χ^2 = 9.72, df = 2 (P = 0.008); I^2 = 79%
Test for overall effect: Z = 3.01 (P = 0.003)



B

Study or Subgroup	Roux-en-Y Mean	SD	Total	Billroth I Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95%CI
Ishikawa *et al*[20]	432	250	24	374	392	26	4.0%	58.00 [-122.85, 238.85]
Lee *et al*[26]	214.7	189.7	47	143.1	177.3	49	24.4%	71.60 [-1.92, 145.12]
Imamura *et al*[27]	220	180	169	210	217	163	71.5%	10.00 [-32.97, 52.97]
Total (95%CI)			240			238	100.0%	26.99 [-9.35, 63.33]

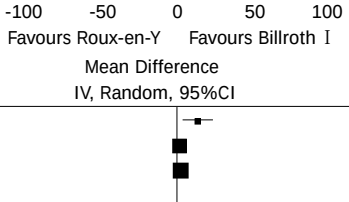
Heterogeneity: χ^2 = 2.13, df = 2 (P = 0.35); I^2 = 6%
Test for overall effect: Z = 1.46 (P = 0.15)



C

Study or Subgroup	Roux-en-Y Mean	SD	Total	Billroth I Mean	SD	Total	Weight	Mean Difference IV, Random, 95%CI
Ishikawa *et al*[20]	31.8	21.7	24	19	6.2	26	9.2%	12.80 [3.80, 21.80]
Lee *et al*[26]	10.8	7.7	47	9.2	3.1	49	42.9%	1.60 [-0.77, 3.97]
Imamura *et al*[27]	16.4	10.4	169	14.1	6.5	163	47.9%	2.30 [0.44, 4.16]
Total (95%CI)			240			238	100.0%	2.96 [-0.00, 5.93]

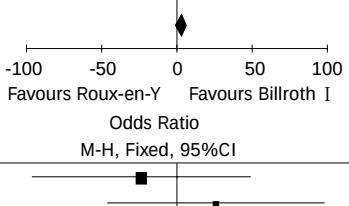
Heterogeneity: τ^2 = 3.87; χ^2 = 5.56, df = 2 (P = 0.06); I^2 = 64%
Test for overall effect: Z = 1.96 (P = 0.05)



D

Study or Subgroup	Roux-en-Y Events	Total	Billroth I Events	Total	Weight	Odds Ratio M-H, Fixed, 95%CI
Ishikawa *et al*[20]	0	24	1	26	31.9%	0.35 [0.01, 8.93]
Lee *et al*[26]	1	47	0	49	10.7%	3.19 [0.13, 80.37]
Imamura *et al*[27]	0	169	2	163	57.3%	0.19 [0.01, 4.00]
Total (95%CI)		240		238	100.0%	0.56 [0.12, 2.66]
Total events	1		3			

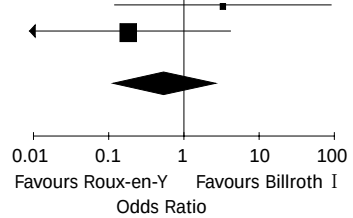
Heterogeneity: χ^2 = 1.68, df = 2 (P = 0.43); I^2 = 0%
Test for overall effect: Z = 0.73 (P = 0.47)



E

Study or Subgroup	Roux-en-Y Events	Total	Billroth I Events	Total	Weight	Odds Ratio M-H, Fixed, 95%CI
Ishikawa *et al*[20]	2	24	0	26	11.1%	5.89 [0.27, 129.15]
Lee *et al*[26]	2	47	0	49	11.9%	5.44 [0.25, 116.35]
Imamura *et al*[27]	2	169	3	163	77.1%	0.64 [0.11, 3.87]
Total (95%CI)		240		238	100.0%	1.79 [0.52, 6.13]
Total events	6		3			

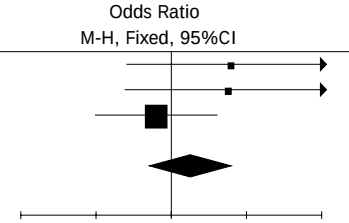
Heterogeneity: χ^2 = 2.33, df = 2 (P = 0.31); I^2 = 14%
Test for overall effect: Z = 0.92 (P = 0.36)



F

Study or Subgroup	Roux-en-Y Events	Total	Billroth I Events	Total	Weight	Odds Ratio M-H, Fixed, 95%CI
Ishikawa *et al*[20]	0	24	8	26	23.4%	0.04 [0.00, 0.82]
Lee *et al*[26]	2	47	28	49	76.6%	0.03 [0.01, 0.15]
Total (95%CI)		71		75	100.0%	0.04 [0.01, 0.14]
Total events	2		36			

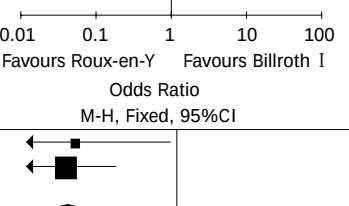
Heterogeneity: χ^2 = 0.03, df = 1 (P = 0.86); I^2 = 0%
Test for overall effect: Z = 4.78 (P < 0.00001)



G

Study or Subgroup	Roux-en-Y Events	Total	Billroth I Events	Total	Weight	Odds Ratio M-H, Fixed, 95%CI
Ishikawa *et al*[20]	7	24	16	26	17.5%	0.26 [0.08, 0.84]
Hirao *et al*[31]	44	157	71	156	82.5%	0.47 [0.29, 0.75]
Total (95%CI)		181		182	100.0%	0.43 [0.28, 0.66]
Total events	51		87			

Heterogeneity: χ^2 = 0.84, df = 1 (P = 0.36); I^2 = 0%
Test for overall effect: Z = 3.80 (P = 0.0001)



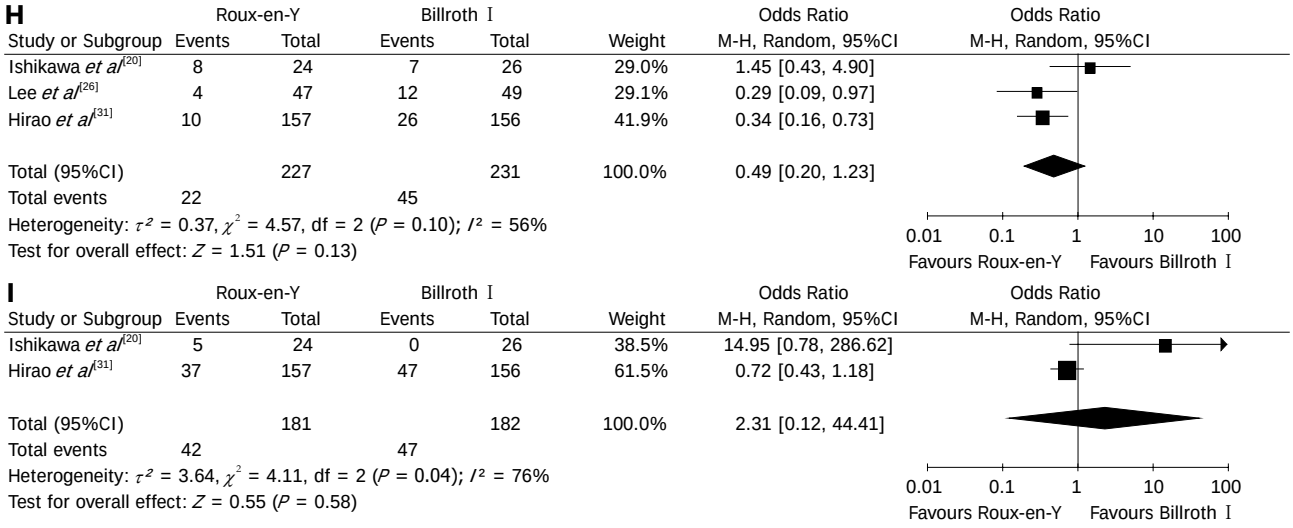


Figure 2 Roux-en-Y versus Billroth I -randomized controlled trials comparison. A: Operation time; B: Intraoperative blood loss; C: Hospital stay; D: Anastomotic leakage; E: Anastomotic stricture; F: Bile reflux; G: Remnant gastritis; H: Reflux esophagitis; I: Delayed gastric emptying. Pooled weighted mean difference (WMD) or odds ratio (OR) with 95%CI was calculated using the fixed-or random effects model.

gitis (OR 0.49, 95%CI: 0.20, 1.23; $P = 0.13$) and delayed gastric emptying (OR 2.31, 95%CI: 0.12, 44.41; $P = 0.58$). However, B- I reconstruction method took significantly less time to perform as compared to R-Y reconstruction (WMD 40.02, 95%CI: 13.93, 66.11; $P = 0.003$).

Only one study[20] reported incidence of dumping symptoms. The incidence of dumping symptoms was not significantly different between the two groups (OR 1.09, 95%CI: 0.14, 8.42; $P = 0.93$).

OCS comparison: Nine OCS were included[9,10,13,14,21-25]. Forest plots are illustrated in Figure 3. Results suggested that R-Y reconstruction had significantly lower incidence of bile reflux (OR 0.21, 95%CI: 0.08, 0.54; $P = 0.001$), remnant gastritis (OR 0.18, 95%CI: 0.11, 0.29; $P < 0.00\,001$) and reflux esophagitis (OR 0.48, 95%CI: 0.26, 0.89; $P = 0.02$). No significant differences were found between the two reconstructive methods in terms of intraoperative blood loss (WMD 26.90, 95%CI: -46.54, 100.34; $P = 0.47$), hospital stay (WMD 1.40, 95%CI: -0.17, 2.97; $P = 0.08$), anastomotic leakage (OR 1.26, 95%CI: 0.40, 3.97; $P = 0.70$), stricture (OR 0.94, 95%CI: 0.31, 2.89; $P = 0.91$), dumping symptoms (OR 0.59, 95%CI: 0.32, 1.12; $P = 0.11$) and delayed gastric emptying (OR 0.95, 95%CI: 0.24, 3.74; $P = 0.94$). Results also suggest that B- I reconstruction require shorter operation time (WMD 31.30, 95%CI: 12.99, 49.60; $P = 0.0008$) as compared to R-Y procedure.

Publication bias

Funnel plot analysis of the studies in the meta-analysis reporting was performed on operation time after DG in RCTs and remnant gastritis in OCS respectively. None of the studies lay outside the limits of the 95%CIs, and there was no evidence of publication bias among the studies (Figure 4).

DISCUSSION

Surgical intervention plays a vital role in the survival of patients with resectable gastric cancer. However, there seems to be lack of consensus within surgeons with regards to the choice of reconstructive procedure after DG. The ideal gastrointestinal reconstruction procedure should minimize postoperative morbidity and improve quality of life[32]. In current surgical practice, B- I and R-Y procedures are the commonly used reconstruction techniques following resection of distal stomach. To the best of our knowledge, B- I reconstruction has commonly been employed after DG for gastric cancer due to its simplicity, physiological advantage of allowing food to pass through the duodenum and ease of postoperative endoscopy allowing access to the papilla of Vater[33,34]. However, two most common drawbacks of the B- I anastomosis, remnant gastritis and reflux esophagitis, as a consequence of the absence of the pyloric sphincter which allows reflux of duodenal contents into the remnant stomach and esophagus have been well reported[35]. Furthermore, unregulated release of chyme into the duodenum results in rapid gastric emptying which manifests as dumping syndrome[36]. It is important to note that reflux of duodenal contents into the esophagus is strongly associated with Barrett's esophagus or esophageal cancer and remnant stomach cancer after gastrectomy[37-39].

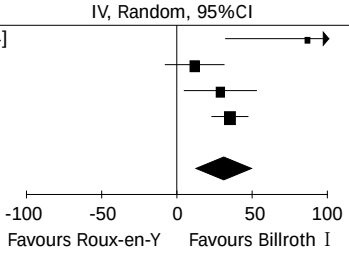
Traditionally R-Y reconstruction has been the reconstruction method of choice in total gastrectomy[34] and is being increasingly used to prevent duodenogastric and gastroesophageal reflux in DG[10,14,21]. The potential advantages of improved postoperative quality of life take precedence over the possible increased risk of postoperative complications due to two gastrointestinal anastomoses and increased operating time, when considering R-Y reconstruction.

Based on our analysis, which only includes high qual-

A

Study or Subgroup	Roux-en-Y Mean	SD	Total	Billroth I Mean	SD	Total	Weight	Mean Difference IV, Random, 95%CI
Shinoto *et al*[10]	333	94	20	247	116	43	9.1%	86.00 [32.16, 139.84]
Kojima *et al*[21]	275.1	54.7	68	263.3	58.7	65	29.2%	11.80 [-7.50, 31.10]
Kim *et al*[25]	202.7	50.5	26	174	59.2	72	25.0%	28.70 [4.96, 52.44]
Kumagai *et al*[24]	260	51	95	225	55	329	36.6%	35.00 [23.15, 46.85]
Total (95%CI)			209			509	100.0%	31.30 [12.99, 49.60]

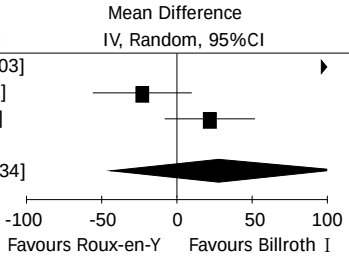
Heterogeneity: τ^2 = 201.62; χ^2 = 8.26, df = 3 (P = 0.04); I^2 = 64%
Test for overall effect: Z = 3.35 (P = 0.0008)



B

Study or Subgroup	Roux-en-Y Mean	SD	Total	Billroth I Mean	SD	Total	Weight	Mean Difference IV, Random, 95%CI
Shinoto *et al*[10]	858	571	20	447	302	43	6.6%	411.00 [144.97, 677.03]
Kojima *et al*[21]	80.5	95.2	68	103.6	97.1	65	46.3%	-23.10 [-55.80, 9.60]
Kumagai *et al*[24]	84	127	95	62	142	329	47.1%	22.00 [-7.79, 51.79]
Total (95%CI)			183			437	100.0%	26.90 [-46.54, 100.34]

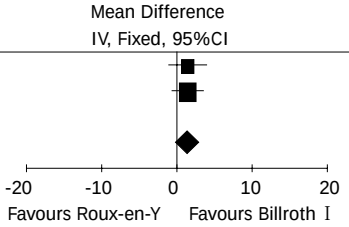
Heterogeneity: τ^2 = 2752.82; χ^2 = 13.03, df = 2 (P = 0.001); I^2 = 85%
Test for overall effect: Z = 0.72 (P = 0.47)



C

Study or Subgroup	Roux-en-Y Mean	SD	Total	Billroth I Mean	SD	Total	Weight	Mean Difference IV, Fixed, 95%CI
Kim *et al*[25]	10.8	4.7	26	9.4	7.2	72	40.6%	1.40 [-1.06, 3.86]
Kumagai *et al*[24]	14	9.5	95	12.6	6.4	329	59.4%	1.40 [-0.63, 3.43]
Total (95%CI)			121			401	100.0%	1.40 [-0.17, 2.97]

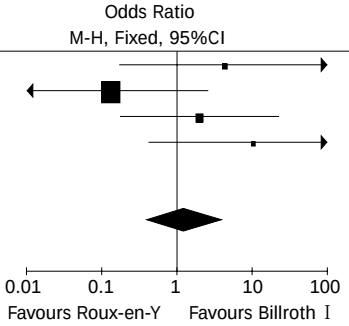
Heterogeneity: χ^2 = 0.00, df = 1 (P = 1.00); I^2 = 0%
Test for overall effect: Z = 1.75 (P = 0.08)



D

Study or Subgroup	Roux-en-Y Events	Total	Billroth I Events	Total	Weight	Odds Ratio M-H, Fixed, 95%CI
Fukuhara *et al*[14]	1	29	0	41	7.7%	4.37 [0.17, 111.09]
Kojima *et al*[21]	0	68	3	65	69.1%	0.13 [0.01, 2.57]
Tanaka *et al*[23]	2	51	1	50	18.9%	2.00 [0.18, 22.78]
Kumagai *et al*[24]	1	95	0	329	4.3%	10.46 [0.42, 258.88]
Namikawa *et al*[22]	0	38	0	47		Not estimable
Total (95%CI)		281		532	100.0%	1.26 [0.40, 3.97]
Total events	4		4			

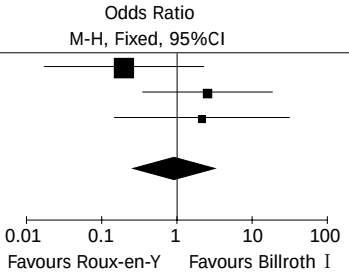
Heterogeneity: χ^2 = 4.60, df = 3 (P = 0.20); I^2 = 35%
Test for overall effect: Z = 0.39 (P = 0.70)



E

Study or Subgroup	Roux-en-Y Events	Total	Billroth I Events	Total	Weight	Odds Ratio M-H, Fixed, 95%CI
Kojima *et al*[21]	1	68	4	65	63.8%	0.23 [0.02, 2.09]
Kumagai *et al*[24]	2	95	3	329	20.8%	2.34 [0.38, 14.19]
Tanaka *et al*[23]	2	51	1	50	15.4%	2.00 [0.18, 22.78]
Total (95%CI)		214		444	100.0%	0.94 [0.31, 2.89]
Total events	5		8			

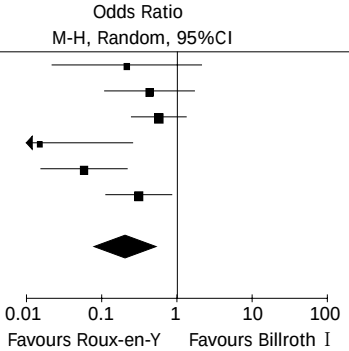
Heterogeneity: χ^2 = 2.92, df = 2 (P = 0.23); I^2 = 31%
Test for overall effect: Z = 0.11 (P = 0.91)



F

Study or Subgroup	Roux-en-Y Events	Total	Billroth I Events	Total	Weight	Odds Ratio M-H, Random, 95%CI
Shinoto *et al*[10]	1	8	8	20	10.7%	0.21 [0.02, 2.09]
Osugi *et al*[9]	4	18	10	25	17.9%	0.43 [0.11, 1.69]
Nunobe *et al*[13]	9	182	17	203	23.5%	0.57 [0.25, 1.31]
Kojima *et al*[21]	0	68	21	65	8.0%	0.02 [0.00, 0.26]
Namikawa *et al*[22]	3	38	28	47	18.4%	0.06 [0.02, 0.22]
Kim *et al*[25]	8	23	38	60	21.6%	0.31 [0.11, 0.84]
Total (95%CI)		337		420	100.0%	0.21 [0.08, 0.54]
Total events	25		122			

Heterogeneity: τ^2 =0.80, χ^2 = 13.84, df = 5 (P = 0.02); I^2 = 64%
Test for overall effect: Z = 3.26 (P = 0.001)



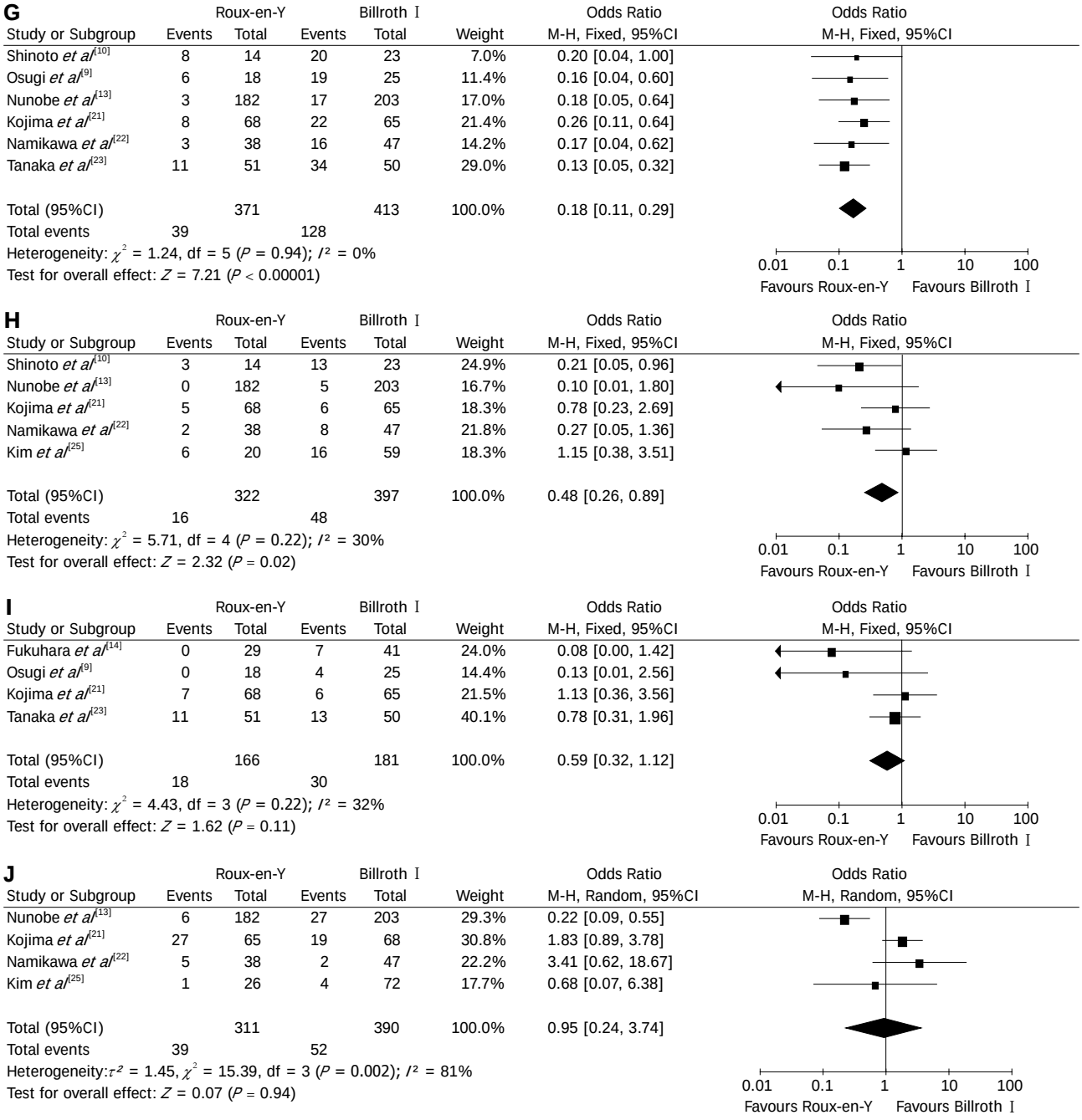


Figure 3 Roux-en-Y versus Billroth I -observational non-randomized clinical studie comparison. A: Operation time; B: Intraoperative blood loss; C: Hospital stay; D: Anastomotic leakage; E: Anastomotic stricture; F: Bile reflux; G: Remnant gastritis; H: Reflux esophagitis; I: Dumping symptoms; J: Delayed gastric emptying. Pooled weighted mean difference (WMD) or odds ratio (OR) with 95%CI was calculated using the fixed-or random effects model.

ity RCTs and OCS, we have addressed this issue, to the best of our effort, of the most appropriate gastrointestinal reconstruction following DG. It is important to note that the results come from a small number of RCTs and therefore, have to be interpreted with caution. Overall analyses for RCTs comparing R-Y with B- I reconstruction favor R-Y method in terms of preventing postoperative bile reflux and remnant gastritis, although we found no significant difference in reflux esophagitis between the two groups. The angle of His is significantly larger in patients who undergo B- I reconstruction compared to those with R-Y and this may be a factor contributing to reduced incidence of reflux symptoms in the latter group[22,28]. We may not have detected an existing difference due to the smaller sample size of the included studies. As only one RCT[20] evaluated incidences of dumping syndrome, descriptive analysis was used, and no difference was found between the two groups with regards to dumping syndrome.

The operating time was significantly shorter in B- I group compared to R-Y group, which can be explained by the additional anastomosis in R-Y reconstruction. Although it has been previously reported that anastomotic leak is higher in B- I reconstruction, possibly due to ex-

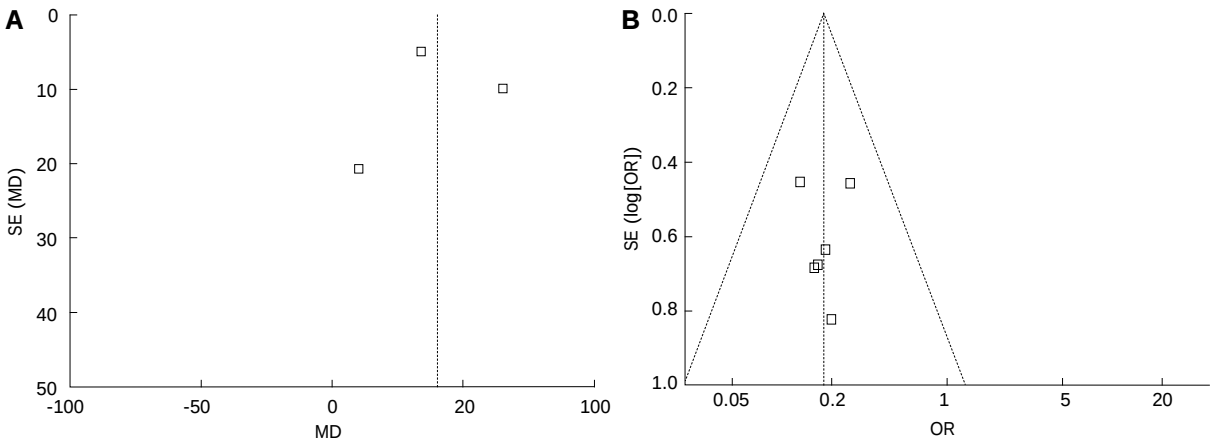


Figure 4 Funnel plot. A: Operation time-randomized controlled trial; B: Remnant gastritis-observational clinical studies. None of the studies lay outside the limits of the 95%CIs, and there was no evidence of publication bias.

cessive devascularization of duodenal stump and tension on the anastomosis[20,21,27], we found no difference in the rate of anastomotic leak within the two groups. It may be largely due to the use of gastrointestinal stapling devices and the refinement of technique.

Similarly, analysis of pooled data from the OCS, revealed a reduced incidence of bile reflux, remnant gastritis, reflux esophagitis and prolonged operative time in the R-Y reconstruction group. Consequently, based on the above findings, we can conclude that R-Y reconstruction following DG is likely to be superior to B- Ⅰ reconstruction not only in preventing bile reflux and remnant gastritis, but also reflux esophagitis (OCS analysis only), as it reduces duodenogastric and gastroesophageal reflux[13,28].

We also analyzed data regarding intraoperative bleeding and duration of hospitalization and found no significant difference in either of these parameters within the two groups, although one would expect an earlier recovery of gastrointestinal function in R-Y reconstruction group.

This review does have some limitations and hence the results should be interpreted with a degree of caution. Firstly, most data are extracted from OCS, with fewer RCT's making it difficult to make firm conclusions. In addition, several important outcomes including remnant gastritis, dumping symptoms and delayed gastric emptying have not been reported adequately in the RCTs. It is important to mention that we were unable to analyze important outcomes including quality of life and incidence of gastric carcinoma in the gastric remnant due to lack of available data. We would therefore propose well-designed RCTs with adequate follow-up and emphasis on assessing important outcomes to clarify ambiguities surrounding the use of these reconstruction methods.

In summary, our systematic review demonstrates that RY reconstruction is likely to be more effective in preventing gastroesophageal reflux or duodenogastric reflux as compared to B- Ⅰ. Furthermore, we have shown that based on results from RCTs and OCS, RY reconstruction may be used safely without increasing anastomotic leakage, anastomotic stricture and intraoperative bleeding.

In conclusion, this systematic review points towards some clinical advantages that are rendered by R-Y compared to B- Ⅰ reconstruction post DG. However there is a need for further adequately powered, well-designed RCTs comparing the same.

COMMENTS

Background

Currently, Billroth Ⅰ (B- Ⅰ) and Roux-en-Y (R-Y) reconstructions are commonly performed after distal gastrectomy for gastric cancer. However, deciding which of these reconstruction procedures is superior, remains controversial. Therefore, in order to help arrive at a possible consensus, the authors conducted a systematic review and meta-analysis to compare the clinical efficacy and safety of B- Ⅰ versus R-Y reconstruction following distal gastrectomy (DG) for gastric cancer.

Research frontiers

In order to compare the safety and effectiveness of the B- Ⅰ and R-Y reconstructions, operative outcomes including operation time, intraoperative blood loss and postoperative outcomes such as anastomotic leakage and stricture, bile reflux, remnant gastritis, reflux esophagitis, dumping symptoms, delayed gastric emptying and hospital stay were included in this study.

Innovations and breakthroughs

Although existing randomized controlled trials (RCTs) and retrospective comparative studies have concluded that R-Y reconstruction could prevent gastroesophageal and duodenogastric reflux following DG, there was a need to further assess the clinical advantages of R-Y and B- Ⅰ reconstruction based on high-level evidence. This meta-analysis reports that the R-Y reconstruction has some clinical advantages in reducing the incidence of bile reflux and remnant gastritis compared to the B- Ⅰ technique. Also, R-Y reconstruction does not significantly increase postoperative complications.

Applications

This study shows that R-Y reconstruction following DG for gastric cancer has some clinical advantages compared with B- Ⅰ reconstruction. However, taking into account the limited number of studies, further adequately powered and well-designed RCTs should be undertaken to investigate the same.

Terminology

Following distal gastrectomy, three methods, namely, (1) gastroduodenal anastomosis or B- Ⅰ; (2) gastrojejunal anastomosis or Billroth-Ⅱ; and (3) R-Y gastrojejunostomy are mainly used for gastrointestinal tract reconstruction.

Peer review

This is an interesting study, which can point out to some extent, the direction in gastrointestinal tract reconstruction for the future gastrointestinal surgeon.

REFERENCES

1 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]
2 **Kim JP**. Current status of surgical treatment of gastric cancer. *J Surg Oncol* 2002; **79**: 79-80 [PMID: 11815992 DOI: 10.1002/jso.10050]
3 **Roukos DH**. Current advances and changes in treatment strategy may improve survival and quality of life in patients with potentially curable gastric cancer. *Ann Surg Oncol* 1999; **6**: 46-56 [PMID: 10030415 DOI: 10.1007/s10434-999-0046-z]
4 **Nakajima T**. Gastric cancer treatment guidelines in Japan. *Gastric Cancer* 2002; **5**: 1-5 [PMID: 12021853 DOI: 10.1007/s101200200000]
5 **Yoshino K**. [History of gastric cancer surgery]. *Nihon Geka Gakkai Zasshi* 2000; **101**: 855-860 [PMID: 11201113]
6 **Weil PH**, Buchberger R. From Billroth to PCV: a century of gastric surgery. *World J Surg* 1999; **23**: 736-742 [PMID: 10390597 DOI: 10.1007/PL00012379]
7 **Pääkkönen M**, Aukee S, Syrjänen K, Mäntyjärvi R. Gastritis, duodenogastric reflux and bacteriology of the gastric remnant in patients operated for peptic ulcer by Billroth I operation. *Ann Clin Res* 1985; **17**: 32-36 [PMID: 4015029]
8 **Richter JE**. Duodenogastric Reflux-induced (Alkaline) Esophagitis. *Curr Treat Options Gastroenterol* 2004; **7**: 53-58 [PMID: 14723838 DOI: 10.1007/s11938-004-0025-1]
9 **Osugi H**, Fukuhara K, Takada N, Takemura M, Kinoshita H. Reconstructive procedure after distal gastrectomy to prevent remnant gastritis. *Hepatogastroenterology* 2004; **51**: 1215-1218 [PMID: 15239282]
10 **Shinoto K**, Ochiai T, Suzuki T, Okazumi S, Ozaki M. Effectiveness of Roux-en-Y reconstruction after distal gastrectomy based on an assessment of biliary kinetics. *Surg Today* 2003; **33**: 169-177 [PMID: 12658381 DOI: 10.1007/s005950300039]
11 **Miwa K**, Hasegawa H, Fujimura T, Matsumoto H, Miyata R, Kosaka T, Miyazaki I, Hattori T. Duodenal reflux through the pylorus induces gastric adenocarcinoma in the rat. *Carcinogenesis* 1992; **13**: 2313-2316 [PMID: 1473239 DOI: 10.1093/carcin/13.12.2313]
12 **Svensson JO**. Duodenogastric reflux after gastric surgery. *Scand J Gastroenterol* 1983; **18**: 729-734 [PMID: 6669936 DOI: 10.3109/00365528309182087]
13 **Nunobe S**, Okaro A, Sasako M, Saka M, Fukagawa T, Katai H, Sano T. Billroth 1 versus Roux-en-Y reconstructions: a quality-of-life survey at 5 years. *Int J Clin Oncol* 2007; **12**: 433-439 [PMID: 18071862 DOI: 10.1007/s10147-007-0706-6]
14 **Fukuhara K**, Osugi H, Takada N, Takemura M, Higashino M, Kinoshita H. Reconstructive procedure after distal gastrectomy for gastric cancer that best prevents duodenogastroesophageal reflux. *World J Surg* 2002; **26**: 1452-1457 [PMID: 12370787 DOI: 10.1007/s00268-002-6363-z]
15 **Adachi Y**, Shiraishi N, Shiromizu A, Bandoh T, Aramaki M, Kitano S. Laparoscopy-assisted Billroth I gastrectomy compared with conventional open gastrectomy. *Arch Surg* 2000; **135**: 806-810 [PMID: 10896374 DOI: 10.1001/archsurg.135.7.806]
16 **Jadad AR**, Moore RA, Carroll D, Jenkinson C, Reynolds DJ, Gavaghan DJ, McQuay HJ. Assessing the quality of reports of randomized clinical trials: is blinding necessary? *Control Clin Trials* 1996; **17**: 1-12 [PMID: 8721797 DOI: 10.1016/0197-2456(95)00134-4]
17 **Stang A**. Critical evaluation of the Newcastle-Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. *Eur J Epidemiol* 2010; **25**: 603-605 [PMID: 20652370 DOI: 10.1007/s10654-010-9491-z]
18 **Higgins JP**, Thompson SG, Deeks JJ, Altman DG. Measuring inconsistency in meta-analyses. *BMJ* 2003; **327**: 557-560 [PMID: 12958120 DOI: 10.1136/bmj.327.7414.557]
19 **DerSimonian R**, Laird N. Meta-analysis in clinical trials. *Control Clin Trials* 1986; **7**: 177-188 [PMID: 3802833 DOI: 10.1016/0197-2456(86)90046-2]
20 **Ishikawa M**, Kitayama J, Kaizaki S, Nakayama H, Ishigami H, Fujii S, Suzuki H, Inoue T, Sako A, Asakage M, Yamashita H, Hatono K, Nagawa H. Prospective randomized trial comparing Billroth I and Roux-en-Y procedures after distal gastrectomy for gastric carcinoma. *World J Surg* 2005; **29**: 1415-1420; discussion 1421 [PMID: 16240061 DOI: 10.1007/s00268-005-7830-0]
21 **Kojima K**, Yamada H, Inokuchi M, Kawano T, Sugihara K. A comparison of Roux-en-Y and Billroth-I reconstruction after laparoscopy-assisted distal gastrectomy. *Ann Surg* 2008; **247**: 962-967 [PMID: 18520223 DOI: 10.1097/SLA.0b013e31816d9526]
22 **Namikawa T**, Kitagawa H, Okabayashi T, Sugimoto T, Kobayashi M, Hanazaki K. Double tract reconstruction after distal gastrectomy for gastric cancer is effective in reducing reflux esophagitis and remnant gastritis with duodenal passage preservation. *Langenbecks Arch Surg* 2011; **396**: 769-776 [PMID: 21448727 DOI: 10.1007/s00423-011-0777-8]
23 **Tanaka S**, Matsuo K, Matsumoto H, Maki T, Nakano M, Sasaki T, Yamashita Y. Clinical outcomes of Roux-en-Y and Billroth I reconstruction after a distal gastrectomy for gastric cancer: What is the optimal reconstructive procedure? *Hepatogastroenterology* 2011; **58**: 257-262 [PMID: 21510325]
24 **Kumagai K**, Hiki N, Nunobe S, Jiang X, Kubota T, Aikou S, Watanabe R, Tanimura S, Sano T, Kitagawa Y, Yamaguchi T. Different features of complications with Billroth-I and Roux-en-Y reconstruction after laparoscopy-assisted distal gastrectomy. *J Gastrointest Surg* 2011; **15**: 2145-2152 [PMID: 21948148 DOI: 10.1007/s11605-011-1683-7]
25 **Kim TG**, Hur H, Ahn CW, Xuan Y, Cho YK, Han SU. Efficacy of Roux-en-Y Reconstruction Using Two Circular Staplers after Subtotal Gastrectomy: Results from a Pilot Study Comparing with Billroth-I Reconstruction. *J Gastric Cancer* 2011; **11**: 219-224 [PMID: 22324013 DOI: 10.5230/jgc.2011.11.4.219]
26 **Lee MS**, Ahn SH, Lee JH, Park do J, Lee HJ, Kim HH, Yang HK, Kim N, Lee WW. What is the best reconstruction method after distal gastrectomy for gastric cancer? *Surg Endosc* 2012; **26**: 1539-1547 [PMID: 22179454 DOI: 10.1007/s00464-011-2064-8]
27 **Imamura H**, Takiguchi S, Yamamoto K, Hirao M, Fujita J, Miyashiro I, Kurokawa Y, Fujiwara Y, Mori M, Doki Y. Morbidity and mortality results from a prospective randomized controlled trial comparing Billroth I and Roux-en-Y reconstructive procedures after distal gastrectomy for gastric cancer. *World J Surg* 2012; **36**: 632-637 [PMID: 22270979 DOI: 10.1007/s00268-011-1408-9]
28 **Namikawa T**, Kitagawa H, Okabayashi T, Sugimoto T, Kobayashi M, Hanazaki K. Roux-en-Y reconstruction is superior to billroth I reconstruction in reducing reflux esophagitis after distal gastrectomy: special relationship with the angle of his. *World J Surg* 2010; **34**: 1022-1027 [PMID: 20143067 DOI: 10.1007/s00268-010-0452-1]
29 **Inokuchi M**, Kojima K, Yamada H, Kato K, Hayashi M, Motoyama K, Sugihara K. Long-term outcomes of Roux-en-Y and Billroth-I reconstruction after laparoscopic distal gastrectomy. *Gastric Cancer* 2013; **16**: 67-73 [PMID: 22467062 DOI: 10.1007/s10120-012-0154-5]
30 **Takiguchi S**, Yamamoto K, Hirao M, Imamura H, Fujita J, Yano M, Kobayashi K, Kimura Y, Kurokawa Y, Mori M, Doki Y. A comparison of postoperative quality of life and dysfunction after Billroth I and Roux-en-Y reconstruction following distal gastrectomy for gastric cancer: results from a multi-institutional RCT. *Gastric Cancer* 2012; **15**: 198-205 [PMID: 21993852 DOI: 10.1007/s10120-011-0098-1]
31 **Hirao M**, Takiguchi S, Imamura H, Yamamoto K, Kurokawa Y, Fujita J, Kobayashi K, Kimura Y, Mori M, Doki Y; Osaka

University Clinical Research Group for Gastroenterological Study. Comparison of Billroth I and Roux-en-Y Reconstruction after Distal Gastrectomy for Gastric Cancer: One-year Postoperative Effects Assessed by a Multi-institutional RCT. *Ann Surg Oncol* 2012 Oct 28 [Epub ahead of print] [PMID: 23104705 DOI: 10.1245/s10434-012-2704-9]

32 **Piessen G**, Triboulet JP, Mariette C. Reconstruction after gastrectomy: which technique is best? *J Visc Surg* 2010; **147**: e273-e283 [PMID: 20934934 DOI: 10.1016/j.jviscsurg.2010.09.004]

33 **Kim BJ**, O'Connell T. Gastroduodenostomy after gastric resection for cancer. *Am Surg* 1999; **65**: 905-907 [PMID: 10515531]

34 **Kumagai K**, Shimizu K, Yokoyama N, Aida S, Arima S, Aikou T. Questionnaire survey regarding the current status and controversial issues concerning reconstruction after gastrectomy in Japan. *Surg Today* 2012; **42**: 411-418 [PMID: 22391980 DOI: 10.1007/s00595-012-0159-z]

35 **Kubo M**, Sasako M, Gotoda T, Ono H, Fujishiro M, Saito D, Sano T, Katai H. Endoscopic evaluation of the remnant stomach after gastrectomy: proposal for a new classification. *Gastric Cancer* 2002; **5**: 83-89 [PMID: 12111583 DOI: 10.1007/s101200200014]

36 **Bühner S**, Ehrlein HJ, Thoma G, Schumpelick V. Canine motility and gastric emptying after subtotal gastrectomy. *Am J Surg* 1988; **156**: 194-200 [PMID: 3421427 DOI: 10.1016/S0002-9610(88)80065-5]

37 **Sato T**, Miwa K, Sahara H, Segawa M, Hattori T. The sequential model of Barrett's esophagus and adenocarcinoma induced by duodeno-esophageal reflux without exogenous carcinogens. *Anticancer Res* 2002; **22**: 39-44 [PMID: 12017320]

38 **Wetscher GJ**, Hinder RA, Smyrk T, Perdikis G, Adrian TE, Profanter C. Gastric acid blockade with omeprazole promotes gastric carcinogenesis induced by duodenogastric reflux. *Dig Dis Sci* 1999; **44**: 1132-1135 [PMID: 10389684 DOI: 10.1023/A:1026615905170]

39 **Goldstein SR**, Yang GY, Curtis SK, Reuhl KR, Liu BC, Mirvish SS, Newmark HL, Yang CS. Development of esophageal metaplasia and adenocarcinoma in a rat surgical model without the use of a carcinogen. *Carcinogenesis* 1997; **18**: 2265-2270 [PMID: 9395230 DOI: 10.1093/carcin/18.11.2265]

P- Reviewer Shi HY **S- Editor** Gou SX
L- Editor A **E- Editor** Lu YJ



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i7.1135

World J Gastroenterol 2013 February 21; 19(7): 1135-1139
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Gastrointestinal sarcoidosis associated with pneumatosis cystoides intestinalis

Hussein Rahim, Mubashir Khan, Jay Hudgins, Kevin Lee, Lei Du, Louis Amorosa

Hussein Rahim, Mubashir Khan, Kevin Lee, Lei Du, Louis Amorosa, Department of Medicine, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, New Brunswick, NJ 08820, United States
Jay Hudgins, Department of Pathology, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, New Brunswick, NJ 08820, United States
Author contributions: Rahim H wrote paper, performed literature review. Amorosa L supervised writing paper, literature review, and patient care. Hudgins J provided pathology images and discussion. Khan M assisted in reviewing paper and literature; all authors participated in care of the patient.
Correspondence to: Louis Amorosa, MD, Department of Medicine, University of Medicine and Dentistry of New Jersey-Robert Wood Johnson Medical School, 125 Paterson St No. 5100, New Brunswick, NJ 08820,
United States. amoroslo@umdnj.edu
Telephone: +1-732-2357219 Fax: +1-732-2358610
Received: September 15, 2012 Revised: December 6, 2012
Accepted: December 15, 2012
Published online: February 21, 2013

Abstract

A 39-year-old male reported fevers, weight loss, watery loose stools, and decreased visual acuity in his right eye over the prior five years. He was pancytopenic, had an elevated American council on exercise level, total bilirubin, and alkaline phosphatase. Computed tomography revealed massive hepatosplenomegaly and emphysematous lung changes. Liver biopsy showed non caseating granulomas. The patient was diagnosed with extrapulmonary sarcoidosis and was treated with prednisone. The patient symptomatically improved but 5 mo later presented with abdominal pain caused by perforation of the cecum. He underwent a cecectomy and pathology revealed pneumatosis cystoides intestinalis. This represents the first reported association between pneumatosis cystoides intestinalis and sarcoidosis. The etiology of pneumatosis cystoides intestinalis in this case was likely multifactorial and involved both effects of the corticosteroids as well as the advanced nature of the gastrointestinal sarcoidosis. Furthermore this case has the unique features of emphysematous lung changes and pancytopenia which are uncommon with sarcoidosis.

© 2013 Baishideng. All rights reserved.

Key words: Sarcoidosis; Pneumatosis cystoides intestinalis; Pancytopenia; Emphysema; Corticosteroids

Rahim H, Khan M, Hudgins J, Lee K, Du L, Amorosa L. Gastrointestinal sarcoidosis associated with pneumatosis cystoides intestinalis. *World J Gastroenterol* 2013; 19(7): 1135-1139 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i7/1135.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i7.1135

INTRODUCTION

Sarcoidosis is an idiopathic multisystem non-caseating granulomatous disease that has been reported to impact almost any organ. It is more common in African Americans and typically patients present with pulmonary manifestations associated with bilateral hilar adenopathy on chest X-ray. Very rarely does it present with predominantly gastrointestinal (GI) symptoms[1]. Pneumatosis intestinalis cystoides is a rare disorder defined as gaseous cysts in the bowel wall. While the etiology remains unclear, it has been associated with multiple conditions including, connective tissue disease, various drugs, colonoscopies, ileal surgeries, and chronic pulmonary disease[2,3]. Here we present a case of an individual with GI sarcoidosis who 5 mo after initial presentation was diagnosed with pneumatosis cystoides intestinalis.

CASE REPORT

A 39-year-old male presented with right lower quadrant abdominal pain that ranged between 5-9/10 severity and was diffusely located in both the right upper and lower

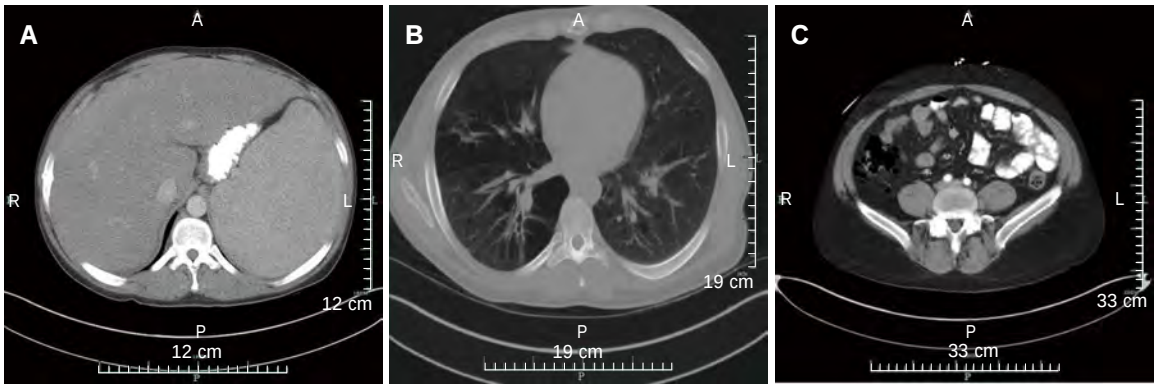


Figure 1 Computed tomography. A: Abdomen and pelvis showing massive hepatosplenomegaly at initial presentation; B: Chest at initial presentation demonstrating extensive bilateral emphysematous changes; C: Abdomen revealing pockets of free air consistent with perforation. No evidence of pneumatosis is seen on computed tomography scan. A: Anterior; P: Posterior; R: Right; L: Left.

quadrants of the abdomen. The pain begin approximately five years ago and has waxed and waned since. The week prior to admission the pain was significantly worsening. It was particularly aggravated by movement and coughing. Since the onset of symptoms five years ago, he reported watery loose bowel movements after meals, fevers up to 102F, night sweats, a 50lb unintentional weight loss, decreased visual acuity in his right eye and associated photophobia. He also described a chronic cough with occasional dark brown phlegm. He reported waking in the middle of the night coughing. He was initially evaluated multiple times at an outside hospital, but could not remember his specific diagnosis. He has a 15 pack year smoking history and denies any illicit drug use. His prior occupation involved working with copper products. His physical exam revealed severe tenderness over the right side of the abdomen. Liverspan was approximately 25 cm and spleen was easily palpable. The right eye showed an irregular opacity and photophobia.

On presentation he was pancytopenic, with an elevated alkaline phosphatase, gamma glutamyl transpeptidase, total bilirubin and angiotensin converting enzyme (ACE) level. Hemoglobin (Hgb) 13.1 g/dL [normal range (NR) 14.1-17.7 g/dL], platelet count 75 thousand/μL (NR 140-440 thousand/μL), white blood cell (WBC) 2.1 thousand/μL (NR 4.0-10 thousand/μL), alkaline phosphatase 383 IU/μL (NR 45-115 IU/μL), total bilirubin 1.7 mg/dL (NR 0.1-1.3 mg/dL), ACE was 97 U/L (NR 9-67 U/L). Anti-mitochondrial antibody, hepatitis panel, anti-nuclear antibody, human immunodeficiency virus, epstein-barr, and rapid plasma reagin serologies were negative. Tuberculin purified protein derivative and sputum acid fast smear were negative.

Abdominal computed tomography (CT) revealed a markedly enlarged liver and spleen each measuring 24 cm with distended portal and splenic veins (Figure 1A). Shotty retroperitoneal, upper abdominal and mesenteric lymphadenopathy was noted. CT chest revealed a subcentimeter enlargement of mediastinal lymphnodes that were pathological in number. Extensive bilateral centrilobular emphysematous changes were noted (Figure 1B). Bone marrow biopsy was normocellular. Immunophenotyping did not show diagnostic abnormalities of the B- or T-cells. Co-expression of abnormal antigens, antigen deletion, or light-chain clonality was not detected. Outside hospital records from 5 years prior to current admission were obtained. Full body positron emission tomography (PET) scan revealed no foci of abnormal uptake. Hgb was 13.9 g/dL, platelet count 220 thousand/μL, and WBC 4.5 thousand/μL. Alkaline phosphatase 388 IU/μL. Total bilirubin 1.2 mg/dL. Bone marrow biopsy showed hypocellular marrow with mild megaloblastic changes but no evidence of infiltrative changes.

The above findings were most consistent with a chronic inflammatory condition. Chronic inflammatory conditions that can cause hepatic noncaseating granulomas include sarcoidosis, Crohn's disease, primary biliary cirrhosis, Whipple's disease, copper poisoning, lymphomas, secondary syphilis. Given the negative full body PET scan, negative bone marrow and retroperitoneal lymph node biopsy, negative flow cytometry results, and long indolent course lymphoma was highly unlikely. While his previous occupation dealt with copper, symptoms started well before employment there and he did not demonstrate any other signs of copper poisoning. Whipple's disease is more common in European Caucasians and usually does not include ocular manifestations. However, it cannot be definitively excluded without a small bowel biopsy. Primary biliary cirrhosis and syphilis were ruled out through serologies. Crohn's disease is possible but is unlikely to cause such marked hepatosplenomegaly or pancytopenia. An extrapulmonary manifestation of sarcoidosis is the most likely explanation.

The patient was started on a 40 mg prednisone and instructed to follow up in the clinic within a mo. At subsequent follow-ups at outpatient clinic 2 wk, 2 mo, and 3 mo after presentation he was having difficult to control steroid induced diabetes requiring increasing doses of metformin and glipizide. He reported home blood sugar measurements ranged from 100-250 mg/dL. He had two separate hospitalizations for hyperglycemia 1 mo and 4 mo after presentation. At each visit his prednisone dose was being tapered. At 4 mo follow up at outpatient

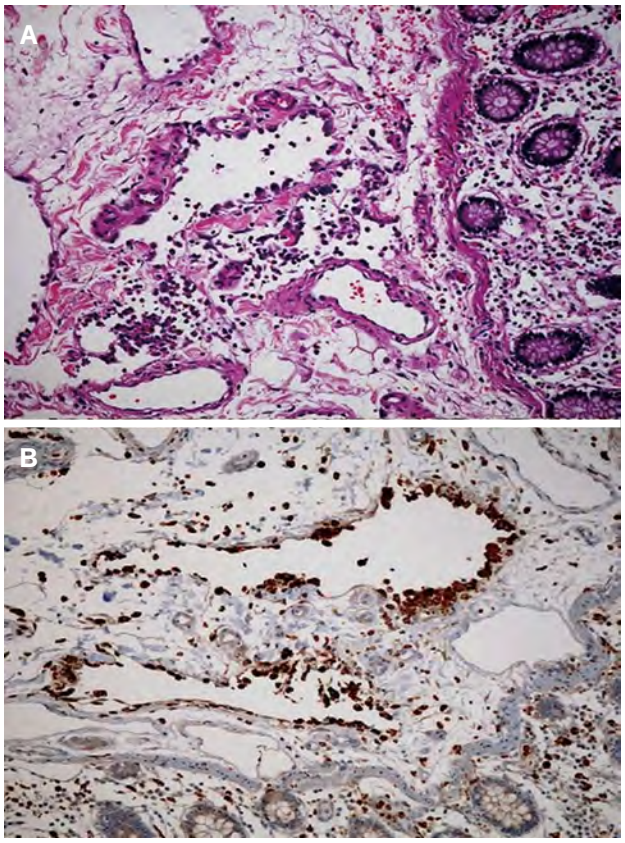


Figure 2 Pathology of the resected tissue revealed portions of large bowel with submucosal and mucosal hemorrhage and submucosa with irregular spaces lined with histiocytes (CD68 positive and CD31 negative) consistent with pneumatosis cystoides intestinalis. A: Cecum specimen stained with hematoxylin and eosin demonstrates partially endotheliolized cystic spaces in the submucosa lined by eosinophilic cells characteristic of pneumatosis intestinalis; B: Immunohistochemical staining for CD 68 which is found in cytoplasmic granules of cells within the monocyte/macrophage lineage confirms presents of histiocytes lining cystic spaces.

clinic the dose was 25 mg. The second hospitalization occurred at an outside hospital 4.5 mo after initial presentation. On discharge the patient reported he was told to increase the dose of glipizide as well as to increase his prednisone dose from 25 mg to 40 mg daily. Overall he was reporting decreased distension and abdominal discomfort. ACE level measured at outside hospital after 4 mo of steroid therapy was down to 29 U/L (97 U/L at initial presentation).

Five mo after initial presentation he came to our institution with headache, dizziness, abdominal pain, and nonbloody diarrhea 1 d. He reported the abdominal pain and discomfort were approximately baseline for him. He was afebrile and normotensive. His abdomen was tender to palpation right greater than left with guarding and rebound. Fecal occult blood was negative. He was found to have blood glucose of 623 mg/dL. Admission labs demonstrated improved pancytopenia, decreased alkaline phosphatase, but an elevated lactate [lactate 5.6 mmol/L (normal 0.5-2.2 mmol/L), Hgb (17.6 g/dL), platelets (114 thousand/μL), WBC (6.2 thousand/μL), alkaline phosphatase (223 IU/L)]. CT of the abdomen revealed pockets of free air distributed circumferentially along the cecum consistent with perforation (Figure 1C). He was taken to the OR for exploratory laparotomy requiring cecectomy and creation of ileostomy and mucous fistula. In the operating room there was no identified perforation externally, but a 2 cm × 2 cm defect within the cecum that did not penetrate the serosal surface of the bowel. The mucosa was hemorrhagic and flattened. Pathology of the resected tissue revealed portions of large bowel with submucosal and mucosal hemorrhage and submucosa with irregular spaces lined with histiocytes (CD68 positive and CD31 negative) consistent with pneumatosis cystoides intestinalis (Figure 2).

DISCUSSION

Sarcoidosis is an idiopathic multisystem non-caseating granulomatous disease that has been reported to impact almost any organ. It is more common in African Americans and typically patients present with pulmonary manifestations associated with bilateral hilar adenopathy on chest X-ray[1]. Other organs frequently involved include skin, lymph nodes, and ocular manifestations. The diagnosis is established by exclusion of other possibilities and can be supported by characteristic physical findings and biopsy showing noncaseating granulomas. ACE levels are elevated in 10% of chronic sarcoidosis and 60% of acute sarcoidosis[1].

The patient's chief complaint related to the abdominal distension and pain secondary to hepatosplenomegaly. Abdominal pain is an infrequent chief complaint in sarcoidosis however, both the liver and spleen have been reported to be involved[4]. Hepatomegaly has been reported to be as high as 21% of patients clinically and as many as 13% of patients with liver involvement had involvement independent of any typical pulmonary manifestations[4]. Splenomegaly has been reported by physical exam in 5%-14% with hypersplenism seen in 15% of those with splenic involvement[4]. While biopsy specimens from the patient presented are not available given the degree of splenomegaly and pancytopenia one could hypothesize involvement.

The patient described presented with pancytopenia likely secondary to hypersplenism. He was severely thrombocytopenic and leukopenic and mildly anemic. Cytopenias have been frequently described in sarcoidosis. However, to our knowledge significantly fewer reports describing pancytopenia. One report describes thrombocytopenia and leukopenia secondary to hypersplenism a second report notes pancytopenia with splenomegaly both were resolved with splenectomy[5,6]. This indicates a potential therapeutic role for the patient described here, but repeat CT after 5 mo on steroids revealed dramatic improvement in spleen size (Figure 3).

Pneumatosis cystoides intestinalis is a rare disorder defined as gas within the bowel wall. The presentation can range from being asymptomatic to acute abdominal pain. The mechanism of pneumatosis cystoides intestinalis is multifactorial. Current theories propose luminal gas which could be pulmonary or bacterial in origin dis-

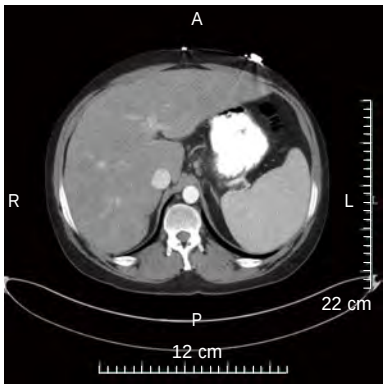


Figure 3 Computed tomography abdomen with contrast demonstrating marked reduction in spleen size and moderate reduction in hepatomegaly after 5 mo of steroid treatment. A: Anterior; P: Posterior; R: Right; L: Left.

sect through mucosa which has been compromised. Mucosa may be compromised by a number of mechanisms including immunologically or physically[7].

While typically diagnosed *via* CT scan[2] certain cases where the pneumatosis is very subtle the finding may be missed and air contrast enema may be useful[8,9]. Furthermore, when diagnosed *via* CT, the majority of patients can be managed nonoperatively as exploratory laparotomy is likely to be of benefit to only a subset of patients. Those patients include those with abdominal distension, peritonitis, and lactic acidemia[10]. The patient described here represents one of these subtle cases as he was only found to have pneumatosis on pathology. He clearly was in need of operative management given the pneumoperitoneum, abdominal distension, and elevated lactic acid.

Pneumatosis cystoides intestinalis has been linked to a number of conditions in the past. One of which is chronic obstructive lung disease[2,3]. Pulmonary function tests obtained 4 mo after presentation at an outside institution revealed moderate obstructive lung disease with abnormal diffusion capacity [forced expiratory volume in 1 second (FEV1)/forced vital capacity = 70% predicted, FEV1 = 69% predicted, single breath diffusion Dsb 38% predicted]. Typically sarcoidosis most commonly affects the lungs, but the typical radiographic presentation was absent. On chest X-ray bihilar and mediastinal lymphadenopathy (75%) and pulmonary infiltrates (50%) are the most common presentations[1]. This usually occurs in an upper lobe and nodular predominance. On CT scan typical features include bilateral perihilar opacities, bilateral symmetric hilar or mediastinal lymphadenopathy, fibrotic changes such as reticular opacities, architectural distortion, or bronchiectasis[1]. The patient presented had severe emphysematous changes which are rarely associated with sarcoidosis. Prior case reports have documented emphysema in patients with sarcoidosis though the mechanism is unclear[11,12]. While he does have a 15 pack year hx it would be unlikely all lung abnormalities are due to past tobacco at only the age of 39, though this does confound the finding.

Pneumatosis cystoides intestinalis has been very frequently linked to medications. Corticosteroids have been reported most often. Many of the other conditions, pneumatosis cystoides intestinalis has classically been linked to are treated with steroids as well, including connective tissue disease and some autoimmune conditions[2,3,13,14]. The hypothesized mechanism suggests the immunosuppressive effects of steroids results in depletion of the lymphoid tissue within the Peyer's patches, this leads to mucosal disruption allowing intraluminal gas diffusion[15,16]. One review indicates that 33% of patients with pneumatosis intestinalis had received prior steroid treatment. Another 32% had received either chemotherapy or methotrexate[15,17]. This hypothesis is supported by the fact that other immunosuppressive or cytotoxic drugs have also been associated with pneumatosis cystoides intestinalis. This includes but is not limited to sunitinib, cisplatin, irinotecan, and docetaxel, methotrexate[17-20].

Alternative mechanisms have been described for other drugs that have been implicated in pneumatosis cystoides intestinalis. The chemotherapeutic agents described above have not only been suggested to contribute to the development of pneumatosis cystoides intestinalis *via* their immunosuppressive effects but also through their apoptotic effects on rapidly dividing cells resulting in compromise of mucosal integrity. Vascular endothelial growth factor and epidermal growth factor inhibitors are hypothesized to damage the microvasculature of the intestinal wall resulting in compromise of mucosal integrity[19,21]. Alpha glucosidase inhibitors, miglitol and acarbose, inhibit the absorption of carbohydrates. It has been suggested that digestion of these carbohydrates by intestinal flora result in gas production. The increased intraluminal pressure could potentially lead to dissection through the mucosa[15,22,23].

The patient was on a steroid taper, but was increased from 25 mg to 40 mg by an outside hospital for reasons that are unclear 1 mo prior to perforation. The 5 mo course of steroids coupled with the recent increased dosage thus could have played a significant role in the pathogenesis through immunosuppression as detailed above. However, given the patients improvement in symptoms and ACE level it seems the initial course of tapering the dosage as symptoms improved was the best course of action.

The patient described here likely had a number of factors that could have additively contributed to the development of pneumatosis cystoides intestinalis. The steroid treatment coupled with his baseline pancytopenia clearly placed him in an immunosuppressed state. The massive hepatomegaly and respiratory disease may have lead to increased intraabdominal pressure producing strain on the mucosal walls. We may also speculate that the 5 year history of untreated GI sarcoidosis may have further weakened the intestinal walls through granulomatosis inflammationa nd disruption of normal architecture. Cumulatively, this created an environment where pneumatosis intestinalis could readily develop.

The described case represents a unique presentation of the difficult to recognize and diagnose GI sarcoidosis. The initial findings of pancytopenia and emphysematous

changes in the lungs are typically not found in sarcoidosis. Furthermore this is the first reported association between pneumatosis intestinalis and sarcoidosis though the exact mechanism of pneumatosis cystoides intestinalis is unclear and likely multifactorial as in many such cases.

REFERENCES

1 **O'Regan A**, Berman JS. Sarcoidosis. *Ann Intern Med* 2012; **156**: ITC5-1, ITC5-2, ITC5-3, ITC5-4, ITC5-5, ITC5-6, ITC5-7, ITC5-8, ITC5-9, ITC5-10, ITC5-11, ITC5-12, ITC5-13, ITC5-14, ITC5-15, quiz ITC5-16 [PMID: 22547486 DOI: 10.1059/0003-4819-156-9-201205010-01005]

2 **Khalil PN**, Huber-Wagner S, Ladurner R, Kleespies A, Siebeck M, Mutschler W, Hallfeldt K, Kanz KG. Natural history, clinical pattern, and surgical considerations of pneumatosis intestinalis. *Eur J Med Res* 2009; **14**: 231-239 [PMID: 19541582 DOI: 10.1186/2047-783X-14-6-231]

3 **Azzaroli F**, Turco L, Ceroni L, Galloni SS, Buonfiglioli F, Calvanese C, Mazzella G. Pneumatosis cystoides intestinalis. *World J Gastroenterol* 2011; **17**: 4932-4936 [PMID: 22171137 DOI: 10.3748/wjg.v17.i44.4932]

4 **Ebert EC**, Kierson M, Hagspiel KD. Gastrointestinal and hepatic manifestations of sarcoidosis. *Am J Gastroenterol* 2008; **103**: 3184-392; quiz 3193 [PMID: 18853979 DOI: 10.1111/j.1572-0241.2008.02202.x]

5 **Haran MZ**, Feldberg E, Miller G, Berrebi A. Sarcoidosis presenting as massive splenomegaly and bicytopenia. *Am J Hematol* 2000; **63**: 232-233 [PMID: 10706772 DOI: 10.1002/(SICI)1096-8652(200004)63]

6 **Thadani U**, Aber CP, Taylor JJ. Massive splenomegaly, pancytopenia and haemolytic anaemia in sarcoidosis. *Acta Haematol* 1975; **53**: 230-240 [PMID: 808933 DOI: 10.1159/000208188]

7 **St Peter SD**, Abbas MA, Kelly KA. The spectrum of pneumatosis intestinalis. *Arch Surg* 2003; **138**: 68-75 [PMID: 12511155 DOI: 10.1001/archsurg.138.1.68]

8 **Kawamoto K**, Ueyama T, Iwashita I, Utsunomiya T, Honda H, Onitsuka H, Haraguchi Y, Kojima N, Takano H, Masuda K. Colonic submucosal tumors: comparison of endoscopic US and target air-enema CT with barium enema study and colonoscopy. *Radiology* 1994; **192**: 697-702 [PMID: 8058936]

9 **Ihara E**, Harada N, Motomura S, Chijiiwa Y. A new approach to Pneumatosis cystoides intestinalis by target air-enema CT. *Am J Gastroenterol* 1998; **93**: 1163-1164 [PMID: 9672353 DOI: 10.1111/j.1572-]

10 **Duron VP**, Rutigliano S, Machan JT, Dupuy DE, Mazzaglia PJ. Computed tomographic diagnosis of pneumatosis intestinalis: clinical measures predictive of the need for surgical intervention. *Arch Surg* 2011; **146**: 506-510 [PMID: 21576602 DOI: 10.1001/archsurg.2011.95]

11 **Judson MA**, Strange C. Bullous sarcoidosis: a report of three cases. *Chest* 1998; **114**: 1474-1478 [PMID: 9824033 DOI: 10.1378/chest.114.5.1474]

12 **Zimmerman I**, Mann N. Boeck's sarcoid; a case of sarcoidosis complicated by pulmonary emphysema and cor pulmonale. *Ann Intern Med* 1949; **31**: 153-162 [PMID: 18134816]

13 **Cabrera GE**, Scopelitis E, Cuellar ML, Silveira LH, Mena H, Espinoza LR. Pneumatosis cystoides intestinalis in systemic lupus erythematosus with intestinal vasculitis: treatment with high dose prednisone. *Clin Rheumatol* 1994; **13**: 312-316 [PMID: 8088081]

14 **Ohara H**, Kato Y, Nakano M, Ishii Y, Serizawa H, Watanabe N, Wakabayashi K, Tsunematsu S, Kumagai N, Tsuchimoto K, Hibi T. [A case of pneumatosis cystoides intestinalis induced by steroid pulse therapy for severe acute hepatitis B]. *Nihon Shokakibyo Gakkai Zasshi* 2011; **108**: 1237-1243 [PMID: 21737976]

15 **Saito M**, Tanikawa A, Nakasute K, Tanaka M, Nishikawa T. Additive contribution of multiple factors in the development of pneumatosis intestinalis: a case report and review of the literature. *Clin Rheumatol* 2007; **26**: 601-603 [PMID: 16404494 DOI: 10.1007/s10067-005-0179-9]

16 **Heng Y**, Schuffler MD, Haggitt RC, Rohrmann CA. Pneumatosis intestinalis: a review. *Am J Gastroenterol* 1995; **90**: 1747-1758 [PMID: 7572888]

17 **Sequeira W**. Pneumatosis cystoides intestinalis in systemic sclerosis and other diseases. *Semin Arthritis Rheum* 1990; **19**: 269-277 [PMID: 2192457 DOI: 10.1016/0049-0172(90)90049-L]

18 **Candelaria M**, Bourlon-Cuellar R, Zubieta JL, Noel-Ettiene LM, Sánchez-Sánchez JM. Gastrointestinal pneumatosis after docetaxel chemotherapy. *J Clin Gastroenterol* 2002; **34**: 444-445 [PMID: 11907358 DOI: 10.1097/00004836-200204000-00012]

19 **Flaig TW**, Kim FJ, La Rosa FG, Breaker K, Schoen J, Russ PD. Colonic pneumatosis and intestinal perforations with sunitinib treatment for renal cell carcinoma. *Invest New Drugs* 2009; **27**: 83-87 [PMID: 18563296 DOI: 10.1007/s10637-008-9146-z]

20 **Kung D**, Ruan DT, Chan RK, Ericsson ML, Saund MS. Pneumatosis intestinalis and portal venous gas without bowel ischemia in a patient treated with irinotecan and cisplatin. *Dig Dis Sci* 2008; **53**: 217-219 [PMID: 17530401 DOI: 10.1007/s10620-007-9846-9]

21 **Coriat R**, Ropert S, Mir O, Billemont B, Chaussade S, Massault PP, Blanchet B, Vignaux O, Goldwasser F. Pneumatosis intestinalis associated with treatment of cancer patients with the vascular growth factor receptor tyrosine kinase inhibitors sorafenib and sunitinib. *Invest New Drugs* 2011; **29**: 1090-1093 [PMID: 20524036 DOI: 10.1007/s10637-010-9458-7]

22 **Kojima K**, Tsujimoto T, Fujii H, Morimoto T, Yoshioka S, Kato S, Yasuhara Y, Aizawa S, Sawai M, Makutani S, Yamamoto K, Mochi T, Fukui H. Pneumatosis cystoides intestinalis induced by the α-glucosidase inhibitor miglitol. *Intern Med* 2010; **49**: 1545-1548 [PMID: 20686288 DOI: 10.2169/internalmedicine.49.3634]

23 **Yanaru R**, Hizawa K, Nakamura S, Yoshimura R, Watanabe K, Nakamura U, Yoshinari M, Matsumoto T. Regression of pneumatosis cystoides intestinalis after discontinuing of alpha-glucosidase inhibitor administration. *J Clin Gastroenterol* 2002; **35**: 204-205 [PMID: 12172373 DOI: 10.1097/00004836-200208000-00020]

P- Reviewer Tosetti C **S- Editor** Huang XZ
L- Editor A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i7.1140

World J Gastroenterol 2013 February 21; 19(7): 1140-1142
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Transjugular intrahepatic portosystemic shunt in refractory chylothorax due to liver cirrhosis

Philipp Lutz, Holger Strunk, Hans Heinz Schild, Tilman Sauerbruch

Philipp Lutz, Tilman Sauerbruch, Department of Internal Medicine I, University of Bonn, 53129 Bonn, Germany
Holger Strunk, Hans Heinz Schild, Department of Radiology, University of Bonn, 53129 Bonn, Germany
Author contributions: All authors were involved in the clinical care of this patient and collaborated in writing this paper.
Correspondence to: Philipp Lutz, MD, Department of Internal Medicine I , University of Bonn, Sigmund-Freud-Str. 25, 53129 Bonn, Germany. philipp.lutz@ukb.uni-bonn.de
Telephone: +49-228-28719169 Fax: +49-228-28714322
Received: October 20, 2012 Revised: November 26, 2012
Accepted: December 15, 2012
Published online: February 21, 2013

Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i7/1140.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i7.1140

Abstract

A pleural effusion containing chylomicrons is termed chylothorax and results from leakage of lymph fluid into the pleural cavity. We report on the case of a 59-year-old woman with severe dyspnea due to a large chylothorax. She was known to have liver cirrhosis but no ascites. There was no history of trauma, cardiac function was normal and thorough diagnostic work-up did not reveal any signs of malignancy. In summary, no other etiology of the chylothorax than portal hypertension could be found. Therapy with diuretics as well as parenteral feeding failed to relieve symptoms. After a transjugular intrahepatic portosystemic shunt (TIPS) had successfully been placed, pleural effusion decreased considerably. Eight months later, TIPS revision had to be performed because of stenosis, resulting in remission from chylothorax. This case shows that even in the absence of ascites, chylothorax might be caused by portal hypertension and that TIPS can be an effective treatment option.

© 2013 Baishideng. All rights reserved.

Key words: Chylothorax; Cirrhosis; Liver; Portal hypertension; Transjugular intrahepatic portosystemic shunt

Lutz P, Strunk H, Schild HH, Sauerbruch T. Transjugular intrahepatic portosystemic shunt in refractory chylothorax due to liver cirrhosis. *World J Gastroenterol* 2013; 19(7): 1140-1142

INTRODUCTION

Chylothorax is defined as a pleural effusion containing chylomicrons. Triglyceride levels of at least 110 mg/dL have been found to reliably indicate chylothorax[1], originating from a lesion in the lymphatic vessels, mostly the thoracic duct. In about 40% of patients with chylothorax, these lesions are caused by trauma or surgery, whereas in about 30%, a malignant tumor is found. The remaining cases are attributed to a variety of conditions, including congenital, infectious and cardiac diseases, or are considered to be idiopathic[2]. Chylothorax rarely occurs in patients with liver cirrhosis. In such cases, concomitant ascites is usually present[3]. We report on the effective treatment of massive chylothorax in a female patient with liver cirrhosis but no ascites by placement of a transjugular intrahepatic portosystemic shunt (TIPS).

CASE REPORT

The 59-year-old woman was referred to our university hospital due to chylothorax which could not be sufficiently managed in a peripheral hospital. Since 2008, a right sided pleural effusion had to be drained occasionally despite diuretic therapy. In recent weeks, however, the pleural effusion had turned milky and had to be drained more frequently. Three weeks prior to admission, nearly 1.5 L/d had to be removed (Figure 1). The patient presented no further symptoms apart from dyspnea. Her past medical history included diagnosis of alcoholic liver cirrhosis (Child B), diabetes mellitus type Ⅱ and resection of a local ovarian carcinoma 15 years ago. The exact tumor staging was unknown. The patient had received adjuvant chemotherapy and the carcinoma has been in remission since then. One year ago, she was treated for bleeding gastric ulcer related to infection with *Helicobacter pylori*. There was no history of trauma.

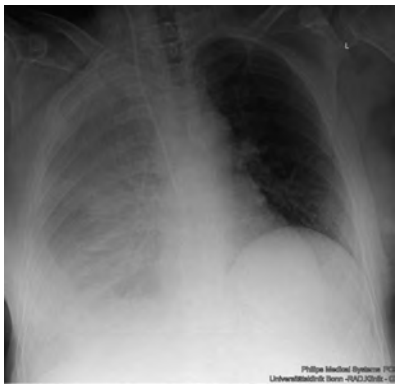


Figure 1 Chest X-ray before transjugular intrahepatic portosystemic shunt placement, showing a large right-sided pleural effusion.

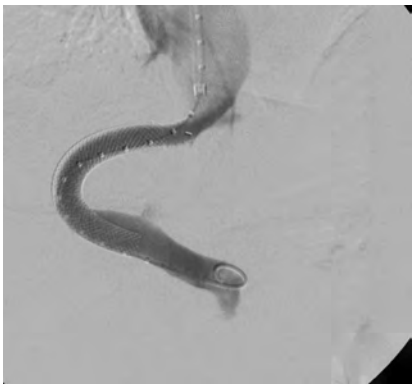


Figure 2 Angiogram after transjugular intrahepatic portosystemic shunt placement, the catheter tip is in the portal vein.

Apart from the signs of right-sided pleural effusion, some spider naevi and a small umbilical hernia, physical examination was unremarkable. Blood analysis showed slightly elevated bilirubin (1.2 mg/dL, reference range up to 1 mg/dL), increased gamma-glutamyl transferase (112 U/L, reference range up to 38 U/L), international normalized ratio (of prothrombin time) above normal (1.3) and decreased serum albumin (31 g/L, reference range 35-52 g/L). Model for End-stage Liver Disease score was 10. Mild hyperglycemia was due to diabetes mellitus. Urine analysis was unremarkable. Analysis of the pleural fluid showed 95 polymorphonuclear cells/μL, 294 mononuclear cells/μL, low albumin of 4.2 g/L, no cholesterol, but markedly increased triglycerides (386 mg/dL). Bacterial cultures were negative, microscopic examination did not reveal malignant cells. No tumor was detected in a computed tomography (CT) scan carried out at the referring hospital. Cardiac evaluation by echocardiography and transthoracic echocardiography revealed no signs of heart failure or pulmonary hypertension. Abdominal ultrasound showed a liver morphology consistent with cirrhosis and concomitant splenomegaly, but neither portal vein thrombosis nor signs of hepatocellular carcinoma nor ascites were present. To further characterize the degree of portal hypertension, we performed endoscopy of the upper gastrointestinal tract. Esophageal varices grade Ⅱ were detected.

In summary, we confirmed the diagnosis of chylothorax with a daily production of 2-4 liters. However, it was uncertain whether this was caused by liver cirrhosis. As diuretic therapy failed to drain the chylothorax, we put the patient on parenteral feeding for a total of nine days to reduce the flow in the lymphatic ducts and to promote healing of a suspected lymphatic lesion. As expected, the milky aspect of the pleural effusion vanished, but the daily amount of secretion from the pleural drainage increased even further (about 1.5-3 L/d). Since high renin blood levels are often associated with decompensated portal hypertension[4], we determined renin blood levels, revealing a value of 1680 μU/mL (reference range up to 46 μU/mL) in conjunction with an elevated level of aldosterone of 946 pg/mL (reference range up to 310 pg/mL). Thus, we proposed TIPS as treatment option and this was placed successfully in March 2010 (Figure 2). Fifteen days later, the pleural drain could be removed; another week later, the patient could be dismissed with a small, stable pleural effusion under diuretic medication. During the following months, the pleural effusion had to be drained about once per month. TIPS revision due to stenosis had to be performed by inserting a new stent in November 2010. Since then, no further pleural drainage was needed (Figure 3). After TIPS placement, the patient had several episodes of hepatic encephalopathy grade Ⅰ-Ⅱ according to the West Haven criteria, but responded to treatment with lactulose and rifaximin. Levels of bilirubin did not increase significantly after the intervention. To date, more than two years after TIPS placement, the patient is still without recurrence of chylothorax (Figure 4).

DISCUSSION

Liver cirrhosis, together with portal hypertension is a rare cause of chylothorax[3]. Diagnosis was difficult in this patient because she presented with almost normal laboratory values and without ascites. However, tiny amounts of abdominal fluid had been present in the past. Conservative management of chylothorax failed. To the best of our knowledge, TIPS as successful treatment of chylothorax in cirrhotic patients has been described before in only two cases: in one patient with traumatic injury of the thoracic duct[5] and in a second patient with concomitant refractory ascites[6]. High renin levels in our patient suggested that activation of the renin angiotensin aldosterone system (RAAS), causing sodium and fluid retention, was due to portal hypertension, complicated by the leakage of chyle in the pleural cavity. This activated RAAS argued against idiopathic etiology, which is described in 10% of patients with chylothorax. Indeed, renin values in our patient decreased considerably from 1680 to 317 μU/mL after TIPS placement, along with regression of excessive chyle formation. Other etiologies of chylothorax were not very plausible: there was no history of recent trauma or surgery and a malignant process - bearing the history of ovarian cancer in mind - was improbable due to negative CT scan and pleural fluid cytology. Furthermore, the patient developed no malignancy during the follow-up of more than two years. In the end, placement of TIPS improved the quality of life

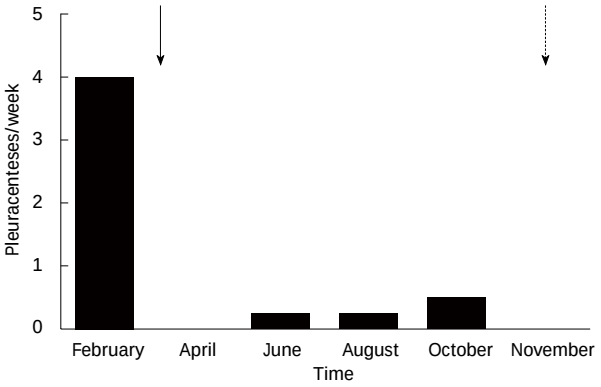


Figure 3 Need for pleurocentesis over time. There is a clear drop after transjugular intrahepatic portosystemic shunt (TIPS) placement in March (arrow) and no need for further pleurocentesis after TIPS revision in November (dotted arrow).



Figure 4 Chest X-ray during follow up without pleural effusion.

of this patient enormously. After failure of conservative treatment, the main alternative to TIPS would have been surgical procedures, such as pleurodesis or ligation/embolization of the thoracic duct[7], which, however, do not address portal hypertension as underlying disease. Fasting and parenteral nutrition have been described as effective in chylothorax particularly following surgery or trauma. In most other forms, lymphatic obstruction or increased venous pressure in the superior vena cava cause lymphangiectasia and chylothorax[7]. In these cases, parenteral feeding is not effective. Somatostatin has been used in chylothorax of various etiologies with some success[8]. Due to its characteristic contents, drainage of a chylothorax can lead to malnutrition and immunosuppression caused by loss of lipids, immunoglobulins and lymphocytes. However, this characteristic content seems to reduce the risk of infection of a pleural drain[9].

The success of the TIPS placement in our patient confirms the hepatic origin of the chylothorax. It has been suggested that in liver cirrhosis, chyle flow increases substantially due to increased formation of hepatic lymph and due to portal hypertension. Since drainage into the venous system is limited by a valve at the junction of the thoracic duct and the subclavian vein, pressure in the lymphatic vessels is increased, leading to an elevated risk of spontaneous rupture[10]. Thus, in our patient, such a spontaneous leak might have persisted as long as portal hypertension was high, maintaining the pleural effusion *via* small gaps in the diaphragm as in hepatic hydrothorax, which occurs in about 5%-12% of liver cirrhosis patients[11]. A recent review described a clinical response rate of about 70% in 198 patients with hepatic hydrothorax after TIPS placement. However, controlled studies are still missing. No case of chylothorax was described in these patients[12].

In summary, this case shows that massive chylothorax can develop in patients with otherwise well compensated liver cirrhosis and that it can be treated effectively by TIPS placement.

REFERENCES

1 **Staats BA**, Ellefson RD, Budahn LL, Dines DE, Prakash UB, Offord K. The lipoprotein profile of chylous and nonchylous pleural effusions. *Mayo Clin Proc* 1980; **55**: 700-704 [PMID: 7442324]

2 **Agrawal V**, Sahn SA. Lipid pleural effusions. *Am J Med Sci* 2008; **335**: 16-20 [PMID: 18195578 DOI: 10.1097/MAJ.0b013e31815d2634]

3 **Romero S**, Martín C, Hernandez L, Verdu J, Trigo C, Perez-Mateo M, Alemany L. Chylothorax in cirrhosis of the liver: analysis of its frequency and clinical characteristics. *Chest* 1998; **114**: 154-159 [PMID: 9674463 DOI: 10.1378/chest.114.1.154]

4 **Arroyo V**, Ginès P. Mechanism of sodium retention and ascites formation in cirrhosis. *J Hepatol* 1993; **17** Suppl 2: S24-S28 [PMID: 8491967 DOI: 10.1016/S0168-8278(05)80451-9]

5 **Vignaux O**, Gouya H, Dousset B, Mazuir E, Buffet C, Calmus Y, Legmann P. Refractory chylothorax in hepatic cirrhosis: successful treatment by transjugular intrahepatic portosystemic shunt. *J Thorac Imaging* 2002; **17**: 233-236 [PMID: 12082377 DOI: 10.1097/00005382-200207000-00010]

6 **Kinney TB**, Ferrara SL, Miller FJ, Roberts AC, Hassanein T. Transjugular intrahepatic portosystemic shunt creation as treatment for refractory chylous ascites and chylothorax in a patient with cirrhosis. *J Vasc Interv Radiol* 2004; **15**: 85-89 [PMID: 14709693 DOI: 10.1097/01.RVI.0000106391.63463.4C]

7 **Doerr CH**, Miller DL, Ryu JH. Chylothorax. *Semin Respir Crit Care Med* 2001; **22**: 617-626 [PMID: 16088705 DOI: 10.1055/s-2001-18797]

8 **Kalomenidis I**. Octreotide and chylothorax. *Curr Opin Pulm Med* 2006; **12**: 264-267 [PMID: 16825878 DOI: 10.1097/01.mcp.0000230629.73139.26]

9 **Nair SK**, Petko M, Hayward MP. Aetiology and management of chylothorax in adults. *Eur J Cardiothorac Surg* 2007; **32**: 362-369 [PMID: 17580118 DOI: 10.1016/j.ejcts.2007.04.024]

10 **Aalami OO**, Allen DB, Organ CH. Chylous ascites: a collective review. *Surgery* 2000; **128**: 761-778 [PMID: 11056439 DOI: 10.1067/msy.2000.109502]

11 **Garcia N**, Mihas AA. Hepatic hydrothorax: pathophysiology, diagnosis, and management. *J Clin Gastroenterol* 2004; **38**: 52-58 [PMID: 14679328]

12 **Rössle M**, Gerbes AL. TIPS for the treatment of refractory ascites, hepatorenal syndrome and hepatic hydrothorax: a critical update. *Gut* 2010; **59**: 988-1000 [PMID: 20581246 DOI: 10.1136/gut.2009.193227]

P- Reviewers Durandy Y, Neuberger J, Bordas JM
S- Editor Gou SX **L- Editor** A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i7.1143

World J Gastroenterol 2013 February 21; 19(7): 1143-1146
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Cytomegalovirus-associated gastric ulcer: A side effect of steroid injections for pyloric stenosis

Hirohito Mori, Shintaro Fujihara, Noriko Nishiyama, Hideki Kobara, Makoto Oryu, Kiyohito Kato, Kazi Rafiq, Tsutomu Masaki

Hirohito Mori, Shintaro Fujihara, Noriko Nishiyama, Hideki Kobara, Makoto Oryu, Kiyohito Kato, Tsutomu Masaki, Departments of Gastroenterology and Neurology, Kagawa Medical University School of Medicine, Kagawa 761-0793, Japan
Kazi Rafiq, Departments of Pharmacology, Kagawa Medical University School of Medicine, Kagawa 761-0793, Japan
Author contributions: Mori H was responsible for conception of the research and for drafting the article; Fujihara S, Nishiyama N, Kobara H, Oryu M, Kato K and Rafiq K participated equally in the work; Masaki T provided critical revision of the manuscript for intellectual content and was responsible for final approval of the manuscript.
Correspondence to: Hirohito Mori, MD, PhD, Department of Gastroenterology and Neurology, Kagawa University School of Medicine, 1750-1 Ikenobe, Miki, Kita, Kagawa 761-0793, Japan. hiro4884@med.kagawa-u.ac.jp
Telephone: +81-87-8912156 Fax: +81-87-8912158
Received: September 24, 2012 Revised: November 13, 2012
Accepted: December 25, 2012
Published online: February 21, 2013

Abstract

The local injection of triamcinolone acetonide (TA) is effective in preventing pyloric stenosis and deformity following large endoscopic submucosal dissection (ESD). However, because of its long-acting nature, TA can induce long-term local immunosuppression and subsequent adverse events. We report a case of a cytomegalovirus (CMV) ulcer that formed only at the TA local injection site. A 68-year-old man underwent ESD to treat early gastric cancer that formed over the pylorus. The lesion extended to the duodenum, and an artificial ulcer covered more than two-thirds of the circumference of the pylorus. To prevent pyloric stenosis, TA was locally injected into the ulcer floor. On day 12, a deeper ulcer 10 mm in diameter was discovered in the center of the post-ESD ulcer. Biopsies revealed large cells with intranuclear inclusion bodies, which stained positive for the anti-CMV antibody. Local TA injections are useful, however, CMV ulcer might occur as adverse events.

© 2013 Baishideng. All rights reserved.

Key words: Pyloric stenosis; Local triamcinolone acetonide injection; Local immunosuppression; Cytomegalovirus-associated ulcer; Adverse events

Mori H, Fujihara S, Nishiyama N, Kobara H, Oryu M, Kato K, Rafiq K, Masaki T. Cytomegalovirus-associated gastric ulcer: A side effect of steroid injections for pyloric stenosis. *World J Gastroenterol* 2013; 19(7): 1143-1146 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i7/1143.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i7.1143

INTRODUCTION

Endoscopic submucosal dissection (ESD) for early gastric cancer was developed to resect larger lesions *en bloc*[1-4]. ESD has an advantage over endoscopic mucosal resection in that it enables *en bloc* tumor removal[5-8]. However, ESD creates a large artificial ulcer that can lead to gastric stenosis[9]. In recent years, the local injection of triamcinolone acetonide (TA) has reportedly prevented post-ESD esophageal stricture, pyloric stenosis, and deformity following large ESDs because TA promotes the formation of granulation tissue at an early stage in the healing process, which leads to gastric mucosa regeneration[10-12]. However, because of its long-acting nature, TA can induce long-term local immunosuppression and can cause subsequent adverse events. We report a case of cytomegalovirus (CMV) ulcer formation that occurred only at the local TA injection site. This is the first case report of a side effect of local TA injection after treatment of an ESD ulcer floor in a non-compromised host.

CASE REPORT

A 68-year-old man underwent ESD to treat early-stage gastric cancer that was located over the pylorus (Figure

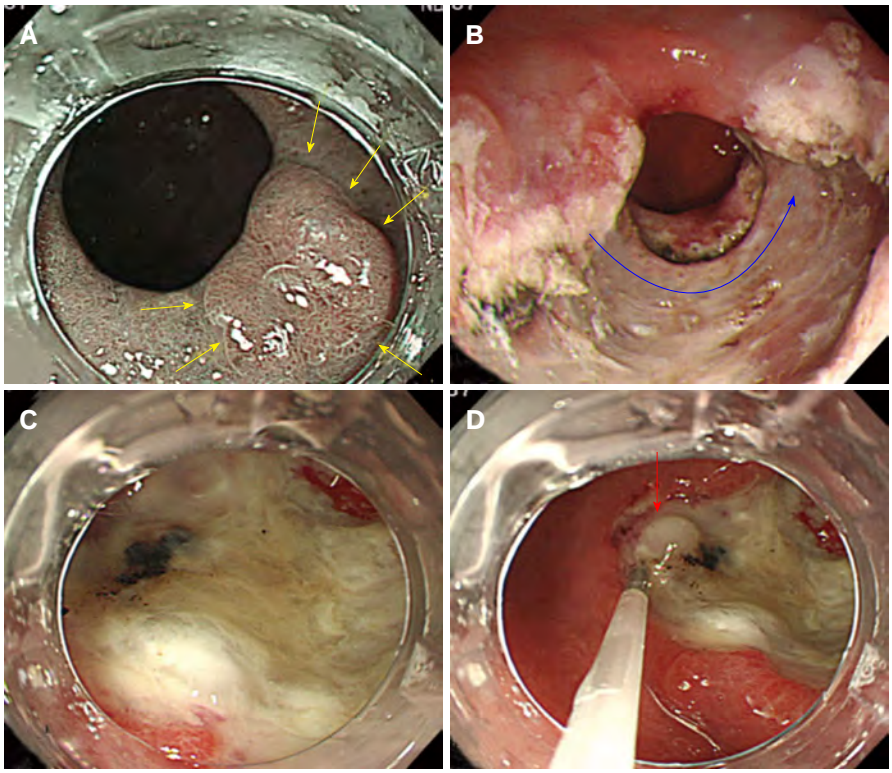


Figure 1 Endoscopic findings of tumor, post-endoscopic submucosal dissection ulcer and triamcinolone acetonide injection. A: An narrow band imaging endoscopic image reveals a flat, early gastric cancer lesion extending over the gastric outlet to the pylorus (yellow arrows); B: A post-endoscopic submucosal dissection artificial ulcer covering two-thirds of the circumference of the pylorus (blue curved arrow); C: The ulcer floor covered by a thick layer of white moss; D: Triamcinolone acetonide (2 mL) was injected locally at each site (red arrow).

1A). The lesion partially extended to the duodenum, and an artificial ulcer that formed after dissection covered over two-thirds of the pylorus circumference (Figure 1B). As routine pre-ESD examination, we conducted serology test, electrocardiogram, respiratory function test, abdominal ultrasound examination and computed tomography. These results indicated no underlying disease. To prevent pyloric stenosis, TA was locally injected into the ulcer, which was covered with white moss (Figure 1C) in 5-mm intervals [0.2 mL (2 mg)] at each site, on postoperative day 5 (Figure 1D). On day 12, abundant granulation tissue had formed over the ulcer, but another deeper ulcer approximately 10 mm in diameter was discovered centered in the post-ESD ulcer (Figure 2A). Biopsies that were conducted from the margin of the deeper ulcer revealed large cells with intranuclear inclusion bodies (Figure 2C) that stained positive for anti-CMV antibody staining (Figure 2D). As the patient wasn't a compromised host and had no other underlying disease, we thought the CMV activity of the ulcer floor was limited and focal. The CMV ulcer was occurred under focal immunosuppressive condition by TA. We considered after TA effect would subside about for 14 d, the CMV activity would decrease and the ulcer healed. We conducted frequent follow up endoscopy at POD12, 20 and 30, and confirmed negative conversion of CMV by serology test and histopathological examination. The deeper ulcer improved gradually (Figure 2B), and on day 20, the biopsies were negative for anti-CMV antibody staining. The post-ESD artificial ulcer healed without any pylorus stricture.

DISCUSSION

Some clinical studies have recommended administration of oral prednisolone[10] and local TA injection into post-ESD artificial ulcers[11] in order to prevent severe esophageal stenosis. The beneficial effects of this procedure were introduced at the Conference of Japan Gastroenterological Endoscopy in April 2009, and some clinical trials reported on the efficacy of local TA injections and oral prednisolone administration. After these reports, we reported that local TA injection into the floor of a post-ESD artificial gastric ulcer promotes the formation of granulation tissue in an early stage of the healing process, which leads to regenerated gastric mucosa without mucosal convergence or gastric deformity[12]. As our previous report, we conducted local steroid injection into post-ESD artificial ulcers to 21 patients and analyzed them according to the protocol, there were no complications. So, this case was the first case of CMV-associated ulcer development related to the side effects of local steroid injection following ESD. Pharmacologically, TA modulates the wound-healing process for post-ESD ulcers by suppressing inflammatory cell infiltration and fibrosis. This wound healing is caused by decreasing the procollagen-proline dioxygenase or prolyl hydroxylase levels. Decreasing the levels of these enzymes reduces the tissue

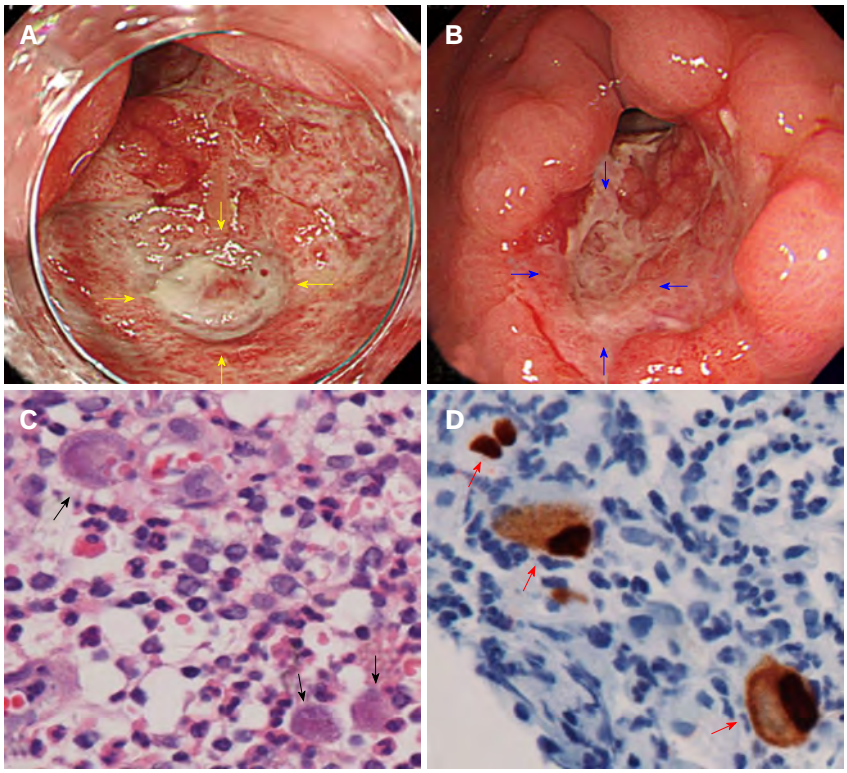


Figure 2 Endoscopic findings of cytomegalovirus associated ulcer and microscopic examination. A: Artificial ulcer on postoperative day 12, showing the formation of abundant granulation tissue and a 10-mm-deep ulcer at the center of the granulation tissue (yellow arrows); B: The healing process of the deep ulcer (blue arrows) on postoperative day 15; C: A biopsy from the deeper ulcer margin revealed large cells with intranuclear inclusion bodies (black arrow, HE staining, × 600); D: Large cells with intranuclear inclusion bodies stained positive for anti-cytomegalovirus (CMV) antibodies (red arrows, anti-CMV antibody immunohistochemical staining, × 600).

collagen component[13]; in this way, the local TA injection promoted the formation of flat and sufficient granulation tissue without fibrotic contraction. However, no infections or adverse effects were reported following local steroid injection into the post-ESD artificial esophageal or gastric ulcer floor. Subclinical CMV infection is high among Japanese infants (about 90%). Latent infection, which is established in the granulocytes and monocytes, can be reactivated by administering potent immunosuppressants, such as steroids[14]. Although the CMV ulcers generally occurred in compromised hosts, in our case, the formation of a CMV ulcer was likely caused by an inflammatory reaction in the post-ESD ulcer and reactivation of the latently infected granulocytes and monocytes, which had migrated to phagocytize the necrotic and granulation tissues on the ulcer floor. Ulcer healing occurred after 14-21 d when the TA effects had subsided. The local injection of TA is effective in preventing post-ESD esophageal stricture or pyloric stenosis; however, after the CMV is reactivated, the long-acting nature of TA may delay the healing process. CMV is recognized an important pathogen of severe infections in immunocompromised hosts, and causes CMV mononucleosis with multi-organ involvements. In general, CMV-related ulcers might make multiple and deeper lesions in digestive tract in upper gastrointestinal endoscopy[15].

In this case, the ulcer located at the center of the post-ESD artificial ulcer floor which was limited and local area under focal immunosuppressive condition by TA.

In conclusion, the local steroid injection might be an etiological factor for CMV-associated ulcers. We advise that clinicians observe the ulcer floor following TA injections and quickly treat CMV-associated ulcers with ganciclovir if needed.

ACKNOWLEDGMENTS

We thank Professor Yasuyuki Suzuki for technical and editorial assistance.

REFERENCES

1 **Ono H**, Hasuike N, Inui T, Takizawa K, Ikehara H, Yamaguchi Y, Otake Y, Matsubayashi H. Usefulness of a novel electrosurgical knife, the insulation-tipped diathermic knife-2, for endoscopic submucosal dissection of early gastric cancer. *Gastric Cancer* 2008; **11**: 47-52 [PMID: 18373177 DOI: 10.1007/s10120-008-0452-0]

2 **Fujishiro M**, Yahagi N, Kakushima N, Kodashima S, Muraki Y, Ono S, Yamamichi N, Tateishi A, Shimizu Y, Oka M, Ogura K, Kawabe T, Ichinose M, Omata M. Endoscopic submucosal dissection of esophageal squamous cell neoplasms. *Clin Gastroenterol Hepatol* 2006; **4**: 688-694 [PMID: 16713746 DOI: 10.1007/s10120-008-0489-0]

3 **Ono H**, Kondo H, Gotoda T, Shirao K, Yamaguchi H, Saito D, Hosokawa K, Shimoda T, Yoshida S. Endoscopic mucosal resection for treatment of early gastric cancer. *Gut* 2001; **48**: 225-229 [PMID: 11156645 DOI: 10.1136/gut.48.2.225]

4 **Gotoda T**, Yamamoto H, Soetikno RM. Endoscopic submuco-

sal dissection of early gastric cancer. *J Gastroenterol* 2006; **41**: 929-942 [PMID: 17096062 DOI: 10.1007/s00535-006-1954-3]
5 **Gotoda T**. A large endoscopic resection by endoscopic submucosal dissection procedure for early gastric cancer. *Clin Gastroenterol Hepatol* 2005; **3**: S71-S73 [PMID: 16013003 DOI: 10.1016/S1542-3565(05)00251-X]
6 **Yoshino J,** Nakazawa S, Wakabayashi T. Endoscopic change of intractable ulcer after eight-weeks treatment. *Stomach and Intestine* 1993; **28:** 263-239
7 **Takizawa K**, Oda I, Gotoda T, Yokoi C, Matsuda T, Saito Y, Saito D, Ono H. Routine coagulation of visible vessels may prevent delayed bleeding after endoscopic submucosal dissection--an analysis of risk factors. *Endoscopy* 2008; **40**: 179-183 [PMID: 18322872 DOI: 10.1055/s-2007-995530]
8 **Saito Y**, Uraoka T, Matsuda T, Emura F, Ikehara H, Mashimo Y, Kikuchi T, Fu KI, Sano Y, Saito D. Endoscopic treatment of large superficial colorectal tumors: a case series of 200 endoscopic submucosal dissections (with video). *Gastrointest Endosc* 2007; **66**: 966-973 [PMID: 17524403 DOI: 10.1111/j.1443-1661.2005.00459.x]
9 **Fujiwara S**, Morita Y, Toyonaga T, Kawakami F, Itoh T, Yoshida M, Kutsumi H, Azuma T. A randomized controlled trial of rebamipide plus rabeprazole for the healing of artificial ulcers after endoscopic submucosal dissection. *J Gastroenterol* 2011; **46**: 595-602 [PMID: 21359522 DOI: 10.1007/s00535-011-0372-3]
10 **Yamaguchi N**, Isomoto H, Nakayama T, Hayashi T, Nishiyama H, Ohnita K, Takeshima F, Shikuwa S, Kohno S, Nakao K. Usefulness of oral prednisolone in the treatment of esophageal stricture after endoscopic submucosal dissection for superficial esophageal squamous cell carcinoma. *Gastrointest Endosc* 2011; **73**: 1115-1121 [PMID: 21492854 DOI: 10.1016/j.gie.2011.02.005]
11 **Hashimoto S**, Kobayashi M, Takeuchi M, Sato Y, Narisawa R, Aoyagi Y. The efficacy of endoscopic triamcinolone injection for the prevention of esophageal stricture after endoscopic submucosal dissection. *Gastrointest Endosc* 2011; **74**: 1389-1393 [PMID: 22136782 DOI: 10.1016/j.gie.2011.07.070]
12 **Mori H**, Rafiq K, Kobara H, Fujihara S, Nishiyama N, Kobayashi M, Himoto T, Haba R, Hagiike M, Izuishi K, Okano K, Suzuki Y, Masaki T. Local steroid injection into the artificial ulcer created by endoscopic submucosal dissection for gastric cancer: prevention of gastric deformity. *Endoscopy* 2012; **44**: 641-648 [PMID: 22696191 DOI: 10.1055/s-0032-1309815]
13 **Carrico TJ**, Mehrhof AI, Cohen IK. Biology of wound healing. *Surg Clin North Am* 1984; **64**: 721-733 [PMID: 6382655]
14 **Limaye AP**, Kirby KA, Rubenfeld GD, Leisenring WM, Bulger EM, Neff MJ, Gibran NS, Huang ML, Santo Hayes TK, Corey L, Boeckh M. Cytomegalovirus reactivation in critically ill immunocompetent patients. *JAMA* 2008; **300**: 413-422 [PMID: 18647984 DOI: 10.1001/jama.300.4.413]
15 **Heininger A**, Jahn G, Engel C, Notheisen T, Unertl K, Hamprecht K. Human cytomegalovirus infections in nonimmunosuppressed critically ill patients. *Crit Care Med* 2001; **29**: 541-547 [PMID: 11373417 DOI: 10.1097/00003246-200103000-00012]

P- Reviewers Enomoto S, Sugimoto M
S- Editor Wen LL **L- Editor** A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i7.1147

World J Gastroenterol 2013 February 21; 19(7): 1147-1149
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Isolated fever induced by mesalamine treatment

Rita Slim, Joseph Amara, Roy Nasnas, Khalil Honein, Joseph Bou Jaoude, Cesar Yaghi, Fady Daniel, Raymond Sayegh

Rita Slim, Joseph Amara, Khalil Honein, Joseph Bou Jaoude, Cesar Yaghi, Fady Daniel, Raymond Sayegh, Department of Gastroenterology, Hotel Dieu de France University Hospital, Saint Joseph University, Beirut 94010, Lebanon
Roy Nasnas, Department of Infectious Diseases, Hotel Dieu de France University Hospital, Saint Joseph University, Beirut 94010, Lebanon
Author contributions: Slim R and Amara J designed the paper; Nasnas R, Honein K, Jaoude JB, Yaghi C, Daniel F and Sayegh R revised the paper.
Correspondence to: Rita Slim, MD, Department of Gastroenterology, Hotel Dieu de France University Hospital, Saint Joseph University, Beirut 94010, Lebanon. rslimkaram@hotmail.com
Telephone: +961-1-615300 Fax: +961-1-615300
Received: September 23, 2012 Revised: December 17, 2012
Accepted: December 22, 2012
Published online: February 21, 2013

Abstract

Adverse reactions to mesalamine, a treatment used to induce and maintain remission in inflammatory bowel diseases, particularly ulcerative colitis, have been described in the literature as case reports. This case illustrates an unusual adverse reaction. Our patient developed an isolated fever of unexplained etiology, which was found to be related to mesalamine treatment. A 22-year-old patient diagnosed with ulcerative colitis developed a fever with rigors and anorexia 10 d after starting oral mesalamine while his colitis was clinically resolving. Testing revealed no infection. A mesalamine-induced fever was considered, and treatment was stopped, which led to spontaneous resolution of the fever. The diagnosis was confirmed by reintroducing the mesalamine. One year later, this side effect was noticed again in the same patient after he was administered topical mesalamine. This reaction to mesalamine seems to be idiosyncratic, and the mechanism that induces fever remains unclear. Fever encountered in the course of a mesalamine treatment in ulcerative colitis must be considered a mesalamine-induced fever when it cannot be explained by the disease activity, an associated extraintestinal manifestation, or an infectious etiology.

© 2013 Baishideng. All rights reserved.

Key words: Mesalamine; 5-aminosalicylic acid; Side effects; Adverse reactions; Fever

Slim R, Amara J, Nasnas R, Honein K, Jaoude JB, Yaghi C, Daniel F, Sayegh R. Isolated fever induced by mesalamine treatment. *World J Gastroenterol* 2013; 19(7): 1147-1149 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i7/1147.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i7.1147

INTRODUCTION

Mesalamine is a well-known treatment for inflammatory bowel diseases (IBDs), particularly ulcerative colitis (UC), in which it is used for remission induction and maintenance therapy. Mesalamine is considered a safe drug, but some side effects have been described in the literature. We report a case of mesalamine-induced isolated fever in a patient with UC.

CASE REPORT

A 22-year-old-man presented with a 1 wk history of apyretic acute bloody diarrhea, which was treated with 500 mg of metronidazole three times a day (TID) without clinical improvement. At this stage, flexible sigmoidoscopy showed diffuse hyperemic mucosa with few erosions. The biopsies indicated infectious colitis. The blood analysis and stool exam were normal. Treatment with 500 mg of ciprofloxacin twice a day and 500 mg of metronidazole TID was prescribed. Ten days later, the patient's clinical condition worsened, with passage of 5 stools per day with mucus and blood. A complete colonoscopy was performed, which demonstrated diffuse pancolitis with superficial ulcerations, loss of vascular

pattern and easy contact bleeding from the mucosa. The terminal ileum was normal. The colonic biopsies were consistent with UC. The blood analysis at this time was also unremarkable except for the C reactive protein (CRP) level, which was 17 mg/dL. Treatment with 4 g of pH-dependent release mesalamine (Asacol®) was started, and a significant clinical improvement was noted, with resolution of the bloody diarrhea and passage of 2 normal stools per day. Ten days later, while the patient was doing well, he presented with a 39 ℃ fever with rigor, myalgia and anorexia. Upon admission, 6 d after the fever onset, his physical examination was normal.

A blood analysis showed a hemoglobin level of 12.2 g/dL, a white blood cell count of 8600/mL (of which 77% were granulocytes), a platelet count of 264 000/mL, and a CRP level of 104 mg/dL, with normal levels of liver and pancreatic enzymes. Serologies for typhoid fever, brucellosis, parvovirus, Epstein Barr virus and cytomegalovirus were negative. Stool, blood and urine cultures were also negative. The tuberculosis skin test was normal as well as the chest X-ray and cardiac ultrasound. The mesalamine was discontinued, and the fever was treated symptomatically with paracetamol. Two days later, the patient's clinical condition improved, with the disappearance of the febrile syndrome and a decrease in the CRP level to 62 mg/dL. After discussing the matter with the patient and because a reactivation of the colitis was feared, particularly after the rapid remission that was obtained with the use of 5-aminosalicylic acid (5-ASA), we decided to reintroduce another time-dependent release mesalamine (Pentasa®) under medical surveillance. After administering the second dose, the patient presented with a 38.5 ℃ fever with rigors. Mesalamine was stopped, and the fever resolved the following day without any intervention. The diagnosis of fever related to mesalamine was confirmed. Two days later, the patient presented with bloody diarrhea, and a treatment with 40 mg of prednisone and 150 mg of azathioprine was started, with rapid improvement. The patient was discharged in good condition. Two months later, the prednisone was stopped after tapering, and the patient was maintained in clinical remission with azathioprine. Eight months later, the patient presented with a flare-up of his colitis. He consulted another gastroenterologist, who treated him with suppositories of Asacol because the rectosigmoidoscopy showed an active proctitis. Two days later, the patient presented with the same symptoms encountered with oral mesalamine (*i.e.*, fever and rigors), which spontaneously resolved after stopping the topical treatment.

DISCUSSION

The 5-ASA agents are known to be safe and remain among the first-line approaches for inducing remission and preventing relapse in UC[1]. These benefits of 5-ASA agents are less impressive in the treatment of mild to moderate Crohn's disease. Their possible role in chemoprevention of colon cancer strengthens the indication for the long-term use of 5-ASA[2].

The efficacy of sulfasalazine in UC has been known since 1940, but the use of this drug was problematic because of its side effects, either dose related or idiosyncratic reactions, such as headache, dyspepsia, nausea, hypersensitivity rash, hemolytic or aplastic anemia, and pulmonary or hepatic dysfunction[3]. The identification in sulfasalazine of the 5-ASA moiety, which is responsible for the therapeutic effects, led to the development of new drugs that delivered 5-ASA directly to the colonic mucosa with fewer side effects.

These new drugs included mesalamine or 5-ASA itself, olsalazine (Dipentum; a dimer of 5-ASA) and balsalazide (Colazal or Colazide; a pro-drug of 5-ASA). In their systematic review of the safety of 5-ASA-based agents, Loftus *et al*[3] concluded that all of these agents appeared to be safe and that the fraction of patients experiencing adverse events was similar to that seen in patients treated with placebo.

However, severe adverse events encountered with mesalamine therapy have been reported as isolated cases. A French pharmacovigilance study of mesalamine microgranules (Pentasa) reported between 6.6 and 9.0 adverse events per million treatment days over a 2-year period, including cases of pancreatitis, hepatitis, pericarditis and hematological disturbances[4,5]. Other side effects have also been reported, such as worsening colitis, renal toxicity (interstitial nephritis and nephrotic syndrome), pulmonary toxicity (interstitial lung disease and fibrosis, bronchiolitis obliterans, pulmonary granulomatosis and eosinophilic pleural effusion) hair loss and Stevens-Johnson syndrome[6]. Some of these drug-related complications must be distinguished from the extraintestinal manifestations of IBD, and they may arise independently of disease activity. The causal relationship between mesalamine administration and the occurrence of the adverse event is established by the appearance of the symptoms after the administration of the 5-ASA compound, the rapid resolution of the symptoms after discontinuation and the reoccurrence of the same symptoms if the treatment is restarted[6].

The mechanism by which mesalamine induces side effects remains unclear. In some cases, the occurrence of a complication seems to be dose related, whereas in other cases it appears to be more of an idiosyncratic reaction[7]. Moreover, the pathogenesis of the fever induced by mesalamine remains unknown.

In cases of acute pancreatitis, the suggested mechanism is an increased permeability of the pancreatic duct directly due to the effects of salicylic acid[8]. In cases of mesalamine-induced exacerbation of UC[9] or myocarditis[10], eosinophilic infiltration of the colon and the myocardium point to an allergic drug reaction.

Mesalamine-related complications are known to occur with oral and topical preparations. Some reports have claimed an absence of cross reactivity among different 5-ASA-based drugs[11].

Our patient presented with an isolated fever while his bloody diarrhea was improving. Mesalamine-induced isolated fever has been reported by Gonzalo *et al*[12] and the causal relationship was proved by a placebo-controlled challenge test and a protocol of desensitization, which was realized successfully. We realized, under medical surveillance, a challenge test with a different 5-ASA drug, and the fever reappeared earlier than the first time (1 d *vs* 10 d). On both occasions, no other causes of fever could be identified, and the fever disappeared spontaneously after stopping the drug. Moreover, reintroducing the mesalamine in suppository form one year later led to the same side effect: an isolated fever that also resolved after stopping the treatment. Although mesalamine seemed to be efficacious, we did not choose to adopt a desensitization protocol at that time because the patient feared a more severe reaction, and we needed another strategy to treat the reactivated colitis.

In conclusion, mesalamine appears to be safe and to achieve good results in IBD patients, particularly those with UC. Side effects of 5-ASA-based drugs are rare, but they should be distinguished from extraintestinal manifestations and should be suspected when the symptoms resolve with the discontinuation of the treatment and reappear with its reintroduction. An isolated fever can reappear on multiple occasions after the reintroduction of various formulations of mesalamine, as illustrated in our case report.

REFERENCES

1 **Ford AC**, Achkar JP, Khan KJ, Kane SV, Talley NJ, Marshall JK, Moayyedi P. Efficacy of 5-aminosalicylates in ulcerative colitis: systematic review and meta-analysis. *Am J Gastroenterol* 2011; **106**: 601-616 [PMID: 21407188 DOI: 10.1038/ajg.2011.67]

2 **Cheng Y,** Desreumaux P. 5-aminosalicylic acid is an attractive candidate agent for chemoprevention of colon cancer in patients with inflammatory bowel disease. *World J Gastroenterol* 2005; **11:** 309-314 [PMID: 156377333]

3 **Loftus EV**, Kane SV, Bjorkman D. Systematic review: short-term adverse effects of 5-aminosalicylic acid agents in the treatment of ulcerative colitis. *Aliment Pharmacol Ther* 2004; **19**: 179-189 [PMID: 14723609]

4 **Marteau P**, Nelet F, Le Lu M, Devaux C. Adverse events in patients treated with 5-aminosalicyclic acid: 1993-1994 pharmacovigilance report for Pentasa in France. *Aliment Pharmacol Ther* 1996; **10**: 949-956 [PMID: 8971293 DOI: 10.1046/j.1365-2036.1996.92264000.x]

5 **Rogler G**. Gastrointestinal and liver adverse effects of drugs used for treating IBD. *Best Pract Res Clin Gastroenterol* 2010; **24**: 157-165 [PMID: 20227029 DOI: 10.1016/j.bpg.2009.10.011]

6 **Schroeder KW**. Is mesalamine safe? *Gastroenterol Hepatol (NY)* 2007; **3**: 878-879 [PMID: 21960801]

7 **Sossai P**, Cappellato MG, Stefani S. Can a drug-induced pulmonary hypersensitivity reaction be dose-dependent? A case with mesalamine. *Mt Sinai J Med* 2001; **68**: 389-395 [PMID: 11687867]

8 **Arai Y**, Arihiro S, Ide D, Odagi I, Itagaki M, Komoike N, Nakao Y, Takakura K, Saruta M, Matsuoka M, Kato T, Tajiri H. Acute Pancreatitis due to pH-Dependent Mesalazine That Occurred in the Course of Ulcerative Colitis. *Case Rep Gastroenterol* 2011; **5**: 610-616 [PMID: 22110423 DOI: 10.1159/000333605]

9 **Gupta MK**, Pollack S, Hutchings JJ. Mesalamine induced symptom exacerbation of ulcerative colitis: Case report and brief discussion. *World J Gastrointest Pharmacol Ther* 2010; **1**: 132-134 [PMID: 21577308 DOI: 10.4292/wjgpt.v1.i6.132]

10 **Stelts S**, Taylor MH, Nappi J, Van Bakel AB. Mesalamine-associated hypersensitivity myocarditis in ulcerative colitis. *Ann Pharmacother* 2008; **42**: 904-905 [PMID: 18430794 DOI: 10.1345/aph.1K288]

11 **Kung SJ**, Choudhary C, McGeady SJ, Cohn JR. Lack of cross-reactivity between 5-aminosalicylic acid-based drugs: a case report and review of the literature. *Ann Allergy Asthma Immunol* 2006; **97**: 284-287 [PMID: 17042131]

12 **Gonzalo MA**, Alcalde MM, García JM, Alvarado MI, Fernández L. Desensitization after fever induced by mesalazine. *Allergy* 1999; **54**: 1224-1225 [PMID: 10604561 DOI: 10.1034/j.1398-9995.1999.00298.x]

P- Reviewers Slomiany BL, Kane SV
S- Editor Huang XZ **L- Editor** A **E- Editor** Xiong L

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i7.1150

World J Gastroenterol 2013 February 21; 19(7): 1150-1151
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

Management of duodenal ulcer bleeding resistant to endoscopy: Surgery is dead!

Romaric Loffroy

Romaric Loffroy, Cardiovascular Imaging Unit, Department of Vascular and Interventional Radiology, Bocage Teaching Hospital, University of Dijon School of Medicine, 21079 Dijon Cedex, France
Author contributions: Loffroy R wrote this letter.
Correspondence to: Romaric Loffroy, MD, PhD, Cardiovascular Imaging Unit, Department of Vascular and Interventional Radiology, Bocage Teaching Hospital, University of Dijon School of Medicine, 14 Rue Paul Gaffarel, BP 77908, 21079 Dijon Cedex, France. romaric.loffroy@chu-dijon.fr
Telephone: +33-380-293677 Fax: +33-380-293455
Received: November 23, 2012 Revised: December 17, 2012
Accepted: January 17, 2013
Published online: February 21, 2013

Abstract

Acute massive duodenal bleeding is one of the most frequent complications of peptic ulcer disease. Endoscopy is the first-line method for diagnosing and treating actively bleeding peptic ulcers because its success rate is high. Of the small group of patients whose bleeding fails to respond to endoscopic therapy, increasingly the majority is referred for embolotherapy. Indeed, advances in catheter-based techniques and newer embolic agents, as well as recognition of the effectiveness of minimally invasive treatment options, have expanded the role of interventional radiology in the management of hemorrhage from peptic ulcers over the past decade. Embolization may be effective for even the most gravely ill patients for whom surgery is not a viable option, even when extravasation is not visualized by angiography. However, it seems that careful selection of the embolic agents according to the bleeding vessel may play a role in a successful outcome. The role of the surgeon in this clinical sphere is dramatically diminishing and will certainly continue to diminish in ensuing years, surgery being typically reserved for patients whose bleeding failed to respond all previous treatments. Such a setting has become extremely rare.

© 2013 Baishideng. All rights reserved.

Key words: Massive hemorrhage; Duodenal ulcer; Angiography; Transcatheter embolization; Surgery

Loffroy R. Management of duodenal ulcer bleeding resistant to endoscopy: Surgery is dead! *World J Gastroenterol* 2013; 19(7): 1150-1151 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i7/1150.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i7.1150

TO THE EDITOR

We read with great interest the recent article by Wang *et al*[1] published in the September issue of the *World Journal of Gastroenterology* evaluating the efficacy and safety of emergency transcatheter arterial embolization (TAE) for patients with acute massive duodenal ulcer hemorrhage. We have several comments and questions.

Transcatheter embolization is now accepted as the salvage treatment of choice for acute bleeding from gastroduodenal ulcers. Many published studies have confirmed the feasibility of this approach and the high technical and clinical success rates, ranging from 91% to 100% and from 63% to 100%, respectively, in all case-series including more than 10 patients over the last decade[2,3].

First, we are surprised in the present study that 19 (65.5%) of the 29 patients had no endoscopic hemostasis prior to TAE. In our experience, endoscopic therapy remains the first treatment modality in the management of bleeding peptic ulcers, even in those presenting with massive bleeding. On the other hand, it seems that the authors performed TAE in the gastroduodenal artery territory in 3 patients who did not undergo preliminary endoscopy and for whom angiography was negative. Does it mean that TAE was carried out in these patients without neither endoscopic nor angiographic data? Could the authors clarify this point? Indeed, several pre-

vious studies found that empiric embolization based on endoscopic findings, in the absence of contrast extravasation, was helpful in achieving bleeding control, with no difference according to whether angiography identified the bleeding site[4,5]. However, accurate endoscopic localization of the bleeding site is a prerequisite to allow empiric embolization for angiographically negative upper gastrointestinal bleeding.

Second, we would like to congratulate the authors on their high clinical success rate of 93% (27 of the 29 patients). However, we are surprised that these results were obtained with the use of sponge particles as the only embolic agent. Although the influence of the type of embolic agent on the clinical outcome is controversial, several authors reported a high rate of bleeding recurrence when gelfoam was used alone[6,7], whereas the clinical success was relatively high in recent series in which glue was used as the only embolic agent[8,9]. In addition, two studies demonstrated a statistically significant association between the use of coils as the only embolic agent and greater rebleeding rates[2,10]. On the other hand, good results were reported with the combination of gelatin sponge and coils[2,11]. Based on our experience and the literature, we do not recommend the use of coils alone but in combination with gelfoam or glue, when using the sandwich technique in areas with rich collaterals like the gastroduodenal artery territory[2,10]. It allows a faster and better hemostasis, especially in patients with coagulopathy.

In addition, the normal collateral pathways after a successful embolization should be systematically checked to avoid retrograde filling through anastomoses as the inferior pancreaticoduodenal artery (IPDA) from the superior mesenteric artery in order to maximize results. Indeed, one explanation for good clinical results in this study might be the systematic use of this technique. It may be worthwhile for readers to know the number of patients in whom additional TAE of the IPDA was performed here. Another plausible explanation for high clinical success rate in this study could be the young age of the study population (36 years), without comorbidities. We know that underlying conditions can contribute to a poor outcome.

In conclusion, we agree with the authors about the safety and efficacy of TAE for the treatment of acute hemorrhage from duodenal ulcers. However, angiography should be performed only after failure of endoscopic hemostasis in such a setting. In most cases, embolization obviates the need for surgery and is associated with lower complications and mortality rates than surgical hemostasis. Although prospective studies are needed to compare these management strategies, the available data suggest that TAE is a good alternative to surgery and could be considered the salvage treatment of choice after failed endoscopic treatment. The role of the surgeon in this clinical sphere will certainly continue to diminish in ensuing years.

REFERENCES

1 **Wang YL**, Cheng YS, Liu LZ, He ZH, Ding KH. Emergency transcatheter arterial embolization for patients with acute massive duodenal ulcer hemorrhage. *World J Gastroenterol* 2012; **18**: 4765-4770 [PMID: 23002347 DOI: 10.3748/wjg.v18.i34.4765]

2 **Loffroy R**, Guiu B, D'Athis P, Mezzetta L, Gagnaire A, Jouve JL, Ortega-Deballon P, Cheynel N, Cercueil JP, Krausé D. Arterial embolotherapy for endoscopically unmanageable acute gastroduodenal hemorrhage: predictors of early rebleeding. *Clin Gastroenterol Hepatol* 2009; **7**: 515-523 [PMID: 19418601 DOI: 10.1016/j.cgh.2009.02.003]

3 **Loffroy R**, Guiu B. Role of transcatheter arterial embolization for massive bleeding from gastroduodenal ulcers. *World J Gastroenterol* 2009; **15**: 5889-5897 [PMID: 20014452 DOI: 10.3748/wjg.15.5889]

4 **Ichiro I**, Shushi H, Akihiko I, Yasuhiko I, Yasuyuki Y. Empiric transcatheter arterial embolization for massive bleeding from duodenal ulcers: efficacy and complications. *J Vasc Interv Radiol* 2011; **22**: 911-916 [PMID: 21571546 DOI: 10.1016/j.jvir.2011.03.001]

5 **Loffroy R**, Lin M, Thompson C, Harsha A, Rao P. A comparison of the results of arterial embolization for bleeding and non-bleeding gastroduodenal ulcers. *Acta Radiol* 2011; **52**: 1076-1082 [PMID: 22006986 DOI: 10.1258/ar.2011.110344]

6 **Encarnacion CE**, Kadir S, Beam CA, Payne CS. Gastrointestinal bleeding: treatment with gastrointestinal arterial embolization. *Radiology* 1992; **183**: 505-508 [PMID: 1561358]

7 **Lang EK**. Transcatheter embolization in management of hemorrhage from duodenal ulcer: long-term results and complications. *Radiology* 1992; **182**: 703-707 [PMID: 1535883]

8 **Jae HJ**, Chung JW, Jung AY, Lee W, Park JH. Transcatheter arterial embolization of nonvariceal upper gastrointestinal bleeding with N-butyl cyanoacrylate. *Korean J Radiol* 2007; **8**: 48-56 [PMID: 17277563 DOI: 10.3348/kjr.2007.8.1.48]

9 **Lee CW**, Liu KL, Wang HP, Chen SJ, Tsang YM, Liu HM. Transcatheter arterial embolization of acute upper gastrointestinal tract bleeding with N-butyl-2-cyanoacrylate. *J Vasc Interv Radiol* 2007; **18**: 209-216 [PMID: 17327553 DOI: 10.1016/j.jvir.2006.12.003]

10 **Aina R**, Oliva VL, Therasse E, Perreault P, Bui BT, Dufresne MP, Soulez G. Arterial embolotherapy for upper gastrointestinal hemorrhage: outcome assessment. *J Vasc Interv Radiol* 2001; **12**: 195-200 [PMID: 11265883 DOI: 10.1016/S1051-0443(07)61825-9]

11 **Morris DC**, Nichols DM, Connell DG, Burhenne HJ. Embolization of the left gastric artery in the absence of angiographic extravasation. *Cardiovasc Intervent Radiol* 1986; **9**: 195-198 [PMID: 3094950 DOI: 10.1007/BF02577940]

P- Reviewers Reddy ND, Kouraklis G
S- Editor Wen LL **L- Editor** A **E- Editor** Xiong L



ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

World Journal of *Gastroenterology*

World J Gastroenterol 2013 February 28; 19(8): 1152-1332



Baishideng Publishing Group
www.wjgnet.com

World Journal of Gastroenterology

Contents

Weekly Volume 19 Number 8 February 28, 2013

EDITORIAL

1152 Staging colorectal cancer with the TNM 7th: The presumption of innocence when applying the M category
Puppa G, Poston G, Jess P, Nash GF, Coenegrachts K, Stang A

TOPIC HIGHLIGHT

1158 From fatty liver to fibrosis: A tale of "second hit"
Basaranoglu M, Basaranoglu G, Sentürk H

1166 Fructose as a key player in the development of fatty liver disease
Basaranoglu M, Basaranoglu G, Sabuncu T, Sentürk H

REVIEW

1173 Histone deacetylase inhibitors and pancreatic cancer: Are there any promising clinical trials?
Koutsounas I, Giaginis C, Theocharis S

1182 DNA and histone methylation in gastric carcinogenesis
Calcagno DQ, Gigek CO, Chen ES, Burbano RR, Smith MAC

MINIREVIEWS

1193 Management of small hepatocellular carcinoma in cirrhosis: Focus on portal hypertension
Hernandez-Gea V, Turon F, Berzigotti A, Villanueva A

ORIGINAL ARTICLE

1200 Involvement of parasympathetic pelvic efferent pathway in psychological stress-induced defecation
Suda K, Setoyama H, Nanno M, Matsumoto S, Kawai M

1210 Inhibition of pacemaker activity in interstitial cells of Cajal by LPS *via* NF-κB and MAP kinase
Zuo DC, Choi S, Shahi PK, Kim MY, Park CG, Kim YD, Lee J, Chang IY, So I, Jun JY

1219 NAFLD fibrosis score: A prognostic predictor for mortality and liver complications among NAFLD patients
Treeprasertsuk S, Björnsson E, Enders F, Suwanwalaikorn S, Lindor KD

1230 *Schistosoma japonicum* egg antigen up-regulates fibrogenesis and inhibits proliferation in primary hepatic stellate cells in a concentration-dependent manner
Liu P, Wang M, Lu XD, Zhang SJ, Tang WX

1239 Effect of silencing of high mobility group A2 gene on gastric cancer MKN-45 cells
Wei CH, Wei LX, Lai MY, Chen JZ, Mo XJ

BRIEF ARTICLE

1247 Predictors of colorectal cancer testing using the California Health Inventory Survey
Modiri A, Makipour K, Gomez J, Friedenberg F

1256 Magnetic resonance imaging: Is there a role in clinical management for acute ischemic colitis?
Mazzei MA, Guerrini S, Cioffi Squitieri N, Imbriaco G, Chieca R, Civitelli S, Savelli V, Mazzei FG, Volterrani L

1264 Ensure preparation and capsule endoscopy: A two-center prospective study
Niv E, Ovadia B, Ron Y, Santo E, Mahajna E, Halpern Z, Fireman Z

1271 Effect of alternative antibiotics in treatment of cefotaxime resistant spontaneous bacterial peritonitis
Badawy AA, Zaher TI, Sharaf SM, Emara MH, Shaheen NE, Aly TF

1278 Radiofrequency ablation during continuous saline infusion can extend ablation margins
Ishikawa T, Kubota T, Horigome R, Kimura N, Honda H, Iwanaga A, Seki K, Honma T, Yoshida T

1283 Ethnicity association of *Helicobacter pylori* virulence genotype and metronidazole susceptibility
Alfizah H, Rukman AH, Norazah A, Hamizah R, Ramelah M

1292 Abnormal splenic artery diameter/hepatic artery diameter ratio in cirrhosis-induced portal hypertension
Zeng DB, Dai CZ, Lu SC, He N, Wang W, Li HJ

1299 Ileal pouch anal anastomosis with modified double-stapled mucosectomy-the experience in China
Zhang YJ, Han Y, Lin MB, He YG, Zhang HB, Yin L, Huang L

1306 Tumor suppressor function of ezrin-radixin-moesin-binding phosphoprotein-50 through β-catenin/E-cadherin pathway in human hepatocellular cancer
Peng XL, Ji MY, Yang ZR, Song J, Dong WG

CASE REPORT

1314 Gastric adenocarcinoma arising in gastritis cystica profunda presenting with selective loss of KCNE2 expression
Kuwahara N, Kitazawa R, Fujiishi K, Nagai Y, Haraguchi R, Kitazawa S

1318 Hepatitis B reactivation in chronic myeloid leukemia patients receiving tyrosine kinase inhibitor
Lai GM, Yan SL, Chang CS, Tsai CY

1322 Multiple endocrine neoplasia type 1 with upper gastrointestinal hemorrhage and perforation: A case report and review
Lu YY, Zhu F, Jing DD, Wu XN, Lu LG, Zhou GQ, Wang XP

1327 Glomus tumor in the stomach: Computed tomography and endoscopic ultrasound findings
Tang M, Hou J, Wu D, Han XY, Zeng MS, Yao XZ

1330 Phlegmonous gastritis after esophagectomy: A case report
Fan JQ, Liu DR, Li C, Chen G

World Journal of Gastroenterology
Volume 19 Number 8 February 28, 2013

Contents

APPENDIX	I-VI	Instructions to authors
ABOUT COVER		Editorial Board Member of *World Journal of Gastroenterology*, Metin Basaranoglu, MD, Gastroenterology and Hepatology, Bezmialem Vakif University, Istanbul 34400, Turkey
AIMS AND SCOPE		*World Journal of Gastroenterology* (*World J Gastroenterol*, *WJG*, print ISSN 1007-9327, online ISSN 2219-2840, DOI: 10.3748) is a peer-reviewed open access journal. *WJG* was established on October 1, 1995. It is published weekly on the 7th, 14th, 21st, and 28th each month. The *WJG* Editorial Board consists of 1352 experts in gastroenterology and hepatology from 64 countries. The primary task of *WJG* is to rapidly publish high-quality original articles, reviews, and commentaries in the fields of gastroenterology, hepatology, gastrointestinal endoscopy, gastrointestinal surgery, hepatobiliary surgery, gastrointestinal oncology, gastrointestinal radiation oncology, gastrointestinal imaging, gastrointestinal interventional therapy, gastrointestinal infectious diseases, gastrointestinal pharmacology, gastrointestinal pathophysiology, gastrointestinal pathology, evidence-based medicine in gastroenterology, pancreatology, gastrointestinal laboratory medicine, gastrointestinal molecular biology, gastrointestinal immunology, gastrointestinal microbiology, gastrointestinal genetics, gastrointestinal translational medicine, gastrointestinal diagnostics, and gastrointestinal therapeutics. *WJG* is dedicated to become an influential and prestigious journal in gastroenterology and hepatology, to promote the development of above disciplines, and to improve the diagnostic and therapeutic skill and expertise of clinicians.
INDEXING/ABSTRACTING		*World Journal of Gastroenterology* is now indexed in Current Contents®/Clinical Medicine, Science Citation Index Expanded (also known as SciSearch®), Journal Citation Reports®, Index Medicus, MEDLINE, PubMed, PubMed Central, Digital Object Identifier, and Directory of Open Access Journals. ISI, Journal Citation Reports®, Gastroenterology and Hepatology, 2011 Impact Factor: 2.471 (32/74); Total Cites: 16951 (7/74); Current Articles: 677 (1/74); and Eigenfactor® Score: 0.06035 (5/74).
FLYLEAF	I-IX	Editorial Board

EDITORS FOR THIS ISSUE

Responsible Assistant Editor: *Shuai Ma*
Responsible Science Editor: *Huan-Huan Zhai*
Responsible Electronic Editor: *Dan-Ni Zhang*
Proofing Editorial Office Director: *Xiu-Xia Song*
Proofing Editor-in-Chief: *Lian-Sheng Ma*

NAME OF JOURNAL
World Journal of Gastroenterology

ISSN
ISSN 1007-9327 (print)
ISSN 2219-2840 (online)

LAUNCH DATE
October 1, 1995

FREQUENCY
Weekly

EDITOR-IN-CHIEF
Ferruccio Bonino, MD, PhD, Professor of Gastroenterology, Director of Liver and Digestive Disease Division, Department of Internal Medicine, University of Pisa, Director of General Medicine 2 Unit University Hospital of Pisa, Via Roma 67, 56124 Pisa, Italy

Myung-Hwan Kim, MD, PhD, Professor, Head, Department of Gastroenterology, Director, Center for Biliary Diseases, University of Ulsan College of Medicine, Asan Medical Center, 388-1 Pungnap-2dong, Songpa-gu, Seoul 138-736, South Korea

Kjell Öberg, MD, PhD, Professor, Department of Endocrine Oncology, Uppsala University Hospital, SE-751 85 Uppsala, Sweden

Matt D Rutter, MBBS, MD, FRCP, Consultant Gastroenterologist, Senior Lecturer, Director, Tees Bowel Cancer Screening Centre, University Hospital of North Tees, Durham University, Stockton-on-Tees, Cleveland TS19 8PE, United Kingdom

Andrzej S Tarnawski, MD, PhD, DSc (Med), Professor of Medicine, Chief Gastroenterology, VA Long Beach Health Care System, University of California, Irvine, CA, 5901 E. Seventh Str., Long Beach, CA 90822, United States

EDITORIAL OFFICE
Jin-Lei Wang, Director
Xiu-Xia Song, Vice Director
World Journal of Gastroenterology
Room 903, Building D, Ocean International Center,
No. 62 Dongsihuan Zhonglu, Chaoyang District,
Beijing 100025, China
Telephone: +86-10-59080039
Fax: +86-10-85381893
E-mail: wjg@wjgnet.com
http://www.wjgnet.com

PUBLISHER
Baishideng Publishing Group Co., Limited
Flat C, 23/F., Lucky Plaza,
315-321 Lockhart Road, Wan Chai, Hong Kong, China
Fax: +852-65557188
Telephone: +852-31779906
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com

PUBLICATION DATE
February 28, 2013

COPYRIGHT
© 2013 Baishideng. Articles published by this Open-Access journal are distributed under the terms of the Creative Commons Attribution Non-commercial License, which permits use, distribution, and reproduction in any medium, provided the original work is properly cited, the use is non commercial and is otherwise in compliance with the license.

SPECIAL STATEMENT
All articles published in this journal represent the viewpoints of the authors except where indicated otherwise.

INSTRUCTIONS TO AUTHORS
Full instructions are available online at http://www.wjgnet.com/1007-9327/g_info_20100315215714.htm

ONLINE SUBMISSION
http://www.wjgnet.com/esps/

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i8.1152

World J Gastroenterol 2013 February 28; 19(8): 1152-1157
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

EDITORIAL

Staging colorectal cancer with the TNM 7th: The presumption of innocence when applying the M category

Giacomo Puppa, Graeme Poston, Per Jess, Guy F Nash, Kenneth Coenegrachts, Axel Stang

Giacomo Puppa, Service de Pathologie Clinique, Hôpitaux Universitaires de Genève, 1211 Geneva, Switzerland
Graeme Poston, Department of Surgery, Aintree University Hospitals, Liverpool L9 7AL, United Kingdom
Per Jess, Department of Surgery, Roskilde Hospital, University of Copenhagen, 2200 Copenhagen, Denmark
Guy F Nash, Department of Colorectal Surgery, Poole General Hospital, Poole BH15 2JB, United Kingdom
Kenneth Coenegrachts, Department of Radiology, AZ St.-Jan Brugge-Oostende AV, B-8000 Bruges, Belgium
Axel Stang, Department of Oncology, Hematology and Palliative Care, Asklepios Hospital Hamburg-Barmbek, 22291 Hamburg, Germany
Author contributions: Puppa G and Stang A designed the study and wrote the paper; Puppa G and Coenegrachts K provided the images from their departments; Poston G, Jess P, Nash GF and Coenegrachts K revised the text for important intellectual content.
Correspondence to: Giacomo Puppa, MD, PhD, Service de Pathologie Clinique, Hôpitaux Universitaires de Genève, 1, rue Michel Servet, 1211 Genève,
Switzerland. giacomo.puppa@hcuge.ch
Telephone: +41-22-3724956 Fax: +41-22-3724924
Received: June 25, 2012 Revised: August 18, 2012
Accepted: September 19, 2012
Published online: February 28, 2013

Abstract

One of the main changes of the current TNM-7 is the elimination of the category MX, since it has been a source of ambiguity and misinterpretation, especially by pathologists. Therefore the ultimate staging would be better performed by the patient's clinician who can classify the disease M0 (no distant metastasis) or M1 (presence of distant metastasis), having access to the completeness of data resulting from clinical examination, imaging workup and pathology report. However this important change doesn't take into account the diagnostic value and the challenge of small indeterminate visceral lesions encountered, in particular, during radiological staging of patients with colorectal cancer. In this article the diagnosis of these lesions with multiple imaging modalities, their frequency, significance and relevance to staging and disease management are described in a multidisciplinary way. In particular the interplay between clinical, radiological and pathological staging, which are usually conducted independently, is discussed. The integrated approach shows that there are both advantages and disadvantages to abandoning the MX category. To avoid ambiguity arising both by applying and interpreting MX category for stage assigning, its abandoning seems reasonable. The recognition of the importance of small lesion characterization raises the need for applying a separate category; therefore a proposal for their categorization is put forward. By using the proposed categorization the lack of consideration for indeterminate visceral lesions with the current staging system will be overcome, also optimizing tailored follow-up.

© 2013 Baishideng. All rights reserved.

Key words: Colorectal cancer; Staging; Indeterminate lesions; Imaging; Metastases

Puppa G, Poston G, Jess P, Nash GF, Coenegrachts K, Stang A. Staging colorectal cancer with the TNM 7th: The presumption of innocence when applying the M category. *World J Gastroenterol* 2013; 19(8): 1152-1157 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i8/1152.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i8.1152

INTRODUCTION

Stage Ⅳ colorectal cancer (CRC) is no longer considered a single entity[1] and after several proposals for stratifying it[2,3] the current TNM-7 subdivides the M1 category into M1a (metastasis confined to one organ: liver, lung,

ovary, non-regional lymph node(s) and M1b (metastasis in more than one organ or the peritoneum): accordingly stage Ⅳ is subdivided in IVA (Any T, Any N, M1a) and IVB (Any T, Any N, M1b)[4]. Population-based studies demonstrate that the prognosis for surgically resected stage Ⅳ disease is near identical for that of patients following potentially curative surgery for stage Ⅲ disease[5].

The prognosis and treatment options (including increasingly used multimodality approaches) within the M1 stages depend largely on the extent and distribution of the metastases. Therefore, the current staging will be able to take into account improvements that have been made in surgical techniques for resectable metastases, and the impact of modern chemotherapy on rendering initially unresectable CRC liver metastases operable, while at the same time distinguishing between patients with a chance of cure at presentation and those for whom only palliative treatment is possible[3].

An other major change with the new TNM-7 is that the category "MX: Distant metastasis cannot be assessed" has been eliminated[4].

In this report, the change with the TNM-7 is discussed in a multidisciplinary setting and the diagnostic significance of small indeterminate visceral lesions encountered during radiological staging of patients with colorectal cancer is presented.

The resulting problems, in particular the ambiguities for stage assigning when applying the MX category and the risk of inaccurate staging and follow-up planning because of its elimination, are discussed. A proposal is presented for the categorization of such small, indeterminate visceral lesions.

CHANGE WITH THE TNM FROM THE PATHOLOGIST POINT OF VIEW

There are basically two reasons for MX elimination. Firstly the MX category has been a source of misinterpretation, especially by pathologists (*i.e.*, pMX) who "may assign MX, meaning that they cannot assess distant metastasis"[6]. This is a clinical, not pathological assessment. "If there are no obvious signs of metastasis, M0 or cM0 classifications are appropriate. In other words, once clinically examined, a patient is M0 until proven otherwise"[6] in analogy with the legal right Presumption of Innocence.

The second reason is that assigning the MX category prevents stage grouping by American cancer registries[6].

The elimination of MX allows only two categories, M0 (cM0): no distant metastasis and M1 (cM1 or pM1): distant metastasis[4] utilizing imaging and/or pathological assessment[7].

This goes against the TNM rule of assigning the X category, since the proper use of X is to denote the absence or uncertainty of assigning a given category[8] but importantly these are two different situations.

A similar clinical-pathological context is encountered when applying the R classification (R for residual as descriptor of "tumour remaining in the patient" after primary surgical resection): the assignment of the R classification must be performed "by a designated individual who has access to the complete data"[9].

Despite extensive clinical assessment of treatment results and careful pathologic examination, in some cases the presence of residual tumour cannot be assessed: the RX category applies[9,10].

If the pathologist hasn't access to the preoperative clinico-radiological work-up, he/she can do only a limited staging (pT and pN), in the dark about these clinical data.

As an accurate pathological examination is the prerequisite for a prognostic staging and a tailored patient treatment, the enhanced search for additional prognostic factors applies in particular cases (problems with resection margins assessment; involvement of the peritoneum and/or adjacent structures or organs; suspicion for vascular invasion; few lymph nodes recovered; pericolonic tumour deposits)[11,12].

In this setting, patient characteristics, treatment-related features and disease extension as evaluated both during surgery and preoperatively are all relevant information.

In other words, the stage grouping, as well the risk assessment of patients belonging to the same stage, are better performed in the multidisciplinary setting than considered in isolation.

RADIOLOGICAL STAGING

The major task of radiological staging is the exclusion (cM0) or detection (cM1) of metastases, particularly in the liver and/or the lung. The most frequently used imaging modalities for staging of CRC cancer patients are ultrasonography (US), computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography (PET)/CT (PET/CT)[13]. In the past 10 years, important advances have been made within all four techniques. While some (CT and PET/CT) will give patient specific information regarding abnormal masses and increased tissue metabolic activity throughout the body, others (MRI) will yield organ (liver) specific information and allow characterization of specific abnormalities to differentiate benign from malignant lesions. However, no single modality will diagnose all metastases, and the optimal imaging strategy to classify cM0 or cM1 depends on the clinical context, the organ site investigated, and the individual aims of oncologic care.

With the introduction of multidetector row CT (MDCT) scanning, CT imaging will continue to play the dominant role in the radiological staging of CRC patients[14]. Improved MDCT technologies have resulted in increased CT detection of small (< 1 cm) indeterminate lesions in the lung and/or liver (in around 10%-40% of CT CRC staging examinations)[15-20].

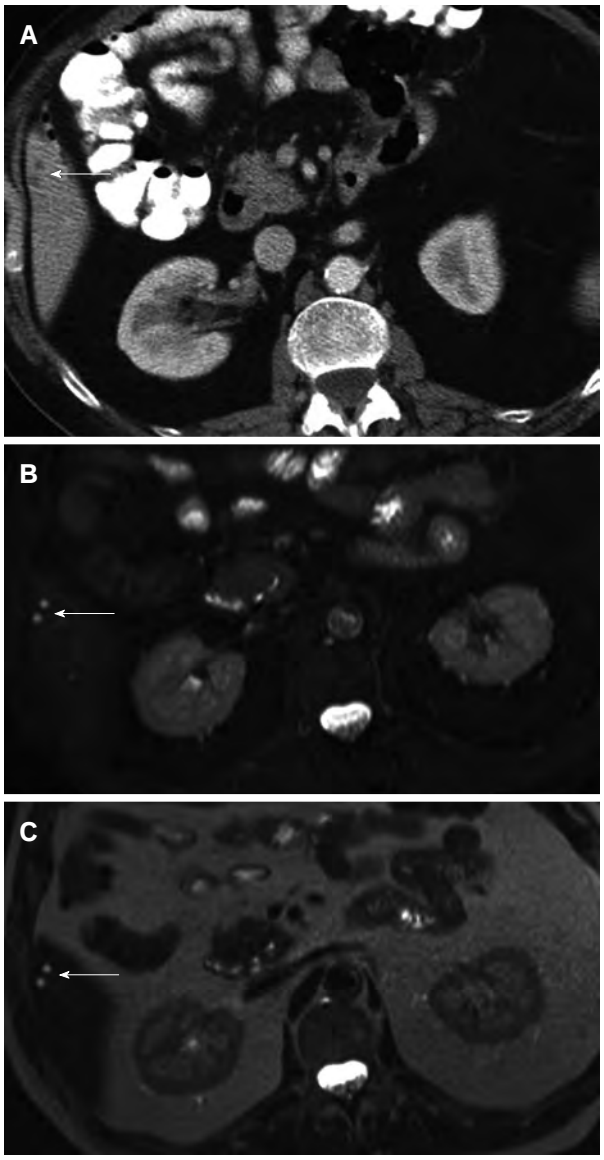


Figure 1 Example of a small lesion which proved to be benign after follow-up. A: Two small (< 10 mm) indeterminate focal liver lesions (white arrow) on contrast-enhanced computed tomography; B: Corresponding magnetic resonance imaging (MRI), images showing a T2w Turbo Spin Echo sequence with fat suppression. Two clearly hyperintense focal liver lesions (white arrow) are displayed compatible with simple liver cysts; C: Corresponding MRI images showing a T2w Turbo Spin Echo sequence without fat suppression. Two clearly hyperintense focal liver lesions (white arrow) are displayed compatible with simple liver cysts.

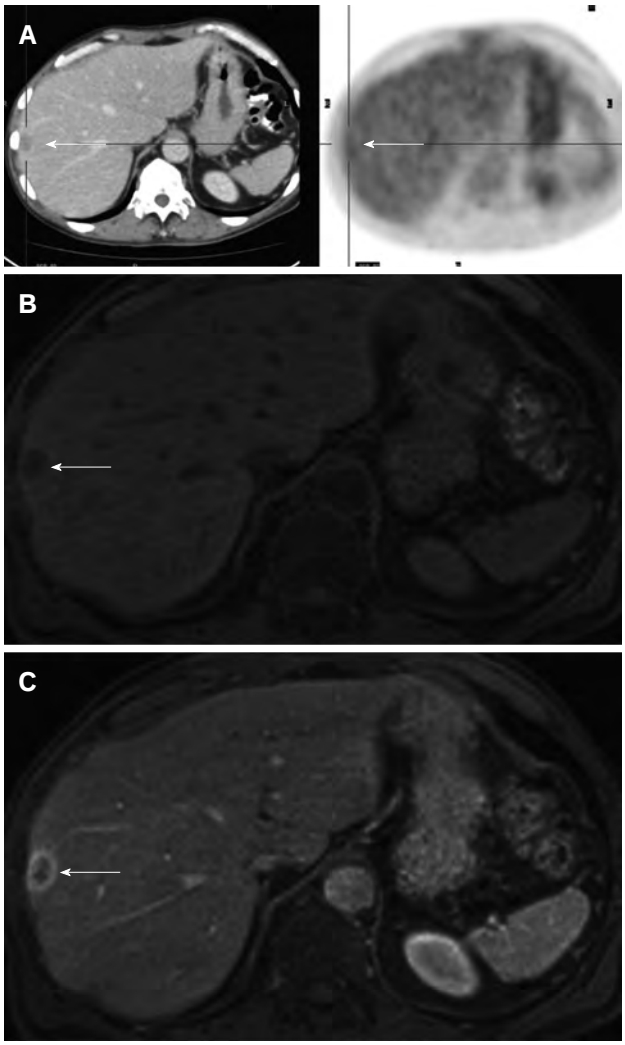


Figure 2 Example of a small lesion which proved to be malignant after follow-up. A: Positron emission tomography (PET)/computed tomography negative (PET-cold) focal liver lesion (white arrow) in a patient who received chemotherapy in the past; B: Corresponding T1w Gradient Echo magnetic resonance imaging (MRI) image before injection of contrast agent shows a rather hypo-intense focal liver lesion (white arrow); C: Corresponding T1w Gradient Echo MRI image in the venous phase after injection of contrast agent shows a hypo-intense focal liver lesion with ring-enhancement compatible with an (active) malignant focal liver lesion (colorectal cancer liver metastasis) (white arrow).

A major drawback of MDCT is that its ability to detect small lesions has outstripped its ability to characterize them. Although most tiny lung and/or liver lesions detected on CT staging of CRC patients are benign (Figure 1), 10%-20% of CT-indeterminate lung and/or liver lesions do develop into definite metastases (Figure 2)[17,18,20,21]. The M stage of CRC patients with CT-indeterminate lung and/or liver lesions, therefore, would require the category MX ("X" meaning uncertainty).

Regarding the definite diagnosis of whether or not these lesions are benign or malignant, consideration should include the T stage and the nodal status of the primary tumour and the distribution patterns of lesions as the probability of malignancy of small lung or liver lesions depends on the stage of the primary tumour (T stage) and the nodal status (N stage)[17,18].

Further evaluation with complementary imaging modalities is needed in patients with small CT-indeterminate lesions if a change of M staging would alter the treatment. In the thorax, additional PET/CT may be helpful in the differentiation of a single < 1 cm pulmonary lesion, but it may not be efficient when more than one small lesion is found on MDCT examination. In the liver, US may be the primary modality of choice in cases of advanced liver metastases, whereas contrast-enhanced US[22] MRI[23,24] and/or US-CT fusion imaging tech-



Figure 3 Restaging the liver after neoadjuvant therapy in a patient with multiple liver metastases from rectal cancer. A: On computed tomography (CT) before intravenous contrast, all the metastases disappeared except for a small lesion suspicious of residual disease (white arrow); B: Corresponding CT after intravenous contrast showing the challenging lesion (white arrow); C: Also on magnetic resonance imaging the lesion (white arrow) remains suspicious for malignancy providing indication for a liver resection.

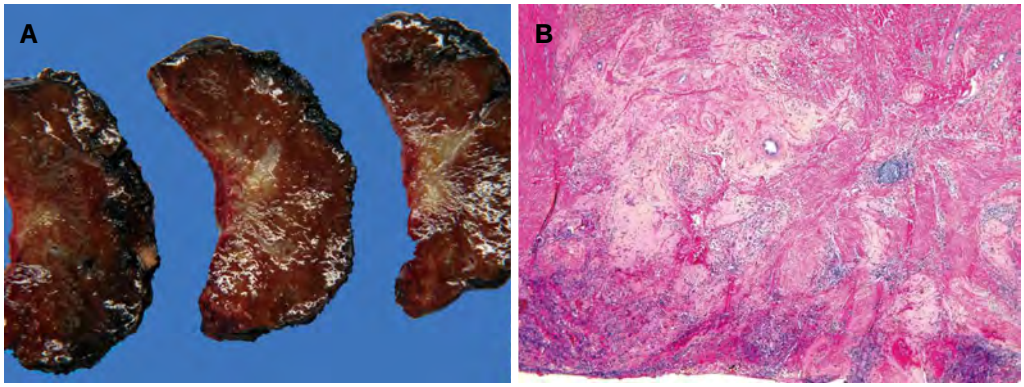


Figure 4 Pathological examination of the resected liver specimen corresponding to the lesion indicated by the white arrow in figure 3. A: Gross specimen: on cut the macroscopic examination shows a star-like whitish lesion; B: At histology (hematoxylin and eosin, x 100) only fibrosis and inflammation are seen indicating a complete response to chemotherapy.

niques[25] may complement the initial CT information in candidates for liver resection.

Follow-up CT imaging may be a reasonable approach in patients in whom a change of M staging would alter the clinical management, as malignant lesions would be expected to grow but benign lesions less so.

During restaging after neoadjuvant chemotherapy, besides the complete clinical remission (disappearance on CT, ycM0) achieved in a small number of patients with metastases (mainly in CRC lung or liver metastases), small lesions, suspected as being residual tumour may be encountered (Figure 3): the difficulties in interpreting these lesions are made harder by the response to chemotherapy which may impact on the sensitivity of preoperative imaging studies in identifying all sites of disease[26].

Either chemotherapy itself, or the fatty infiltration it commonly causes, can affect the ability of CT to effectively restage patients, resulting in both false negative and false positive results; thus, in patients with known hepatic steatosis, or in patients receiving neo-adjuvant chemotherapy, liver MRI is often recommended for staging or restaging of hepatic disease[14].

However, only pathologic examination can determine the actual nature of these lesions (Figure 4).

CONCLUSION

Advantages and disadvantages of the revised TNM classification of metastatic status

Disease management for CRC has evolved in recent years into a multidisciplinary setting and is essentially based on tumour stage.

Cancer staging represents the operational basis for choosing the most appropriate therapy and for evaluating the efficacy of different therapeutic methods; it is an essential component of patient care, cancer research, and control activities, even in light of the impressive progress that has been attained in the fields of clinical strategies and molecular medicine.

The TNM system is subjected to continuous updating through an ongoing expert review of existing data.

Proposals for changes are made in different situations, including when the classification is poorly accepted, poorly used, or criticized in the literature[27]: here comes the decision for MX category elimination[6].

As the MX category results in ambiguity (lack of information or uncertainty in assigning a given category) both in applying and in interpreting it for stage assigning, it seems reasonable to abandon it.

This is also in accord with the general rule of the

TNM system which states that, "if there is doubt concerning the correct category to which a particular patient should be allotted (T, N, or M), then the lower (*i.e.*, less advanced) category should be used"[8].

However, the increased detection of small indeterminate lesions due to improved imaging technologies, particularly with MDCT imaging[20], suggests that a separate category is needed.

This is to avoid the application of M0 category to all indeterminate/suspicious lesions, with consequent reduced diagnostic accuracy and staging errors.

Proposal

Cancer patients with small indeterminate lesions (*e.g.*, in the lung and/or liver) are at risk of developing metastases and therefore need continued follow-up with imaging. The TNM staging system uses an extensive number of prefixes and suffixes, as additional descriptors, their presence indicating cases needing separate analysis[4] and ongoing investigation. We propose to add a suffix (*e.g.*, iPUL, iHEP) to cM0 to indicate the presence of "small indeterminate lesions" considered to be benign but to be surveyed on further follow-up imaging to confirm that they are benign by their unchanged size and radiologic characteristics.

REFERENCES

1 **Poston G**, Adam R, Vauthey JN. Downstaging or downsizing: time for a new staging system in advanced colorectal cancer? *J Clin Oncol* 2006; **24**: 2702-2706 [PMID: 16782909 DOI: 10.1200/JCO.2006.05.8404]

2 **Nagashima I**, Takada T, Nagawa H, Muto T, Okinaga K. Proposal of a new and simple staging system of colorectal liver metastasis. *World J Gastroenterol* 2006; **12**: 6961-6965 [PMID: 17109517]

3 **Poston GJ**, Figueras J, Giuliante F, Nuzzo G, Sobrero AF, Gigot JF, Nordlinger B, Adam R, Gruenberger T, Choti MA, Bilchik AJ, Van Cutsem EJ, Chiang JM, D'Angelica MI. Urgent need for a new staging system in advanced colorectal cancer. *J Clin Oncol* 2008; **26**: 4828-4833 [PMID: 18711170 DOI: 10.1200/JCO.2008.17.6453]

4 **Sobin LH**, Gospodarowicz MK, Wittekind CH. TNM Classification of Malignant Tumors. New York: Wiley-Blackwell, 2009

5 **Morris EJ**, Forman D, Thomas JD, Quirke P, Taylor EF, Fairley L, Cottier B, Poston G. Surgical management and outcomes of colorectal cancer liver metastases. *Br J Surg* 2010; **97**: 1110-1118 [PMID: 20632280 DOI: 10.1002/bjs.7032]

6 **Sobin LH**, Compton CC. TNM seventh edition: what's new, what's changed: communication from the International Union Against Cancer and the American Joint Committee on Cancer. *Cancer* 2010; **116**: 5336-5339 [PMID: 20665503 DOI: 10.1002/cncr.25537]

7 **Jessup JM**, Gunderson LL, Greene FL, Washington MK, Compton CC, Sobin LH, Minsky B, Goldberg RM, Hamilton SR. 2010 Staging System for Colon and Rectal Carcinoma. *Ann Surg Oncol* 2011; **18:** 1513-1517 [DOI: 10.1245/s10434-010-1360-1]

8 **Greene FL**, Brierley J, O'Sullivan B, Sobin LH, Wittekind C. On the use and abuse of X in the TNM classification. *Cancer* 2005; **103**: 647-649 [PMID: 15612025 DOI: 10.1002/cncr.20808]

9 **Wittekind C**, Compton CC, Greene FL, Sobin LH. TNM residual tumor classification revisited. *Cancer* 2002; **94**: 2511-2516 [PMID: 12015777 DOI: 10.1002/cncr.10492]

10 **Puppa G**, Bortolasi L, Colombari R, Sheahan K. Residual tumor (R) classification in colorectal cancer: reduced, expanded, or not uniform? *Arch Pathol Lab Med* 2011; **135**: 288; author reply 289 [PMID: 21366444]

11 **Puppa G**, Sonzogni A, Colombari R, Pelosi G. TNM staging system of colorectal carcinoma: a critical appraisal of challenging issues. *Arch Pathol Lab Med* 2010; **134**: 837-852 [PMID: 20524862]

12 **Puppa G**. TNM staging system of colorectal carcinoma: surgical pathology of the seventh edition. *Diagn Histopathol* 2011; **17**: 243-262 [DOI: 10.1016/j.mpdhp.2011.03.006]

13 **Bipat S**, van Leeuwen MS, Comans EF, Pijl ME, Bossuyt PM, Zwinderman AH, Stoker J. Colorectal liver metastases: CT, MR imaging, and PET for diagnosis--meta-analysis. *Radiology* 2005; **237**: 123-131 [PMID: 16100087 DOI: 10.1148/radiol.2371042060]

14 **Grand DJ**, Beland M, Noto RB, Mayo-Smith W. Optimum imaging of colorectal metastases. *J Surg Oncol* 2010; **102**: 909-913 [PMID: 21165992 DOI: 10.1002/jso.21656]

15 **Kronawitter U**, Kemeny NE, Heelan R, Fata F, Fong Y. Evaluation of chest computed tomography in the staging of patients with potentially resectable liver metastases from colorectal carcinoma. *Cancer* 1999; **86**: 229-235 [PMID: 10421258]

16 **McIntosh J**, Sylvester PA, Virjee J, Callaway M, Thomas MG. Pulmonary staging in colorectal cancer--is computerised tomography the answer? *Ann R Coll Surg Engl* 2005; **87**: 331-333 [PMID: 16176690 DOI: 10.1308/003588405X60579]

17 **Brent A**, Talbot R, Coyne J, Nash G. Should indeterminate lung lesions reported on staging CT scans influence the management of patients with colorectal cancer? *Colorectal Dis* 2007; **9**: 816-818 [PMID: 17931171 DOI: 1463-1318.2007.01229.x]

18 **Christoffersen MW**, Bulut O, Jess P. The diagnostic value of indeterminate lung lesions on staging chest computed tomographies in patients with colorectal cancer. *Dan Med Bull* 2010; **57**: A4093 [PMID: 20175945]

19 **Grossmann I**, Klaase JM, Avenarius JK, de Hingh IH, Mastboom WJ, Wiggers T. The strengths and limitations of routine staging before treatment with abdominal CT in colorectal cancer. *BMC Cancer* 2011; **11**: 433 [PMID: 21982508 DOI: 10.1186/1471-2407-11-433]

20 **McQueen AS**, Scott J. CT staging of colorectal cancer: what do you find in the chest? *Clin Radiol* 2012; **67**: 352-358 [PMID: 22169348 DOI: 10.1016/j.crad.2011.10.005]

21 **Lim GH**, Koh DC, Cheong WK, Wong KS, Tsang CB. Natural history of small, "indeterminate" hepatic lesions in patients with colorectal cancer. *Dis Colon Rectum* 2009; **52**: 1487-1491 [PMID: 19617765 DOI: 10.1007/DCR.0013-e3181a74d5e]

22 **Wilson SR**, Burns PN. Microbubble-enhanced US in body imaging: what role? *Radiology* 2010; **257**: 24-39 [PMID: 20851938]

23 **Motosugi U**, Ichikawa T, Nakajima H, Sou H, Sano M, Sano K, Araki T, Iino H, Fujii H, Nakazawa T. Imaging of small hepatic metastases of colorectal carcinoma: how to use superparamagnetic iron oxide-enhanced magnetic resonance imaging in the multidetector-row computed tomography age? *J Comput Assist Tomogr* 2009; **33**: 266-272 [PMID: 19346857 DOI: 10.1097/RCT.0b013e31817f5c82]

24 **Coenegrachts K**. Magnetic resonance imaging of the liver: New imaging strategies for evaluating focal liver lesions. *World J Radiol* 2009; **1**: 72-85 [PMID: 21160723 DOI: 10.4329/wjr.v1.i1.72]

25 **Stang A**, Keles H, Hentschke S, Seydewitz C, Keuchel M,

Pohland C, Dahlke J, Weilert H, Wessling J, Malzfeldt E. Real-time ultrasonography-computed tomography fusion imaging for staging of hepatic metastatic involvement in patients with colorectal cancer: initial results from comparison to US seeing separate CT images and to multidetector-row CT alone. *Invest Radiol* 2010; **45**: 491-501 [PMID: 20458251 DOI: 10.1097/RLI.0b013e3181ddd3da]

26 **Charnsangavej C**, Clary B, Fong Y, Grothey A, Pawlik TM, Choti MA. Selection of patients for resection of hepatic colorectal metastases: expert consensus statement. *Ann Surg Oncol* 2006; **13**: 1261-1268 [PMID: 16947009 DOI: 10.1245/s10434-006-9023-y]

27 **Gospodarowicz MK**, Miller D, Groome PA, Greene FL, Logan PA, Sobin LH. The process for continuous improvement of the TNM classification. *Cancer* 2004; **100**: 1-5 [PMID: 14692017 DOI: 10.1002/cncr.11898]

P- Reviewer Sipos F **S- Editor** Gou SX **L- Editor** O'Neill M **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i8.1158

World J Gastroenterol 2013 February 28; 19(8): 1158-1165
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

TOPIC HIGHLIGHT

Metin Basaranoglu, MD, PhD, Associate Professor, *Series Editor*

From fatty liver to fibrosis: A tale of "second hit"

Metin Basaranoglu, Gökcen Basaranoglu, Hakan Sentürk

Metin Basaranoglu, Hakan Sentürk, Gastroenterology and Hepatology, Bezmialem Vakif University, Istanbul 34400, Turkey
Gökcen Basaranoglu, Department of Anaesthesiology, Bezmialem Vakif University, Istanbul 34400, Turkey
Author contributions: Basaranoglu M contributed extensively to the work, performed the literature search and designed and wrote the paper; Sentürk H and Basaranoglu G commented on the paper.
Correspondence to: Metin Basaranoglu, MD, Gastroenterology and Hepatology, Bezmialem Vakif University, Istanbul 34400, Turkey. metin_basaranoglu@yahoo.com
Telephone: +90-312-5878030 Fax: +90-312-5540570
Received: April 5, 2012 Revised: June 13, 2012
Accepted: June 28, 2012
Published online: February 28, 2013

Abstract

Although much is known about how fat accumulates in the liver, much remains unknown about how this causes sustained hepatocellular injury. The consequences of injury are recognized as nonalcoholic steatohepatitis (NASH) and progressive fibrosis. The accumulation of fat within the hepatocytes sensitizes the liver to injury from a variety of causes and the regenerative capacity of a fatty liver is impaired. An additional stressor is sometimes referred to as a "second hit" in a paradigm that identifies the accumulation of fat as the "first hit". Possible candidates for the second hit include increased oxidative stress, lipid peroxidation and release of toxic products such as malondialdehyde and 4-hydroxynonenal, decreased antioxidants, adipocytokines, transforming growth factor (TGF)-β, Fas ligand, mitochondrial dysfunction, fatty acid oxidation by CYPs (CYP 2E1, 4A10 and 4A14), and peroxisomes, excess iron, small intestinal bacterial overgrowth, and the generation of gut-derived toxins such as lipopolysaccharide and ethanol. Oxidative stress is one of the most popular proposed mechanisms of hepatocellular injury. Previous studies have specifically observed increased plasma and tissue levels of oxidative stress markers and lipid peroxidation products, with reduced hepatic and plasma levels of antioxidants. There is also some indirect evidence of the benefit of antioxidants such as vitamin E, S-adenosylmethionine, betaine, phlebotomy to remove iron, and N-acetylcysteine in NASH. However, a causal relationship or a pathogenic link between NASH and oxidative stress has not been established so far. A number of sources of increased reactive oxygen species production have been established in NASH that include proinflammatory cytokines such as tumor necrosis factor (TNF)-α, iron overload, overburdened and dysfunctional mitochondria, CYPs, and peroxisomes. Briefly, the pathogenesis of NASH is multifactorial and excess intracellular fatty acids, oxidant stress, ATP depletion, and mitochondrial dysfunction are important causes of hepatocellular injury in the steatotic liver.

© 2013 Baishideng. All rights reserved.

Key words: Fatty liver; Oxidative stress; Fibrosis; Nonalcoholic fatty liver diseases; Nonalcoholic steatohepatitis

Basaranoglu M, Basaranoglu G, Sentürk H. From fatty liver to fibrosis: A tale of "second hit". *World J Gastroenterol* 2013; 19(8): 1158-1165 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i8/1158.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i8.1158

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is one of the most prevalent forms of chronic liver disease in the United States[1]. Contributing factors to this may include the increasingly sedentary lifestyle of the population and increased consumption of a high-fat (HF) diet and high fructose corn syrup (HFCS)[2]. In the setting of excessive

central adiposity, insulin resistance is the major underlying cause of fat accumulation in the liver[3-7]. Nonalcoholic steatohepatitis (NASH), as a subgroup of NAFLD, is characterized by chronic and progressive liver pathology and may lead to advanced fibrosis, cirrhosis, end-stage liver disease, hepatocellular carcinoma and liver-related death[8-10]. One of the important and unresolved problems in NASH is the pathogenesis of hepatocyte injury. One hypothesis for the pathogenesis of NAFLD is the "two-hit" hypothesis[11]. According to this paradigm, the primary abnormality ("first hit") is most likely insulin resistance, which leads to the accumulation of triglycerides within the hepatocytes. Then, a "second hit" induces hepatocyte injury and inflammation (NASH). The mechanisms that cause hepatocyte injury in fatty liver have not been fully elucidated to date. Oxidative stress is one of the most popular proposed mechanisms of hepatocellular injury (Figure 1). It was reported that obesity correlated with systemic oxidative stress in humans and mice[12-14]. Obese adults with metabolic syndrome (MS) have higher plasma concentration of oxidative stress biomarkers than obese adults without MS. Increased reactive oxygen species (ROS) production has been selectively shown in adipose tissue of obese mice[15,16]. Previous studies also specifically observed increased plasma and tissue levels of oxidative stress markers and lipid peroxidation products with reduced hepatic and plasma levels of antioxidants in patients with NASH[2-6,12-16].

MOLECULAR SIGNAL OF DEVELOPMENT OF FATTY LIVER IN OBESITY

Obesity is associated with low-grade chronic inflammation in humans, and this chronic inflammation is a link between obesity and insulin resistance[17-19]. Indeed, obesity is strongly associated with chronic macrophage accumulation within increased adipose tissue in obese humans. Xu *et al*[16] showed that inflamed macrophages are active within white adipose tissue and this activation occurs after increased adiposity and before insulin resistance. Macrophages secrete cytokines which promote insulin resistance in adipose tissue and eventually increase adipose tissue lipolysis, which causes insulin resistance in both muscle and the liver, besides significant amount of inducible NO synthase and interleukin-6[15]. Moreover, a positive correlation between adipocyte size and the content of accumulated macrophages in adipose has been reported. Increased fatty acids or accumulated macrophages might be the reason for this increased ROS production within adipose tissue. These data indicate localized inflammation and systemic consequences such as insulin resistance and increased circulating free fatty acids. Additional evidence that this chronic inflammation causes insulin resistance comes from restoring insulin sensitivity by various anti-inflammatory agents, such as high-dose salicylates *via* IKK-β inhibition or anti-tumor necrosis factor (TNF)-α antibody infusion[20].

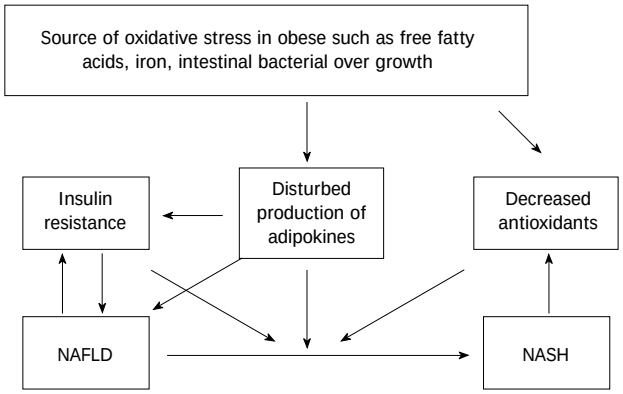


Figure 1 Oxidative stress is one of the most popular proposed mechanisms of hepatocellular injury and possible source of the oxidant stress as follows: increased free fatty acids supply, iron, intestinal bacterial over growth. NASH: Nonalcoholic steatohepatitis; NAFLD: Nonalcoholic fatty liver disease.

OXIDATIVE STRESS AND LIPID PEROXIDATION FOR DEVELOPMENT OF HEPATIC FIBROSIS

A logical and attractive hypothesis is that oxidative stress in TG-loaded hepatocytes is the cause of sustained injury with consequent NASH, fibrosis and cirrhosis[11] (Figure 2). The imbalance between the increased ROS and decreased antioxidants leads to lipid peroxidation of polyunsaturated fatty acids, cellular membranes, mitochondrial membranes, and DNA[21]. Lipid peroxidation products have longer half-lives and the capability to reach extracellular targets. Lipid peroxidation produces cytotoxic aldehydes such as malondialdehyde (MDA) and 4-hydroxynonenal. ROS and these aldehydes further contribute to oxidative stress, decreased ATP production, and increased proinflammatory cytokine release. These events promote hepatocyte injury, necroinflammation and hepatocytes apoptosis. Despite the attractiveness of this hypothesis, most clinical studies only provide correlations between the presence of NASH and elevated indices of oxidant stress, without establishing a causal relationship[22-27]. The lipid peroxidation product 4-hydroxynonenal is found more in perivenular zone (zone 3), correlating with the histological lesions of NASH that are predominantly in zone 3[21]. Lipid peroxidation is greater in patients with NASH than in patients with simple steatosis. The same study has also shown that increased 4-hydroxynonenal strongly correlates with both the grade of necroinflammation and the stage of NASH, but not with the grade of steatosis, while increased evidence of oxidant damage to DNA as measured by 8-hydroxydeoxyguanosine only correlates with the grade of necroinflammation in patients with NASH.

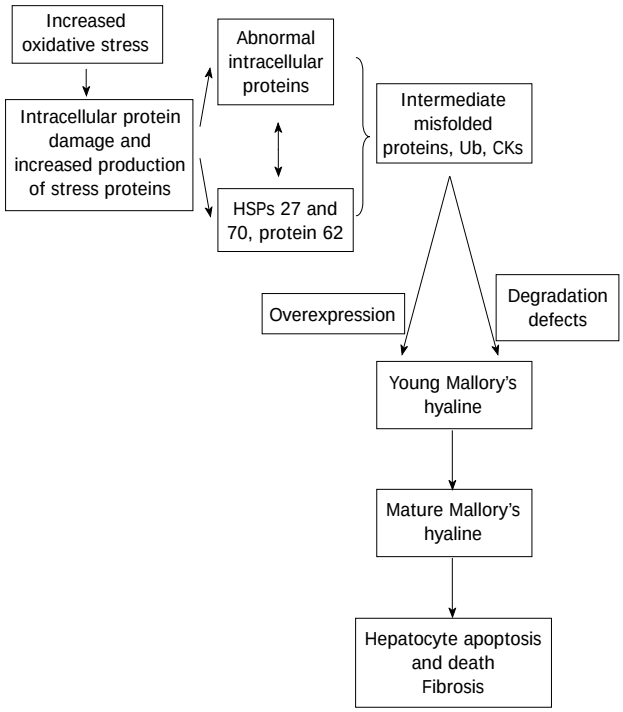


Figure 2 Logical and attractive hypothesis is that oxidative stress in TG-loaded hepatocytes is the cause of sustained injury with consequent **NASH, fibrosis and cirrhosis.** HSP: Heat shock proteins.

UNDERSTANDING THE MOLECULAR MECHANISIMS OF NASH

Oxidative stress

Oxidative stress might play a role in the pathogenesis of hepatocyte dysfunction and inflammation in NAFLD. Oxidative stress can result from either excess ROS production and/or deficient antioxidant capacity[12-15,21]. The enzyme NAD(P)H oxidase catalyzes the transfer of a single electron to molecular oxygen to produce superoxide. Upregulation of NAD(P)H oxidase can raise ROS production, and thereby contribute to the pathogenesis of oxidative stress in HF and high-sucrose-fed mice. Formation of ROS is enhanced by different mechanisms, including xanthine oxidase activation, NADH auto-oxidation, and superoxide dismutase (SOD) inactivation. Experimental evidence supports the concept of diverse ROS, such as superoxide anion, hydrogen peroxide, hydroxyl radical, nitric oxide and peroxynitrite.

Assessment of: (1) NAD(P)H oxidase (a major source of ROS) in liver; (2) downregulation of the main antioxidant enzymes, SOD, glutathione peroxidase (GPX), catalase, and heme oxygenase (HO) in liver tissue; (3) level of lipid peroxidation products, MDA + 4-hydroxyalkenals in the liver; (4) plasma concentration of 8-isoprostanes, lipoperoxides in plasma samples by measuring MDA *via* HPLC; and (5) immunoblotting to quantify NADPH oxidase, Mn SOD, Cu Zn SOD, GPX, catalase, and HO-2 levels in liver will help to understand the underlying mechanisms of oxidative stress in the pathogenesis of NASH[12,13,22-28]. A number of sources of increased ROS production have been established in NASH that include proinflammatory cytokines, such as TNF-α, iron overload, overburdened and dysfunctional mitochondria, CYPs, and peroxisomes.

Sources of oxidants

Mitochondria and mitochondrial β-oxidation: The hepatocyte is a cell rich in mitochondria. Each hepatocyte contains approximately 800 mitochondria[28]. The hepatocyte mitochondria are the main site of β-oxidation of free fatty acids. The electrons removed from free fatty acids during β-oxidation are shuttled through the mitochondrial respiratory chain (MRC), eventually leading to ATP synthesis and generation of CO_2 and water. Inherent in this process is the dissociation of partially reduced molecular oxygen in the form of superoxide, hydrogen peroxide and the hydroxyl radical, species collectively termed ROS. About 1%-5% of oxygen consumed during cellular respiration is not fully reduced to water during this process under physiological conditions, and the production of these ROS is further increased in dysfunctional mitochondria. Thus, mitochondria have been proposed to play a central role in the pathogenesis of NASH[29]. Mitochondria also increase their oxidation capacity for the increased fatty acid flux as observed in obesity and insulin resistant states in humans. However, this increase has its limits and excess free fatty acids are metabolized at other sites in hepatocytes such as peroxisomes (β-oxidation) and the smooth endoplasmic reticulum (ω-oxidation). Acyl-CoA oxidase catalyzes the initial reaction of fatty acid oxidation in peroxisomes; a process that generates hydrogen peroxide and thus may contribute to oxidant stress.

Mitochondrial β-oxidation of short-, medium- and long-chain fatty acids involves multiple steps that include entry of long-chain fatty acids into the mitochondria; a process dependent on carnitine shuttle enzymes carnitine palmitoyltransferase (CPT)-I (an outer membrane enzyme) and CPT-II, and the β-oxidation of fatty acids to form progressively shorter acyl-CoA moieties, acetyl-CoA[29]. These oxidation processes are associated with the reduction of oxidized NAD+ and FAD to NADH and $FADH_2$. Reoxidation of NADH and $FADH_2$ to NAD+ and FAD produces electrons that transfer to the MRC[29,30]. Partially reduced oxygen molecules, termed ROS, are constitutively generated during this process when the electrons of NADH and $FADH_2$ directly react with oxygen and may contribute to oxidant stress if endogenous protective mechanisms are overwhelmed[30]. Consistent with the increased flux of nonesterified fatty acid to the liver in obese patients with NAFLD, mitochondrial β-oxidation of fatty acids in the liver is also increased and as such may contribute to increased generation of ROS and oxidant stress.

Excessive fatty acids might use alternative pathways other than mitochondrial β-oxidation to be metabolized and cause mitochondrial injury. These include

peroxisomal and cytochrome P450 (microsomal CYP) oxidation systems regulated by mainly fatty acids and insulin[31]. These alternative fatty acid oxidation systems produce more ROS and thus their utilization may be a source of oxidant stress.

Peroxisomal fatty acid β-oxidation: Peroxisomal oxidation of fatty acids is the normal route of metabolism of very long chain fatty acids and dicarboxylic acids[31]. Peroxisomal oxidation is a four-step pathway in which electrons from $FADH_2$ and NADH are transferred directly to oxygen. Although this increases the production of hydrogen peroxide, peroxisomes are uniquely endowed with the enzyme catalase that eliminates this reactive oxygen molecule. Fatty acids not oxidized by mitochondria are mainly oxidized by CYP2E1; a process that further increases ROS production within the hepatocytes[32]. Increased endogenous substrate burden such as increased levels of free fatty acids and ketone bodies induce CYP2E1 expression in humans. In normal conditions, CYP2E1 oxidation produces oxygen radicals, but the balance between these ROS and the abundance of endogenous antioxidants determines the extent of resulting oxidant stress. Increased hepatic CYP2E1 expression has been demonstrated by immunostaining of paraffin-embedded liver biopsy sections in patients with NASH. In contrast, hepatic content of CYP3A was decreased in all liver sections from patients with NASH. These studies also showed that weight loss decreased hepatic CYP2E1 activity. In parallel, Leclercq *et al*[33] previously had reported that dietary sugar restriction decreased CYP2E1 activity in humans. These novel studies pointed out that insulin rather than ketone bodies, with or without glucose contribution, regulate the expression and activity of hepatic CYP2E1. These metabolic abnormalities increase hepatic CYP2E1 activity and subsequent pro-oxidant production in patients with NAFLD.

Cytochrome P450 fatty acid ω (omega)-oxidation: Fatty acids can undergo oxidation by the CYP enzymes of the smooth endoplasmic reticulum which is a relatively minor pathway. CYP2E1 and CYP4A isoforms are involved in fatty acid oxidation in conditions with substrate overload such as increased ketone bodies in type 2 diabetes mellitus. CYP4A upregulation particularly occurs in conditions with decreased CYP2E1 activity. The expression of both CYP2E1 and CYP4A mRNA and their protein levels are increased in both obese and diabetic humans[32,33]. Their hepatic activity and expression are also reported to be increased in patients with NASH due to the increased substrates, mainly fatty acids and ketone bodies, irrespective of the underlying clinical condition of diabetes or obesity. Nonetheless, the capacity of this enzyme system is very low to handle fatty acids. Oxidation reactions by the CYP enzymes can be major producers of ROS because of a low degree of coupling between substrate binding and their weak affinity to molecular oxygen, leading to the release of species such as superoxide anion radical, hydroxyl radicals, and hydrogen peroxide.

Mitochondrial dysfunction and ATP depletion: Mitochondria are the organelles primarily responsible for fatty acid β-oxidation and oxidative phosphorylation; the process responsible for the production of ATP[30]. Several observations including decreased mitochondrial enzyme activities and increased fat concentration of skeletal muscle cells in obese or diabetic patients have suggested mitochondrial dysfunction in these disorders. Such abnormalities may increase ROS production and promote both oxidative stress and lipid peroxidation within hepatocytes. Mitochondrial dysfunction is frequently due to a combination of genetic abnormalities, physical inactivity, aging, lipotoxicity (free fatty acids), lipid peroxidation (mitochondrial DNA alterations), and TNF-α[29,30,34]. Hepatic mitochondrial abnormalities have been identified in NAFLD, suggesting that mitochondria may be the source or target of injury and that ineffective mitochondrial function resulting in cellular ATP depletion may be important pathophysiological processes in NAFLD and NASH. The presence of megamitochondria, or mitochondrial swelling, is a microscopically detectable structural abnormality of hepatocyte mitochondria found in a variety of liver diseases including NAFLD[28]. Crystalline inclusions within the mitochondrial matrix have been documented by electron microscopy in patients with NASH. Hepatic mitochondrial DNA levels and the protein products of the mitochondrial genes are also decreased in patients with NASH. Impaired hepatic MRC function increases ROS production, and if ROS production exceeds antioxidant capabilities, oxidative stress and injury, lipid peroxidation of macromolecules and cellular membranes, mitochondrial DNA damage, direct damage of several mitochondrial enzymes, and further MRC dysfunction with more pro-oxidant production are observed. Mixed macro- and microvesicular steatosis due to β-oxidation defects in the mitochondria was the predominant type of steatosis in this study, and CYP 2E1 expression was upregulated, and levels of the antioxidant glutathione were decreased. Carnitine and CPT-I and CPT-II are required to transfer long-chain free fatty acids into the mitochondria for β-oxidation. Some investigators have reported the role of carnitine deficiency in NAFLD development, while others have observed normal hepatic content of total and free carnitine in patients with NASH.

Iron, oxidant stress and NASH: Iron can play a central role in promoting oxidant stress and this is proposed to be the mechanism of progressive liver disease. A large-population based study reported a correlation between elevated serum alanine aminotransferase levels and increased serum transferrin and iron concentrations[35,36]. After initial measurements, investigators in-

duced iron depletion to a level of near-iron deficiency by phlebotomy. Interestingly, they observed improvements in both insulin sensitivity and serum alanine aminotransferase activity in some of the patients, indicating that iron may play a role not only in oxidant stress but also in the initial predisposing factor of insulin resistance.

Free fatty acid toxicity: In addition to insulin resistance and hyperinsulinemia, obesity and type 2 diabetes mellitus are strongly associated with increased concentrations of free fatty acids in the circulation[24,26]. Fatty acids are involved in many important cellular events such as synthesis of cellular membranes, energy storage, and intracellular signaling pathways. However, chronically elevated free fatty acids have the capability to disturb diverse metabolic pathways and induce insulin resistance in many organ systems[37-39]. In addition to their metabolic effects, fatty acids could induce cellular apoptosis, also called lipotoxicity, in two ways: direct toxicity and an indirect effect. One proposed mechanism of fatty acid toxicity in hepatocytes is that fatty acids induce translocation of Bax (which is a mitochondrial protein and a member of the Bcl-2 family) to lysosomes and cause lysosomal destabilization, which promotes the release of cathepsin B (a specific lysosomal enzyme), from lysosomes to the cytosol. Subsequently, a cathepsin-B-dependent process induces nuclear factor (NF)-κB activation and TNF-α overexpression in the liver[26]. TNF-α might further increase lysosomal destabilization and cathepsin-B-dependent hepatocyte apoptosis. Then, cytochrome c release from dysfunctional mitochondria may occur. Mitochondrial dysfunction causes energy depletion, which activates proteolytic caspases and induces DNA fragmentation and chromatin condensation. NF-κB is a transcriptional factor and has both apoptotic and antiapoptotic effects. In healthy hepatocytes, activation of NF-κB by TNF-α induces Bcl-2 synthesis, which prevents the release of cytochrome c from the mitochondria and subsequent apoptosis. Moreover, while cathepsin B has been demonstrated in hepatocyte lysosomes of healthy control individuals, the majority of hepatocytes in patients with NAFLD show diffuse distribution of cathepsin B in the cytosol, with a positive correlation with the stage of NASH[40-44].

HEPATIC FIBROGENESIS IN NASH

Hepatic steatosis is the most frequent and initially observed morphological feature of these processes[45-53]. Steatosis, inflammation, glycogen nuclei, lipogranulomas, ballooning of hepatocytes, Mallory bodies, and fibrosis are the major features of NAFLD.

Genetic and environmental factors may affect the development of liver fibrosis in NAFLD[54-62]. Age, severity of obesity, presence of diabetes, and hyperglycemia are the major nongenetic factors. Elevated plasma glucose, free fatty acids and adipocytokines activate both Kupffer cells and hepatic stellate cells (HSCs) and stimulate fibrogenesis[63-65]. Hepatic connective tissue growth factor (CTGF) mRNA was overexpressed in all NASH patients, while hepatic CTGF mRNA and its protein were upregulated in *fa/fa* rats (obese and diabetic) compared with their lean littermates. The same study also demonstrated upregulation of both CTGF mRNA and its protein in HSCs after exposure to high concentrations of either glucose or insulin. After activation, HSCs proliferate and express α-smooth muscle actin. Activated HSCs express myogenic markers such as c-myb and myocyte enhancer factor-2, exhibit proinflammatory and profibrogenic properties, migrate and secrete extracellular matrix components (ECM) such as collagen, and regulate the degradation of ECM. Activation of HSCs is the crucial step in liver fibrogenesis. A study of NAFLD patients (16 patients with steatosis alone and 60 with NASH) demonstrated that activation of HSCs was positive in almost all cases, and markedly in two thirds of patients, and it was correlated with the degree and location of hepatic fibrosis. HSC activation and upregulation of profibrogenic genes were observed in rats on an HF diet. Lipid-peroxidation-associated inflammation and HSC activation with increased TGF-β1 mRNA expression in methionine-choline-deficient steatohepatitis models have also been reported.

Oxidative stress may also participate in the activation of HSCs and the development of fibrosis in NAFLD. The intracellular NADPH oxidase pathway produces ROS, and the disruption of NADPH oxidase protects mice from developing severe liver injury. Lipid peroxidation products enhance the production of both TGF-β and collagen.

Currently, proposed mechanisms for the transformation from NASH to NASH-associated hepatocellular carcinoma are severe and cumulative oxidative stress to the hepatocytes, production of damaged DNA, defective or inhibited DNA repair systems, chronic continued hepatocyte injury and inflammatory infiltration, impaired antioxidant systems, and increased cell cycle of hepatocytes[65-67]. Animal and human studies have also indicated that a connection between age, sex and the disease might be possible[66-70].

In conclusion, a liver with excess fat may be more vulnerable to stressors than a normal liver. The factors that play key roles in the development of NASH from NAFLD remain uncertain.

REFERENCES

1 **Matteoni CA**, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 1999; **116**: 1413-1419 [PMID: 10348825]

2 **Tetri LH**, Basaranoglu M, Brunt EM, Yerian LM, Neuschwander-Tetri BA. Severe NAFLD with hepatic necroinflammatory changes in mice fed trans fats and a high-fructose corn syrup equivalent. *Am J Physiol Gastrointest*

Liver Physiol 2008; **295**: G987-G995 [PMID: 18772365]
3 **Canbakan B**, Tahan V, Balci H, Hatemi I, Erer B, Ozbay G, Sut N, Hacibekiroglu M, Imeryuz N, Senturk H. Leptin in nonalcoholic fatty liver disease. *Ann Hepatol* 2008; **7**: 249-254 [PMID: 18753993]
4 **Canbakan B**, Senturk H, Tahan V, Hatemi I, Balci H, Toptas T, Sonsuz A, Velet M, Aydin S, Dirican A, Ozgulle S, Ozbay G. Clinical, biochemical and histological correlations in a group of non-drinker subjects with non-alcoholic fatty liver disease. *Acta Gastroenterol Belg* 2007; **70**: 277-284 [PMID: 18074737]
5 **Basaranoglu M**, Basaranoglu G. Pathophysiology of insulin resistance and steatosis in patients with chronic viral hepatitis. *World J Gastroenterol* 2011; **17**: 4055-4062 [PMID: 22039318]
6 **Basaranoglu M**, Turhan N, Sonsuz A, Basaranoglu G. Mallory-Denk Bodies in chronic hepatitis. *World J Gastroenterol* 2011; **17**: 2172-2177 [PMID: 21633525]
7 **Sonsuz A**, Basaranoglu M, Bilir M, Senturk H, Akin P. Hyperinsulinemia in nondiabetic, both obese and nonobese patients with nonalcoholic steatohepatitis. *Am J Gastroenterol* 2002; **97**: 495 [PMID: 11866302]
8 **Caldwell SH**, Oelsner DH, Iezzoni JC, Hespenheide EE, Battle EH, Driscoll CJ. Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. *Hepatology* 1999; **29**: 664-669 [PMID: 10051466 DOI: 10.1002/hep.510290347]
9 **Poonawala A**, Nair SP, Thuluvath PJ. Prevalence of obesity and diabetes in patients with cryptogenic cirrhosis: a case-control study. *Hepatology* 2000; **32**: 689-692 [PMID: 11003611 DOI: 10.1053/jhep.2000.17894]
10 **Charlton M**, Kasparova P, Weston S, Lindor K, Maor-Kendler Y, Wiesner RH, Rosen CB, Batts KP. Frequency of nonalcoholic steatohepatitis as a cause of advanced liver disease. *Liver Transpl* 2001; **7**: 608-614 [PMID: 11460228 DOI: 10.1053/jlts.2001.25453]
11 **Day CP**, James OF. Steatohepatitis: a tale of two "hits"? *Gastroenterology* 1998; **114**: 842-845 [PMID: 9547102]
12 **Keaney JF**, Larson MG, Vasan RS, Wilson PW, Lipinska I, Corey D, Massaro JM, Sutherland P, Vita JA, Benjamin EJ. Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. *Arterioscler Thromb Vasc Biol* 2003; **23**: 434-439 [PMID: 12615693]
13 **Hansel B**, Giral P, Nobecourt E, Chantepie S, Bruckert E, Chapman MJ, Kontush A. Metabolic syndrome is associated with elevated oxidative stress and dysfunctional dense high-density lipoprotein particles displaying impaired antioxidative activity. *J Clin Endocrinol Metab* 2004; **89**: 4963-4971 [PMID: 15472192]
14 **Furukawa S**, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 2004; **114**: 1752-1761 [PMID: 15599400]
15 **Weisberg SP**, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 2003; **112**: 1796-1808 [PMID: 14679176]
16 **Xu H**, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 2003; **112**: 1821-1830 [PMID: 14679177]
17 **Ratziu V**, Bonyhay L, Di Martino V, Charlotte F, Cavallaro L, Sayegh-Tainturier MH, Giral P, Grimaldi A, Opolon P, Poynard T. Survival, liver failure, and hepatocellular carcinoma in obesity-related cryptogenic cirrhosis. *Hepatology* 2002; **35**: 1485-1493 [PMID: 12029634 DOI: 10.1053/jhep.2002.33324]
18 **Ratziu V**, Giral P, Charlotte F, Bruckert E, Thibault V, Theodorou I, Khalil L, Turpin G, Opolon P, Poynard T. Liver fibrosis in overweight patients. *Gastroenterology* 2000; **118**: 1117-1123 [PMID: 10833486]
19 **Stein CJ**, Colditz GA. The epidemic of obesity. *J Clin Endocrinol Metab* 2004; **89**: 2522-2525 [PMID: 15181019]
20 **Hundal RS**, Petersen KF, Mayerson AB, Randhawa PS, Inzucchi S, Shoelson SE, Shulman GI. Mechanism by which high-dose aspirin improves glucose metabolism in type 2 diabetes. *J Clin Invest* 2002; **109**: 1321-1326 [PMID: 12021247]
21 **Seki S**, Kitada T, Sakaguchi H. Clinicopathological significance of oxidative cellular damage in non-alcoholic fatty liver diseases. *Hepatol Res* 2005; **33**: 132-134 [PMID: 16198621]
22 **Marchesini G**, Ridolfi V, Nepoti V. Hepatotoxicity of fast food? *Gut* 2008; **57**: 568-570 [PMID: 18408097 DOI: 10.1136/gut.2007.143958]
23 **Milagro FI**, Campión J, Martínez JA. Weight gain induced by high-fat feeding involves increased liver oxidative stress. *Obesity (Silver Spring)* 2006; **14**: 1118-1123 [PMID: 16899792 DOI: 10.1038/oby.2006.128]
24 **Mozaffarian D**, Katan MB, Ascherio A, Stampfer MJ, Willett WC. Trans fatty acids and cardiovascular disease. *N Engl J Med* 2006; **354**: 1601-1613 [PMID: 16611951 DOI: 10.1056/NEJMra054035]
25 **Lieber CS**, Leo MA, Mak KM, Xu Y, Cao Q, Ren C, Ponomarenko A, DeCarli LM. Model of nonalcoholic steatohepatitis. *Am J Clin Nutr* 2004; **79**: 502-509 [PMID: 14985228]
26 **Feldstein AE**, Werneburg NW, Canbay A, Guicciardi ME, Bronk SF, Rydzewski R, Burgart LJ, Gores GJ. Free fatty acids promote hepatic lipotoxicity by stimulating TNF-alpha expression *via* a lysosomal pathway. *Hepatology* 2004; **40**: 185-194 [PMID: 15239102 DOI: 10.1002/hep.20283]
27 **Harrison SA**, Torgerson S, Hayashi P, Ward J, Schenker S. Vitamin E and vitamin C treatment improves fibrosis in patients with nonalcoholic steatohepatitis. *Am J Gastroenterol* 2003; **98**: 2485-2490 [PMID: 14638353]
28 **Caldwell SH**, Chang CY, Nakamoto RK, Krugner-Higby L. Mitochondria in nonalcoholic fatty liver disease. *Clin Liver Dis* 2004; **8**: 595-617, x [PMID: 15331066]
29 **Pessayre D**, Fromenty B. NASH: a mitochondrial disease. *J Hepatol* 2005; **42**: 928-940 [PMID: 15885365]
30 **Wei Y**, Rector RS, Thyfault JP, Ibdah JA. Nonalcoholic fatty liver disease and mitochondrial dysfunction. *World J Gastroenterol* 2008; **14**: 193-199 [PMID: 18186554]
31 **Bradbury MW**, Berk PD. Lipid metabolism in hepatic steatosis. *Clin Liver Dis* 2004; **8**: 639-671, xi [PMID: 15331068]
32 **Lieber CS**. CYP2E1: from ASH to NASH. *Hepatol Res* 2004; **28**: 1-11 [PMID: 14734144]
33 **Leclercq I**, Horsmans Y, Desager JP, Pauwels S, Geubel AP. Dietary restriction of energy and sugar results in a reduction in human cytochrome P450 2E1 activity. *Br J Nutr* 1999; **82**: 257-262 [PMID: 10655974]
34 **Pérez-Carreras M**, Del Hoyo P, Martín MA, Rubio JC, Martín A, Castellano G, Colina F, Arenas J, Solis-Herruzo JA. Defective hepatic mitochondrial respiratory chain in patients with nonalcoholic steatohepatitis. *Hepatology* 2003; **38**: 999-1007 [PMID: 14512887]
35 **Ruhl CE**, Everhart JE. Relation of elevated serum alanine aminotransferase activity with iron and antioxidant levels in the United States. *Gastroenterology* 2003; **124**: 1821-1829 [PMID: 12806616]
36 **Bugianesi E**, Manzini P, D'Antico S, Vanni E, Longo F, Leone N, Massarenti P, Piga A, Marchesini G, Rizzetto M. Relative contribution of iron burden, HFE mutations, and insulin resistance to fibrosis in nonalcoholic fatty liver. *Hepatology* 2004; **39**: 179-187 [PMID: 14752836]
37 **Neuschwander-Tetri BA**. Hepatic lipotoxicity and the pathogenesis of nonalcoholic steatohepatitis: the central role

of nontriglyceride fatty acid metabolites. *Hepatology* 2010; **52**: 774-788 [PMID: 20683968]
38 **Tarcin O**, Basaranoglu M, Tahan V, Tahan G, Sücüllü I, Yilmaz N, Sood G, Snyder N, Hilman G, Celikel C, Tözün N. Time course of collagen peak in bile duct-ligated rats. *BMC Gastroenterol* 2011; **11**: 45 [PMID: 21527001]
39 **Canbakan B**, Senturk H, Canbakan M, Toptas T, Tuncer M. Reliability of caspase activity as a biomarker of hepatic apoptosis in nonalcoholic fatty liver disease. *Biomark Med* 2011; **5**: 813-815 [PMID: 22103615]
40 **Canbakan B**, Senturk H, Canbakan M, Toptas T, Tabak O, Balci H, Olgac V, Ozbay G. Is alanine aminotransferase level a surrogate biomarker of hepatic apoptosis in nonalcoholic fatty liver disease? *Biomark Med* 2010; **4**: 205-214 [PMID: 20406065]
41 **Tahan V**, Canbakan B, Balci H, Dane F, Akin H, Can G, Hatemi I, Olgac V, Sonsuz A, Ozbay G, Yurdakul I, Senturk H. Serum gamma-glutamyltranspeptidase distinguishes nonalcoholic fatty liver disease at high risk. *Hepatogastroenterology* 2008; **55**: 1433-1438 [PMID: 18795706]
42 **Ipekci SH**, Basaranoglu M, Sonsuz A. The fluctuation of serum levels of aminotransferase in patients with nonalcoholic steatohepatitis. *J Clin Gastroenterol* 2003; **36**: 371 [PMID: 12642751]
43 **Sonsuz A**, Basaranoglu M, Ozbay G. Relationship between aminotransferase levels and histopathological findings in patients with nonalcoholic steatohepatitis. *Am J Gastroenterol* 2000; **95**: 1370-1371 [PMID: 10811364]
44 **Basaranoglu M**, Acbay O, Sonsuz A. A controlled trial of gemfibrozil in the treatment of patients with nonalcoholic steatohepatitis. *J Hepatol* 1999; **31**: 384 [PMID: 10453959]
45 **Bataller R**, Brenner DA. Liver fibrosis. *J Clin Invest* 2005; **115**: 209-218 [PMID: 15690074]
46 **Friedman SL**. Hepatic stellate cells: protean, multifunctional, and enigmatic cells of the liver. *Physiol Rev* 2008; **88**: 125-172 [PMID: 18195085 DOI: 10.1152/physrev.00013.2007]
47 **Trinchet JC**, Hartmann DJ, Pateron D, Laarif M, Callard P, Ville G, Beaugrand M. Serum type I collagen and N-terminal peptide of type III procollagen in chronic hepatitis. Relationship to liver histology and conventional liver tests. *J Hepatol* 1991; **12**: 139-144 [PMID: 1711064 DOI: 10.1016/0168-8278(91)90929-6]
48 **Teare JP**, Sherman D, Greenfield SM, Simpson J, Bray G, Catterall AP, Murray-Lyon IM, Peters TJ, Williams R, Thompson RP. Comparison of serum procollagen III peptide concentrations and PGA index for assessment of hepatic fibrosis. *Lancet* 1993; **342**: 895-898 [PMID: 8105167 DOI: 10.1016/0140-6736(93)91946-J]
49 **Gallorini A**, Plebani M, Pontisso P, Chemello L, Masiero M, Mantovani G, Alberti A. Serum markers of hepatic fibrogenesis in chronic hepatitis type C treated with alfa-2A interferon. *Liver* 1994; **14**: 257-264 [PMID: 7997085 DOI: 10.1111/j.1600-0676.1994.tb00085.x]
50 **Yabu K**, Kiyosawa K, Mori H, Matsumoto A, Yoshizawa K, Tanaka E, Furuta S. Serum collagen type IV for the assessment of fibrosis and resistance to interferon therapy in chronic hepatitis C. *Scand J Gastroenterol* 1994; **29**: 474-479 [PMID: 7518613 DOI: 10.3109/00365529409096841]
51 **Walsh KM**, Timms P, Campbell S, MacSween RN, Morris AJ. Plasma levels of matrix metalloproteinase-2 (MMP-2) and tissue inhibitors of metalloproteinases -1 and -2 (TIMP-1 and TIMP-2) as noninvasive markers of liver disease in chronic hepatitis C: comparison using ROC analysis. *Dig Dis Sci* 1999; **44**: 624-630 [PMID: 10080160]
52 **Mas VR**, Fisher RA, Archer KJ, Maluf DG. Proteomics and liver fibrosis: identifying markers of fibrogenesis. *Expert Rev Proteomics* 2009; **6**: 421-431 [PMID: 19681677 DOI: 10.1586/epr.09.59]
53 **Juran BD**, Lazaridis KN. Applying genomics to the study of complex disease. *Semin Liver Dis* 2007; **27**: 3-12 [PMID: 17295173 DOI: 10.1055/s-2006-960167]
54 **Hamid S**, Aquilina JW, Davidson W, Dhabuwala CB. Arteriovenous malformation of scrotum: a case report. *J Urol* 1992; **147**: 160-162 [PMID: 1729517 DOI: 10.1055/s-2006-960169]
55 **Juran BD**, Lazaridis KN. Genomics and complex liver disease: Challenges and opportunities. *Hepatology* 2006; **44**: 1380-1390 [PMID: 17133459 DOI: 10.1002/hep.21453]
56 **Boess F**, Kamber M, Romer S, Gasser R, Muller D, Albertini S, Suter L. Gene expression in two hepatic cell lines, cultured primary hepatocytes, and liver slices compared to the in vivo liver gene expression in rats: possible implications for toxicogenomics use of in vitro systems. *Toxicol Sci* 2003; **73**: 386-402 [PMID: 12657743 DOI: 10.1093/toxsci/kfg064]
57 **Kuramitsu Y**, Nakamura K. Current progress in proteomic study of hepatitis C virus-related human hepatocellular carcinoma. *Expert Rev Proteomics* 2005; **2**: 589-601 [PMID: 16097891 DOI: 10.1586/14789450.2.4.589]
58 **Tian Q**, Stepaniants SB, Mao M, Weng L, Feetham MC, Doyle MJ, Yi EC, Dai H, Thorsson V, Eng J, Goodlett D, Berger JP, Gunter B, Linseley PS, Stoughton RB, Aebersold R, Collins SJ, Hanlon WA, Hood LE. Integrated genomic and proteomic analyses of gene expression in Mammalian cells. *Mol Cell Proteomics* 2004; **3**: 960-969 [PMID: 15238602 DOI: 10.1074/mcp.M400055-MCP200]
59 **Pollack JR**, Perou CM, Alizadeh AA, Eisen MB, Pergamenschikov A, Williams CF, Jeffrey SS, Botstein D, Brown PO. Genome-wide analysis of DNA copy-number changes using cDNA microarrays. *Nat Genet* 1999; **23**: 41-46 [PMID: 10471496 DOI: 10.1038/14385]
60 **Sellick GS**, Longman C, Tolmie J, Newbury-Ecob R, Geenhalgh L, Hughes S, Whiteford M, Garrett C, Houlston RS. Genomewide linkage searches for Mendelian disease loci can be efficiently conducted using high-density SNP genotyping arrays. *Nucleic Acids Res* 2004; **32**: e164 [PMID: 15561999 DOI: 10.1093/nar/gnh163]
61 **Wang DG**, Fan JB, Siao CJ, Berno A, Young P, Sapolsky R, Ghandour G, Perkins N, Winchester E, Spencer J, Kruglyak L, Stein L, Hsie L, Topaloglou T, Hubbell E, Robinson E, Mittmann M, Morris MS, Shen N, Kilburn D, Rioux J, Nusbaum C, Rozen S, Hudson TJ, Lipshutz R, Chee M, Lander ES. Large-scale identification, mapping, and genotyping of single-nucleotide polymorphisms in the human genome. *Science* 1998; **280**: 1077-1082 [PMID: 9582121 DOI: 10.1126/science.280.5366.1077]
62 **Johnson JM**, Castle J, Garrett-Engele P, Kan Z, Loerch PM, Armour CD, Santos R, Schadt EE, Stoughton R, Shoemaker DD. Genome-wide survey of human alternative pre-mRNA splicing with exon junction microarrays. *Science* 2003; **302**: 2141-2144 [PMID: 14684825 DOI: 10.1126/science.1090100]
63 **Kristensen DB**, Kawada N, Imamura K, Miyamoto Y, Tateno C, Seki S, Kuroki T, Yoshizato K. Proteome analysis of rat hepatic stellate cells. *Hepatology* 2000; **32**: 268-277 [PMID: 10915733 DOI: 10.1053/jhep.2000.9322]
64 **Kawada N**, Kristensen DB, Asahina K, Nakatani K, Minamiyama Y, Seki S, Yoshizato K. Characterization of a stellate cell activation-associated protein (STAP) with peroxidase activity found in rat hepatic stellate cells. *J Biol Chem* 2001; **276**: 25318-25323 [PMID: 11320098 DOI: 10.1074/jbc.M102630200]
65 **Deng X**, Liang J, Lin ZX, Wu FS, Zhang YP, Zhang ZW. Natural taurine promotes apoptosis of human hepatic stellate cells in proteomics analysis. *World J Gastroenterol* 2010; **16**: 1916-1923 [PMID: 20397272 DOI: 10.3748/wjg.v16.i15.1916]
66 **Villanueva A**, Newell P, Chiang DY, Friedman SL, Llovet JM. Genomics and signaling pathways in hepatocellular carcinoma. *Semin Liver Dis* 2007; **27**: 55-76 [PMID: 17295177

DOI: 10.1055/s-2006-960171]
67 **Makridakis M**, Vlahou A. Secretome proteomics for discovery of cancer biomarkers. *J Proteomics* 2010; **73**: 2291-2305 [PMID: 20637910 DOI: 10.1016/j.jprot.2010.07.001]
68 **Friedman SL**. Mechanisms of hepatic fibrogenesis. *Gastroenterology* 2008; **134**: 1655-1669 [PMID: 18471545 DOI: 10.1053/j.gastro.2008.03.003]
69 **Hammel P**, Couvelard A, O'Toole D, Ratouis A, Sauvanet A, Fléjou JF, Degott C, Belghiti J, Bernades P, Valla D, Ruszniewski P, Lévy P. Regression of liver fibrosis after biliary drainage in patients with chronic pancreatitis and stenosis of the common bile duct. *N Engl J Med* 2001; **344**: 418-423 [PMID: 11172178 DOI: 10.1056/NEJM200102083440604]
70 **Bonis PA**, Friedman SL, Kaplan MM. Is liver fibrosis reversible? *N Engl J Med* 2001; **344**: 452-454 [PMID: 11172184 DOI: 10.1056/NEJM200102083440610]

P- Reviewer Liu HK **S- Editor** Lv S **L- Editor** Kerr C
E- Editor Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i8.1166

World J Gastroenterol 2013 February 28; 19(8): 1166-1172
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

TOPIC HIGHLIGHT

Metin Basaranoglu, MD, PhD, Associate Professor, *Series Editor*

Fructose as a key player in the development of fatty liver disease

Metin Basaranoglu, Gokcen Basaranoglu, Tevfik Sabuncu, Hakan Sentürk

Metin Basaranoglu, Hakan Sentürk, Department of Gastroenterology and Hepatology, Bezmialem Vakif University, Istanbul 34400, Turkey
Gokcen Basaranoglu, Department of Anaesthesiology, Bezmialem Vakif University, Istanbul 34400, Turkey
Tevfik Sabuncu, Department of Endocrinology, Harran University, Sanliurfa 68000, Turkey
Author contributions: Basaranoglu M designed the research, performed the literature search and wrote the paper; Basaranoglu G, Sabuncu T and Sentürk H commented on the paper.
Correspondence to: Metin Basaranoglu, MD, Department of Gastroenterology and Hepatology, Bezmialem Vakif University, Istanbul 34400, Turkey. metin_basaranoglu@yahoo.com
Telephone: +90-312-5878030 Fax: +90-312-5540570
Received: July 20, 2012 Revised: September 20, 2012
Accepted: November 14, 2012
Published online: February 28, 2013

Abstract

We aimed to investigate whether increased consumption of fructose is linked to the increased prevalence of fatty liver. The prevalence of nonalcoholic steatohepatitis (NASH) is 3% and 20% in nonobese and obese subjects, respectively. Obesity is a low-grade chronic inflammatory condition and obesity-related cytokines such as interleukin-6, adiponectin, leptin, and tumor necrosis factor-α may play important roles in the development of nonalcoholic fatty liver disease (NAFLD). Additionally, the prevalence of NASH associated with both cirrhosis and hepatocellular carcinoma was reported to be high among patients with type 2 diabetes with or without obesity. Our research group previously showed that consumption of fructose is associated with adverse alterations of plasma lipid profiles and metabolic changes in mice, the American Lifestyle-Induced Obesity Syndrome model, which included consumption of a high-fructose corn syrup in amounts relevant to that consumed by some Americans. The observation reinforces the concerns about the role of fructose in the obesity epidemic. Increased availability of fructose (*e.g.*, high-fructose corn syrup) increases not only abnormal glucose flux but also fructose metabolism in the hepatocyte. Thus, the anatomic position of the liver places it in a strategic buffering position for absorbed carbohydrates and amino acids. Fructose was previously accepted as a beneficial dietary component because it does not stimulate insulin secretion. However, since insulin signaling plays an important role in central mechanisms of NAFLD, this property of fructose may be undesirable. Fructose has a selective hepatic metabolism, and provokes a hepatic stress response involving activation of c-Jun N-terminal kinases and subsequent reduced hepatic insulin signaling. As high fat diet alone produces obesity, insulin resistance, and some degree of fatty liver with minimal inflammation and no fibrosis, the fast food diet which includes fructose and fats produces a gene expression signature of increased hepatic fibrosis, inflammation, endoplasmic reticulum stress and lipoapoptosis. Hepatic *de novo* lipogenesis (fatty acid and triglyceride synthesis) is increased in patients with NAFLD. Stable-isotope studies showed that increased *de novo* lipogenesis (DNL) in patients with NAFLD contributed to fat accumulation in the liver and the development of NAFLD. Specifically, DNL was responsible for 26% of accumulated hepatic triglycerides and 15%-23% of secreted very low-density lipoprotein triglycerides in patients with NAFLD compared to an estimated less than 5% DNL in healthy subjects and 10% DNL in obese people with hyperinsulinemia. In conclusion, understanding the underlying causes of NAFLD forms the basis for rational preventive and treatment strategies of this major form of chronic liver disease.

© 2013 Baishideng. All rights reserved.

Key words: Nonalcoholic; Fatty liver; Diabetes; Insulin resistance; Cytokines; Obesity; Fructose

Basaranoglu M, Basaranoglu G, Sabuncu T, Sentürk H. Fructose as a key player in the development of fatty liver disease. *World J Gastroenterol* 2013; 19(8): 1166-1172 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i8/1166.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i8.1166

INTRODUCTION

Excessive accumulation of triglycerides in hepatocytes in the absence of significant alcohol consumption occurs in about 20%-30% of adults[1-5]. Excessive fat in the liver, called nonalcoholic fatty liver disease (NAFLD), predisposes to the development of nonalcoholic steatohepatitis (NASH). NASH constitutes the subset of NAFLD that is most worrisome because it is a significant risk factor for developing cirrhosis and its complications, including hepatocellular carcinoma (HCC)[6-9]. Because the accumulation of excess fat in the liver is a prerequisite for the development of NASH, understanding the underlying causes of NAFLD forms the basis for rational preventive and treatment strategies of this major form of chronic liver disease.

Obesity is a low-grade chronic inflammatory condition and obesity-related cytokines such as interleukin-6 (IL-6), adiponectin, leptin, and tumor necrosis factor (TNF) α may play important roles in the development of NAFLD. The prevalence of NASH is 3% and 20% in nonobese and obese subjects, respectively. Additionally, the prevalence of NASH associated with both cirrhosis and HCC was reported to be high among patients with type-2 diabetes with or without obesity.

OBESITY EPIDEMIC

A balance exists between energy demand and intake in the human body. Obesity is one of the major abnormalities of this well preserved equilibrium. Obesity, and its consequences such as insulin resistance and the metabolic syndrome, is a growing threat to the health of people in developed nations[10]. A diet based on high cholesterol, high saturated fat, and high fructose (cafeteria or fast food type) recapitulates features of the metabolic syndrome and NASH with progressive fibrosis (Figure 1).

"FAST FOOD" OR "CAFETERIA" TYPE DIET COMPOSED OF HIGH SATURATED FATS, CHOLESTEROL, AND FRUCTOSE

The basis of the composition of "fast food" or "cafeteria" style food is high saturated fats, cholesterol, and fructose[11]. As the high fat diet produces obesity, insulin resistance, and some hepatic steatosis with minimal inflammation and no fibrosis, the fast food diet produces a gene expression signature of increased hepatic fibrosis, inflammation, endoplasmic reticulum stress and lipoapoptosis (Figure 2). Our research group previously

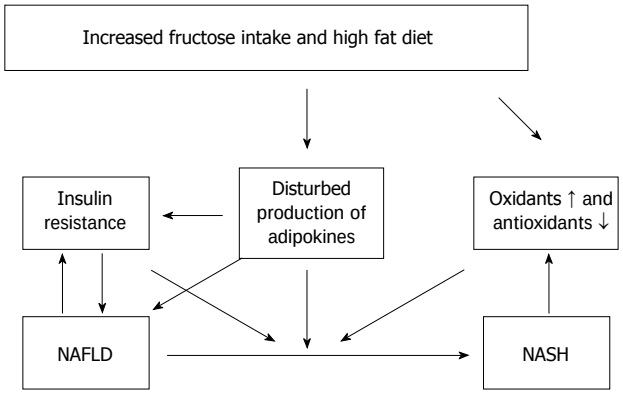


Figure 1 Diet based on high cholesterol, high saturated fat, and high fructose (cafeteria or fast food type) recapitulates features of the metabolic syndrome and nonalcoholic fatty liver disease and nonalcoholic steatohepatitis with progressive fibrosis in human and mice. NAFLD: Nonalcoholic fatty liver disease; NASH: Nonalcoholic steatohepatitis.

showed that consumption of fructose is associated with adverse alterations of plasma lipid profiles and metabolic changes in mice, the American Lifestyle-Induced Obesity Syndrome (ALIOS) model, which included consumption of a high-fructose corn syrup (HFCS) in amounts relevant to that consumed by some Americans[11]. The observation that the ALIOS mice indeed consumed a greater quantity of food beyond the additional calories consumed from the HFCS when fed HFCS compared with control water supports this observation and reinforces the concerns about the role of fructose in the obesity epidemic[12-15]. In adolescents, higher fructose consumption is associated with multiple markers of cardiometabolic risk, but it appears that these relationships are mediated by visceral obesity.

The most commonly used HFCS in soft drinks and other carbohydrate-sweetened beverages is a blend composed of 55% fructose, 41% glucose, and 4% complex polysaccharides. Fructose has increasingly been used as a sweetener since the introduction of high-fructose corn syrups in the 1960s[10-13,16] and is now an abundant source of dietary carbohydrate in the United States. The annual per capita consumption of extrinsic or added fructose was approximately 0.2 kg in 1970 to approximately 28 kg in 1997. This increased consumption has been linked to the increased prevalence of obesity, type 2 diabetes and fatty liver in the United States.

The liver is exquisitely sensitive to changes in nutrient delivery and is uniquely suited to metabolize ingested simple sugars, such as fructose and glucose[13,14]. Stress-activated protein kinases, principally the c-Jun N-terminal kinases (JNK), are activated by cell stress-inducing stimuli. Increased fructose supply provokes a hepatic stress response involving activation of JNK and subsequent reduced hepatic insulin signaling.

UNIQUE METABOLISM OF FRUCTOSE

Fructose, glucose, and galactose are the 3 major dietary

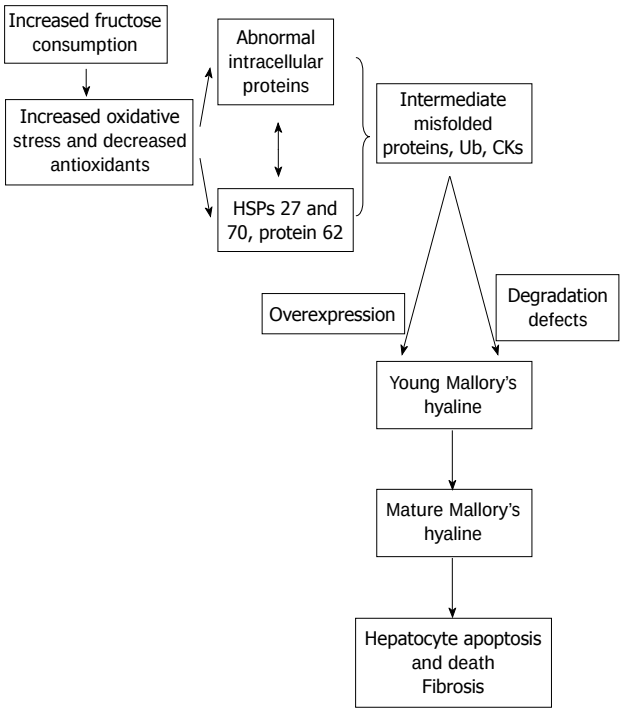


Figure 2 As the high fat diet produces obesity, insulin resistance, and some hepatic steatosis with minimal inflammation with no fibrosis, the fast food diet produces a gene expression signature of increased hepatic fibrosis, inflammation, and endoplasmic reticulum stress and lipoapoptosis. HSP: Heat shock proteins.

monosaccharides. Sucrose (glucose-fructose), lactose (glucose-galactose), and maltose (glucose-glucose) are the major disaccharides. Dietary fructose occurs in 2 forms: mono- or disaccharide. The rate of fructose absorption appears to be between that of mannose and glucose[12-15]. Fructose is absorbed by carrier-mediated facilitated diffusion, an energy-dependent process. The fructose carrier is a member of the glucose transport family and is referred to as glucose transporter 5. Sucrose is cleaved to glucose and fructose by sucrase, an enzyme located in the brush border of small intestine enterocytes.

Fructose was previously accepted as a beneficial dietary component because it does not stimulate insulin secretion. However, since insulin signaling plays an important role in the central mechanisms of NAFLD, this property of fructose may be undesirable[13-15]. Additionally, fructose may prevent suppression of ghrelin secretion, resulting in impaired satiety mechanisms[14]. In large quantities, fructose can also stress the liver by depleting hepatic energy supplies. Normal subjects and patients with NASH exhibited a similar depletion of hepatic ATP levels after an injection of fructose, but recovery of ATP levels after depletion was slower in NASH patients compared with healthy controls. A mixture of fructose and glucose might induce metabolic abnormalities that differ from sucrose, a disaccharide cleaved to fructose and glucose in the small intestine.

Phosphorylation of glucose by glucokinase is a rate-determining step in hepatic glucose metabolism. In contrast to glucose, phosphorylation of fructose in the liver occurs *via* the enzyme fructokinase. In addition, the metabolism of fructose 1-phosphate in the liver occurs independently of phosphofructokinase, a second rate-determining step in glucose metabolism[13-15]. As a result, the liver is the primary site of fructose extraction and metabolism, with extraction approaching 50% to 70% of fructose delivery. Therefore, increased availability of fructose (*e.g.*, high-fructose corn syrup) will increase not only abnormal glucose flux but also fructose metabolism in the hepatocyte. Thus, the anatomic position of the liver places it in a strategic buffering position for absorbed carbohydrates and amino acids.

Fructose extraction and metabolism by the liver are exceptionally high compared to glucose due both to the extensive amount of fructokinase that phosphorylates fructose to fructose 1-phosphate in the liver and to the subsequent metabolism of fructose 1-phosphate at the triose phosphate level, which bypasses flux control at phosphofructokinase[13-16]. Previous studies comparing the metabolism of fructose and glucose in postabsorptive humans over short intervals have shown that fructose is used faster than glucose and that more is converted to liver glycogen. Fructose oxidation represented a significant portion of fructose metabolism, accounting for 56% to 59% of the ingested fructose and approximately 33% of the infused fructose. It is likely that extrahepatic lactate oxidation subsequent to hepatic fructolysis contributed significantly to the estimated rate of fructose oxidation. Thus, increments in fructose after infusion produced immediate changes in hepatic and extrahepatic substrate metabolism, but did not induce changes in overall glucose production. An immediate fructose infusion in humans induced both hepatic and extrahepatic insulin resistance. These data are consistent with the notion that high concentrations of fructose elicit adaptations in the liver that include metabolic intermediates, gene expression, and insulin action.

SYSTEMIC AND HEPATIC INSULIN RESISTANCE IN NAFLD

While insulin receptor defects cause severe insulin resistance, most patients with insulin resistance have impaired post-receptor intracellular insulin signaling. Insulin binds α-subunits of its receptor, which is a cell surface receptor on the major insulin sensitive cells such as skeletal muscle, adipocytes, and hepatocytes, leading to autophosphorylation of the cytoplasmic domains (β-subunits) of the receptor[2-5,17]. The insulin receptor has intrinsic tyrosine kinase activity activated by insulin binding and the autophosphorylated receptor activates its substrates that include insulin receptor substrate (IRS)-1, IRS-2, Src homology collagen, and adaptor protein with a pleckstrin homology and Src homology 2 domain by tyrosine phosphorylation. These phosphory-

lated docking proteins bind and activate several downstream components of the insulin signaling pathways. Activated IRS-1 associates with phosphatidyl inositol 3-kinase, which then activates Akt. These events and insulin-dependent inhibition of hepatic glucose output maintain glucose homeostasis. Insulin also affects glucose homeostasis indirectly by its regulatory effect on lipid metabolism. Any interference in this insulin signaling pathway causes glucotoxicity, insulin resistance and, when islet beta cells are capable of responding, compensatory hyperinsulinemia.

Hepatic expression of insulin receptor protein in humans and the levels of both IRS-1 and IRS-2 in animals were decreased in chronic hyperinsulinemic states[11]. IRS-1 was more closely linked to glucose homeostasis with the regulation of glucokinase expression while IRS-2 was more closely linked to lipogenesis with the regulation of lipogenic enzymes sterol regulatory element-binding protein-1c (SREBP-1c) and fatty acid synthase[18,19]. Additional physiological roles of insulin include regulating the metabolism of macronutrients and stimulating cellular growth. Insulin activates synthesis and inhibits catabolism of lipids while shutting off the synthesis of glucose in the liver.

Adipose tissue is one of the major insulin sensitive organs in the human body and the process of differentiation of preadipocytes to adipocytes is induced by insulin[17,18]. Within the adipose tissue, insulin stimulates triglyceride synthesis and inhibits lipolysis by upregulating lipoprotein lipase activity which is the most sensitive pathway in insulin action, facilitating free fatty acid uptake and glucose transport, inhibiting hormone sensitive lipase, and increasing gene expression of lipogenic enzymes.

PROINFLAMMATORY SIGNALING IN INSULIN RESISTANCE

Protein kinase C theta (PKCθ) and inhibitor κB kinase β (IKK-β) are two proinflammatory kinases involved in insulin downstream signaling[17,18]. They are activated by lipid metabolites such as high plasma free fatty acid concentrations and there is a positive relationship between the activation of PKCθ and the concentration of intermediate fatty acid products. PKCθ activates both IKK-β and JNK, leading to increased Ser 307 phosphorylation of IRS-1 and insulin resistance. Activation or overexpression of IKK-β diminishes insulin signaling and causes insulin resistance whereas inhibition of IKK-β improves insulin sensitivity. Inhibition of IKK-β activity prevented insulin resistance due to TNF-α in cultured cells. IKK-β phosphorylates the inhibitor of nuclear factor kappa B (NF-κB), leading to the activation of NF-κB by the translocation of NF-κB to the nucleus. NF-κB is an inducible transcription factor and promotes specific gene expression in the nucleus. For example, NF-κB regulates the production of multiple inflammatory mediators, such as TNF-α and IL-6. TNF-α and reactive oxygen species could also activate NF-κB[19-22]. In contrast, antioxidants inhibit this activation. NF-κB has both apoptotic and anti-apoptotic effects. The finding that NF-κB deficient mice were protected from high-fat diet-induced insulin resistance suggests that NF-κB directly participates in processes that impair insulin signaling. High-dose salicylates also inhibit NF-κB and subsequently improve insulin sensitivity. These subsequently promote hepatic and systemic insulin resistance. The study group also showed that these results were reversed by curcumin which inhibits NF-κB activity. Curcumin also has the ability to induce antioxidant enzymes and scavenge ROS.

Suppressors of cytokine signaling (SOCS) and inducible nitric oxide synthase are two inflammatory mediators recently recognized to play a role in insulin signaling[23-25]. Induction of SOCS proteins (SOCS 1-7 and cytokine-inducible src homology 2 domain-containing protein) by proinflammatory cytokines might contribute to the cytokine-mediated insulin resistance in obese subjects[26-30]. In fact, the isoforms of SOCS are the members of a negative feedback loop of cytokine signaling, regulated by both phosphorylation and transcription events. SOCS-1, and particularly SOCS-3, are involved in the inhibition of insulin signaling either by interfering with IRS-1 and IRS-2 tyrosine phosphorylation or by the degradation of their substrates. SOCS-3 might also regulate central leptin action and play a role in the leptin resistance of obese human subjects. SOCS might be a link between leptin and insulin resistance because insulin levels are increased in leptin resistant conditions due to the diminished insulin suppression effect of leptin because of insufficient leptin levels. Moreover, SOCS proteins might involve insulin/insulin like growth factor-1 signaling. SOCS-1 knockout mice showed low glucose concentrations and increased insulin sensitivity. SREBP-1c is one of the key mediators of lipid synthesis from glucose and other precursors (*de novo* lipogenesis) in the liver. Indeed, SOCS proteins markedly induce *de novo* fatty acid synthesis in the liver by both the up-regulation of SREBP-1c and persistent insulin resistance with hyperinsulinemia which stimulates SREBP-1c-mediated gene expression. Liver is the insulin clearance organ. Thus, decreased insulin clearance in patients with NAFLD further elevates insulin levels in the circulation and *de novo* lipogenesis in the liver. SOCS-1 and SOCS-3 may exert these effects by inhibiting signal transduction and activator of transcription proteins (STAT), particularly STAT-3, *via* binding Janus tyrosine Kinase (JAK) tyrosine kinase because this binding diminishes the phosphorylation ability of JAK kinase to STAT-3. STAT-3 inhibits the activation of SREBP-1c. Specific STAT-3 knockout mice showed markedly increased expression of SREBP-1c and subsequently increased fat content in the liver. Conversely, inhibition of SOCS proteins, particularly SOCS-3, improved both insulin sensitivity and the activation of

SREBP-1c which eventually reduced liver steatosis and hypertriglyceridemia in db/db mice.

Nitric oxide synthase-2 (NOS2) or inducible nitric oxide synthase (iNOS) production are also induced by proinflammatory cytokines[31]. A high-fat diet in rats causes up-regulation of iNOS mRNA expression and increases iNOS protein activity. Increased production of NOS2 might reduce insulin action in both muscle and pancreas and decreased iNOS activity protects muscles from the high-fat diet induced insulin resistance. It was also shown that leptin deficient ob/ob mice without iNOS were more insulin sensitive than ob wild-type mice. Thus, the production of nitric oxide may be one link between inflammation and insulin resistance.

SOURCES OF LIVER FAT

Accumulation of triglycerides as fat droplets within the cytoplasm of hepatocytes is a prerequisite for subsequent events of NASH. Accumulation of excess triglyceride in hepatocytes is generally the result of increased delivery of non-esterified fatty acids (NEFAs), increased synthesis of NEFAs, impaired intracellular catabolism of NEFAs, impaired secretion as triglyceride, or a combination of these abnormalities[32]. Recent techniques, such as isotope methodologies, multiple-stable-isotope approach and gas chromatography/mass spectrometry, provided valuable information regarding the fate of fatty acids during both fasting and fed states[33] such as the relative contribution of three fatty acid sources to the accumulated fat in NAFLD: adipose tissue, *de novo* lipogenesis, and dietary fat. Additionally, these studies reported that the plasma NEFA pool is the main contributor of both hepatic triglycerides in the fasting state and very low-density lipoproteins (VLDL)-triglycerides in both fasting and fed states.

DYSREGULATED PERIPHERAL LIPOLYSIS

A study showed that adipose tissue makes a major contribution to the plasma NEFA pool, contributing 81.7% in the fasted state and 61.7% in the fed state[33]. Additionally, the contribution of dietary lipids to the plasma NEFA pool was found to be only 26.2% and 10.4% in fed and fasted states, respectively, in the same study. Finally, the contribution of newly made fatty acids (originating from the adipose tissue and liver) to the plasma NEFA pool was 7.0% and 9.4% for the fasted and fed states, respectively.

The liver takes up free fatty acids from the circulating NEFA pool and the rate of uptake depends only on the plasma free fatty acid concentrations. Hepatic NEFA uptake continues despite increased hepatic content of fatty acids and triglycerides[34]. The concentration of free fatty acids is increased in the portal circulation rapidly when lipolysis occurs in visceral adipose tissue. These products directly flux to the liver *via* the splanchnic circulation and contribute to hepatic triglyceride synthesis, NAFLD, and hepatic insulin resistance.

HEPATIC *DE NOVO* LIPOGENESIS

Hepatic *de novo* lipogenesis (fatty acid and triglyceride synthesis) is increased in patients with NAFLD[35-39]. Stable-isotope studies showed that increased *de novo* lipogenesis (DNL) in patients with NAFLD contributed to fat accumulation in the liver and the development of NAFLD[33]. Specifically, DNL was responsible for 26% of accumulated hepatic triglycerides and 15%-23% of secreted VLDL triglycerides in patients with NAFLD compared to an estimated less than 5% DNL in healthy subjects and 10% DNL in obese people with hyperinsulinemia. Interestingly, Donnelly and colleagues demonstrated the similarity between VLDL-triglycerides and hepatic-triglycerides regarding contributions of fatty acid sources (62% *vs* 59% for NEFA contribution, respectively; 23% *vs* 26% for DNL, respectively; and 15% *vs* 15% for dietary fatty acids, respectively) in NAFLD patients. Substrates used for the synthesis of newly made fatty acids by DNL are primarily glucose, fructose, and amino acids; oleic acid (18:1, a ω-6 monounsaturated fatty acid, which is relatively resistant to peroxidation) is the major end product of *de novo* fatty acid synthesis[40-42]. Moreover, simple sugars have the ability to stimulate lipogenesis[33]. Ingested carbohydrates are a major stimulus for hepatic delayed neuronal loss and are thus more likely to directly contribute to NAFLD than dietary fat intake[43-46].

In conclusion, fructose has increasingly been used as a sweetener since the introduction of high-fructose corn syrups in the 1960s and is now an abundant source of dietary carbohydrate in the United States[47-50]. The most commonly used HFCS in soft drinks and other carbohydrate-sweetened beverages is a blend composed of 55% fructose, 41% glucose, and 4% complex polysaccharides[51-55]. This increased consumption has been linked to the increased prevalence of obesity and type 2 diabetes and fatty liver in the United States by increased fructose supply, which provokes a hepatic stress response involving activation of JNK and subsequent reduced hepatic insulin signaling[56-59]. Understanding the underlying causes of NAFLD forms the basis for rational preventive and treatment strategies of this major form of chronic liver disease.

REFERENCES

1 **Matteoni CA**, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 1999; **116**: 1413-1419 [PMID: 10348825]

2 **Marchesini G**, Brizi M, Morselli-Labate AM, Bianchi G, Bugianesi E, McCullough AJ, Forlani G, Melchionda N. Association of nonalcoholic fatty liver disease with insulin resistance. *Am J Med* 1999; **107**: 450-455 [PMID: 10569299 DOI: 10.1016/S0002-9343(99)00271-5]

3 **Marchesini G**, Brizi M, Bianchi G, Tomassetti S, Bugianesi E, Lenzi M, McCullough AJ, Natale S, Forlani G, Melchionda N.

Nonalcoholic fatty liver disease: a feature of the metabolic syndrome. *Diabetes* 2001; **50**: 1844-1850 [PMID: 11473047 DOI: 10.2337/diabetes.50.8.1844]
4 **Seppälä-Lindroos A**, Vehkavaara S, Häkkinen AM, Goto T, Westerbacka J, Sovijärvi A, Halavaara J, Yki-Järvinen H. Fat accumulation in the liver is associated with defects in insulin suppression of glucose production and serum free fatty acids independent of obesity in normal men. *J Clin Endocrinol Metab* 2002; **87**: 3023-3028 [PMID: 12107194 DOI: 10.1210/jc.87.7.3023]
5 **Pagano G**, Pacini G, Musso G, Gambino R, Mecca F, Depetris N, Cassader M, David E, Cavallo-Perin P, Rizzetto M. Nonalcoholic steatohepatitis, insulin resistance, and metabolic syndrome: further evidence for an etiologic association. *Hepatology* 2002; **35**: 367-372 [PMID: 11826410 DOI: 10.1053/jhep.2002.30690]
6 **Marchesini G**, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, Natale S, Vanni E, Villanova N, Melchionda N, Rizzetto M. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. *Hepatology* 2003; **37**: 917-923 [PMID: 12668987 DOI: 10.1053/jhep.2003.50161]
7 **Caldwell SH**, Oelsner DH, Iezzoni JC, Hespenheide EE, Battle EH, Driscoll CJ. Cryptogenic cirrhosis: clinical characterization and risk factors for underlying disease. *Hepatology* 1999; **29**: 664-669 [PMID: 10051466 DOI: 10.1002/hep.510290347]
8 **Poonawala A**, Nair SP, Thuluvath PJ. Prevalence of obesity and diabetes in patients with cryptogenic cirrhosis: a case-control study. *Hepatology* 2000; **32**: 689-692 [PMID: 11003611 DOI: 10.1053/jhep.2000.17894]
9 **Charlton M**, Kasparova P, Weston S, Lindor K, Maor-Kendler Y, Wiesner RH, Rosen CB, Batts KP. Frequency of nonalcoholic steatohepatitis as a cause of advanced liver disease. *Liver Transpl* 2001; **7**: 608-614 [PMID: 11460228 DOI: 10.1053/jlts.2001.25453]
10 **Keaney JF**, Larson MG, Vasan RS, Wilson PW, Lipinska I, Corey D, Massaro JM, Sutherland P, Vita JA, Benjamin EJ. Obesity and systemic oxidative stress: clinical correlates of oxidative stress in the Framingham Study. *Arterioscler Thromb Vasc Biol* 2003; **23**: 434-439 [PMID: 12615693]
11 **Tetri LH**, Basaranoglu M, Brunt EM, Yerian LM, Neuschwander-Tetri BA. Severe NAFLD with hepatic necroinflammatory changes in mice fed trans fats and a high-fructose corn syrup equivalent. *Am J Physiol Gastrointest Liver Physiol* 2008; **295**: G987-G995 [PMID: 18772365 DOI: 10.1152/ajpgi.90272.2008]
12 **Elliott SS**, Keim NL, Stern JS, Teff K, Havel PJ. Fructose, weight gain, and the insulin resistance syndrome. *Am J Clin Nutr* 2002; **76**: 911-922 [PMID: 12399260]
13 **James J**, Thomas P, Cavan D, Kerr D. Preventing childhood obesity by reducing consumption of carbonated drinks: cluster randomised controlled trial. *BMJ* 2004; **328**: 1237 [PMID: 15107313]
14 **Teff KL**, Elliott SS, Tschöp M, Kieffer TJ, Rader D, Heiman M, Townsend RR, Keim NL, D'Alessio D, Havel PJ. Dietary fructose reduces circulating insulin and leptin, attenuates postprandial suppression of ghrelin, and increases triglycerides in women. *J Clin Endocrinol Metab* 2004; **89**: 2963-2972 [PMID: 15181085]
15 **Faeh D**, Minehira K, Schwarz JM, Periasamy R, Park S, Tappy L. Effect of fructose overfeeding and fish oil administration on hepatic de novo lipogenesis and insulin sensitivity in healthy men. *Diabetes* 2005; **54**: 1907-1913 [PMID: 15983189]
16 **Dhingra R**, Sullivan L, Jacques PF, Wang TJ, Fox CS, Meigs JB, D'Agostino RB, Gaziano JM, Vasan RS. Soft drink consumption and risk of developing cardiometabolic risk factors and the metabolic syndrome in middle-aged adults in the community. *Circulation* 2007; **116**: 480-488 [PMID: 17646581]
17 **Weisberg SP**, McCann D, Desai M, Rosenbaum M, Leibel RL, Ferrante AW. Obesity is associated with macrophage accumulation in adipose tissue. *J Clin Invest* 2003; **112**: 1796-1808 [PMID: 14679176]
18 **Xu H**, Barnes GT, Yang Q, Tan G, Yang D, Chou CJ, Sole J, Nichols A, Ross JS, Tartaglia LA, Chen H. Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J Clin Invest* 2003; **112**: 1821-1830 [PMID: 14679177]
19 **Hansel B**, Giral P, Nobecourt E, Chantepie S, Bruckert E, Chapman MJ, Kontush A. Metabolic syndrome is associated with elevated oxidative stress and dysfunctional dense high-density lipoprotein particles displaying impaired antioxidative activity. *J Clin Endocrinol Metab* 2004; **89**: 4963-4971 [PMID: 15472192]
20 **Furukawa S**, Fujita T, Shimabukuro M, Iwaki M, Yamada Y, Nakajima Y, Nakayama O, Makishima M, Matsuda M, Shimomura I. Increased oxidative stress in obesity and its impact on metabolic syndrome. *J Clin Invest* 2004; **114**: 1752-1761 [PMID: 15599400]
21 **Ratziu V**, Bonyhay L, Di Martino V, Charlotte F, Cavallaro L, Sayegh-Tainturier MH, Giral P, Grimaldi A, Opolon P, Poynard T. Survival, liver failure, and hepatocellular carcinoma in obesity-related cryptogenic cirrhosis. *Hepatology* 2002; **35**: 1485-1493 [PMID: 12029634 DOI: 10.1053/jhep.2002.33324]
22 **Ratziu V**, Giral P, Charlotte F, Bruckert E, Thibault V, Theodorou I, Khalil L, Turpin G, Opolon P, Poynard T. Liver fibrosis in overweight patients. *Gastroenterology* 2000; **118**: 1117-1123 [PMID: 10833486]
23 **Stein CJ**, Colditz GA. The epidemic of obesity. *J Clin Endocrinol Metab* 2004; **89**: 2522-2525 [PMID: 15181019]
24 **Hundal RS**, Petersen KF, Mayerson AB, Randhawa PS, Inzucchi S, Shoelson SE, Shulman GI. Mechanism by which high-dose aspirin improves glucose metabolism in type 2 diabetes. *J Clin Invest* 2002; **109**: 1321-1326 [PMID: 12021247]
25 **Seki S**, Kitada T, Sakaguchi H. Clinicopathological significance of oxidative cellular damage in non-alcoholic fatty liver diseases. *Hepatol Res* 2005; **33**: 132-134 [PMID: 16198621]
26 **Marchesini G**, Ridolfi V, Nepoti V. Hepatotoxicity of fast food? *Gut* 2008; **57**: 568-570 [PMID: 18408097 DOI: 10.1136/gut.2007.143958]
27 **Milagro FI**, Campión J, Martínez JA. Weight gain induced by high-fat feeding involves increased liver oxidative stress. *Obesity (Silver Spring)* 2006; **14**: 1118-1123 [PMID: 16899792 DOI: 10.1038/oby.2006.128]
28 **Mozaffarian D**, Katan MB, Ascherio A, Stampfer MJ, Willett WC. Trans fatty acids and cardiovascular disease. *N Engl J Med* 2006; **354**: 1601-1613 [PMID: 16611951 DOI: 10.1056/NEJMra054035]
29 **Feldstein AE**, Werneburg NW, Canbay A, Guicciardi ME, Bronk SF, Rydzewski R, Burgart LJ, Gores GJ. Free fatty acids promote hepatic lipotoxicity by stimulating TNF-alpha expression via a lysosomal pathway. *Hepatology* 2004; **40**: 185-194 [PMID: 15239102 DOI: 10.1002/hep.20283]
30 **Bradbury MW**, Berk PD. Lipid metabolism in hepatic steatosis. *Clin Liver Dis* 2004; **8**: 639-671 [PMID: 15331068]
31 **Wellen KE**, Hotamisligil GS. Inflammation, stress, and diabetes. *J Clin Invest* 2005; **115**: 1111-1119 [PMID: 15864338]
32 **Neuschwander-Tetri BA**, Caldwell SH. Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. *Hepatology* 2003; **37**: 1202-1219 [PMID: 12717402]
33 **Donnelly KL**, Smith CI, Schwarzenberg SJ, Jessurun J, Boldt MD, Parks EJ. Sources of fatty acids stored in liver and secreted via lipoproteins in patients with nonalcoholic fatty liver disease. *J Clin Invest* 2005; **115**: 1343-1351 [PMID: 15864352]
34 **Tamura S**, Shimomura I. Contribution of adipose tissue and de novo lipogenesis to nonalcoholic fatty liver disease. *J Clin*

Invest 2005; **115**: 1139-1142 [PMID: 15864343]
35 **Neuschwander-Tetri BA**, Ford DA, Acharya S, Gilkey G, Basaranoglu M, Tetri LH, Brunt EM. Dietary trans-fatty acid induced NASH is normalized following loss of trans-fatty acids from hepatic lipid pools. *Lipids* 2012; **47**: 941-950 [PMID: 22923371]
36 **Henkel J**, Frede K, Schanze N, Vogel H, Schürmann A, Spruss A, Bergheim I, Püschel GP. Stimulation of fat accumulation in hepatocytes by PGE_2-dependent repression of hepatic lipolysis, β-oxidation and VLDL-synthesis. *Lab Invest* 2012; **92**: 1597-1606 [PMID: 22964849 DOI: 10.1038/labinvest.2012.128]
37 **Arai T**, Kim HJ, Hirako S, Nakasatomi M, Chiba H, Matsumoto A. Effects of dietary fat energy restriction and fish oil feeding on hepatic metabolic abnormalities and insulin resistance in KK mice with high-fat diet-induced obesity. *J Nutr Biochem* 2013; **24**: 267-273 [PMID: 22901684]
38 **Flannery C**, Dufour S, Rabøl R, Shulman GI, Petersen KF. Skeletal muscle insulin resistance promotes increased hepatic de novo lipogenesis, hyperlipidemia, and hepatic steatosis in the elderly. *Diabetes* 2012; **61**: 2711-2717 [PMID: 22829450]
39 **Tappy L**, Lê KA. Does fructose consumption contribute to non-alcoholic fatty liver disease? *Clin Res Hepatol Gastroenterol* 2012; **36**: 554-560 [PMID: 22795319]
40 **Sashidhara KV**, Kumar M, Sonkar R, Singh BS, Khanna AK, Bhatia G. Indole-based fibrates as potential hypolipidemic and antiobesity agents. *J Med Chem* 2012; **55**: 2769-2779 [PMID: 22339404]
41 **Kok BP**, Kienesberger PC, Dyck JR, Brindley DN. Relationship of glucose and oleate metabolism to cardiac function in lipin-1 deficient (fld) mice. *J Lipid Res* 2012; **53**: 105-118 [PMID: 22058427]
42 **Herrema H**, Meissner M, van Dijk TH, Brufau G, Boverhof R, Oosterveer MH, Reijngoud DJ, Müller M, Stellaard F, Groen AK, Kuipers F. Bile salt sequestration induces hepatic de novo lipogenesis through farnesoid X receptor- and liver X receptor alpha-controlled metabolic pathways in mice. *Hepatology* 2010; **51**: 806-816 [PMID: 19998408]
43 **Sevastianova K**, Santos A, Kotronen A, Hakkarainen A, Makkonen J, Silander K, Peltonen M, Romeo S, Lundbom J, Lundbom N, Olkkonen VM, Gylling H, Fielding BA, Rissanen A, Yki-Järvinen H. Effect of short-term carbohydrate overfeeding and long-term weight loss on liver fat in overweight humans. *Am J Clin Nutr* 2012; **96**: 727-734 [PMID: 22952180]
44 **Carvalhana S**, Machado MV, Cortez-Pinto H. Improving dietary patterns in patients with nonalcoholic fatty liver disease. *Curr Opin Clin Nutr Metab Care* 2012; **15**: 468-473 [PMID: 22878240]
45 **Tsuchiya H**, Ebata Y, Sakabe T, Hama S, Kogure K, Shiota G. High-fat, high-fructose diet induces hepatic iron overload via a hepcidin-independent mechanism prior to the onset of liver steatosis and insulin resistance in mice. *Metabolism* 2013; **62**: 62-69 [PMID: 22854109]
46 **Utzschneider KM**, Bayer-Carter JL, Arbuckle MD, Tidwell JM, Richards TL, Craft S. Beneficial effect of a weight-stable, low-fat/low-saturated fat/low-glycaemic index diet to reduce liver fat in older subjects. *Br J Nutr* 2012; 1-9 [PMID: 22849970]
47 **Johnson RJ**, Lanaspa MA, Roncal-Jimenez C, Sanchez-Lozada LG. Effects of excessive fructose intake on health. *Ann Intern Med* 2012; **156**: 905; author reply 905-906 [PMID: 22711095]
48 **Li M**, Feng F, Cheng L. Expression patterns of genes involved in sugar metabolism and accumulation during apple fruit development. *PLoS One* 2012; **7**: e33055 [PMID: 22412983]
49 **Mellouk Z**, Zhang Y, Bulur N, Louchami K, Sener A, Ait Yahia D, Malaisse WJ. The metabolic syndrome of fructose-fed rats: effects of long-chain polyunsaturated ω3 and ω6 fatty acids. IV. D-glucose metabolism by isolated pancreatic islets. *Int J Mol Med* 2012; **29**: 291-293 [PMID: 22076599 DOI: 10.3892/ijmm.2011.824]
50 **Liu J**, Litt L, Segal MR, Kelly MJ, Pelton JG, Kim M. Metabolomics of oxidative stress in recent studies of endogenous and exogenously administered intermediate metabolites. *Int J Mol Sci* 2011; **12**: 6469-6501 [PMID: 22072900]
51 **Bray GA**. Fructose and risk of cardiometabolic disease. *Curr Atheroscler Rep* 2012; **14**: 570-578 [PMID: 22949106]
52 **Caporaso N**, Morisco F, Camera S, Graziani G, Donnarumma L, Ritieni A. Dietary approach in the prevention and treatment of NAFLD. *Front Biosci* 2012; **17**: 2259-2268 [PMID: 22652776]
53 **Zelber-Sagi S**, Ratziu V, Oren R. Nutrition and physical activity in NAFLD: an overview of the epidemiological evidence. *World J Gastroenterol* 2011; **17**: 3377-3389 [PMID: 21876630]
54 **Nseir W**, Nassar F, Assy N. Soft drinks consumption and nonalcoholic fatty liver disease. *World J Gastroenterol* 2010; **16**: 2579-2588 [PMID: 20518077]
55 **Alegret M**, Laguna JC. Opposite fates of fructose in the development of metabolic syndrome. *World J Gastroenterol* 2012; **18**: 4478-4480 [PMID: 22969219]
56 **Sahebkar A**. Potential efficacy of ginger as a natural supplement for nonalcoholic fatty liver disease. *World J Gastroenterol* 2011; **17**: 271-272 [PMID: 21246004]
57 **Mathes AM**. Hepatoprotective actions of melatonin: possible mediation by melatonin receptors. *World J Gastroenterol* 2010; **16**: 6087-6097 [PMID: 21182223]
58 **Ha HL**, Shin HJ, Feitelson MA, Yu DY. Oxidative stress and antioxidants in hepatic pathogenesis. *World J Gastroenterol* 2010; **16**: 6035-6043 [PMID: 21182217]
59 **Grattagliano I**, Bonfrate L, Diogo CV, Wang HH, Wang DQ, Portincasa P. Biochemical mechanisms in drug-induced liver injury: certainties and doubts. *World J Gastroenterol* 2009; **15**: 4865-4876 [PMID: 19842215]

P- Reviewers Koutsilieris M, Lee SY **S- Editor** Gou SX
L- Editor O'Neill M **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i8.1173

World J Gastroenterol 2013 February 28; 19(8): 1173-1181
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

REVIEW

Histone deacetylase inhibitors and pancreatic cancer: Are there any promising clinical trials?

Ioannis Koutsounas, Constantinos Giaginis, Stamatios Theocharis

Ioannis Koutsounas, Constantinos Giaginis, Stamatios Theocharis, Department of Forensic Medicine and Toxicology, Medical School, University of Athens, GR-11527 Athens, Greece
Constantinos Giaginis, Department of Food Science and Nutrition, University of the Aegean, GR-81400 Lemnos, Greece
Author contributions: Koutsounas I, Giaginis C and Theocharis S contributed to this paper equally.
Correspondence to: Stamatios Theocharis, MD, PhD, Pathologist, Associate Professor of the Department of Forensic Medicine and Toxicology, Medical School, University of Athens, 75, Mikras Asias Street, Goudi, GR11527 Athens, Greece. theocharis@ath.forthnet.gr
Telephone: +30-21-7462413 Fax: +30-21-7716098
Received: June 9, 2011 Revised: October 18, 2011
Accepted: August 15, 2012
Published online: February 28, 2013

Abstract

Pancreatic cancer, although not very frequent, has an exceptionally high mortality rate, making it one of the most common causes of cancer mortality in developed countries. Pancreatic cancer is difficult to diagnose, allowing few patients to have the necessary treatment at a relatively early stage. Despite a marginal benefit in survival, the overall response of pancreatic cancer to current systemic therapy continues to be poor, and new therapies are desperately needed. Histone deacetylase (HDAC) enzymes play an important role in the development and progression of cancer and HDAC inhibitors (HDACIs) have been shown to induce differentiation and cell cycle arrest, activate the extrinsic or intrinsic pathways of apoptosis, and inhibit invasion, migration and angiogenesis in different cancer cell lines. As a result of promising preclinical data, various HDACIs are being tested as either monotherapeutic agents or in combination regimens for both solid and hematological malignancies. Vorinostat was the first HDACI approved by the Food and Drug Administration for patients with cutaneous T-cell lymphoma. The use of HDACIs in clinical trials, in pretreated and relapsed patients suffering from advanced pancreatic cancer is discussed. Unfortunately, clinical data for HDACIs in patients with pancreatic cancer are inadequate, because only a few studies have included patients suffering from this type of neoplasm and the number of pancreatic cancer patients that entered HDACIs phase Ⅱ/Ⅲ trials, among others with advanced solid tumors, is very limited. More studies recruiting patients with pancreatic cancer remain to determine the efficiency of these therapies.

© 2013 Baishideng. All rights reserved.

Key words: Pancreatic cancer; Histone deacetylases; Histone deacetylase inhibitors; Clinical trials

Koutsounas I, Giaginis C, Theocharis S. Histone deacetylase inhibitors and pancreatic cancer: Are there any promising clinical trials? *World J Gastroenterol* 2013; 19(8): 1173-1181 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i8/1173.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i8.1173

INTRODUCTION

Worldwide, over 200 000 people die annually of pancreatic cancer. The incidence of pancreatic cancer varies greatly across regions, with the highest incidence and mortality rates found in developed countries, which suggests roles for lifestyle and environmental factors. Deaths from pancreatic cancer rank fourth among cancer-related deaths in the United States. Risk factors for pancreatic cancer include, among others, high-fat diet, smoking, chronic pancreatitis, primary sclerosing cholangitis, hereditary pancreatitis, family history of pancreatic cancer, and diabetes mellitus. Age seems to be a significant risk factor, with incidence increasing with age.

Due to few early illness indicators and lack of screening tests, pancreatic cancer is difficult to diagnose and often at the time of presentation, the cancer has already become advanced. The overall 5-year survival rate among

patients with pancreatic cancer is < 5%. Approximately 20% of patients present with localized, potentially curable tumors. The majority (95%) of cases of pancreatic cancer are adenocarcinomas, resembling the pancreatic ductal cell. Metastasis of this cancer can be either local, most often involving the spleen, adrenal glands and transverse colon, or distant[1,2].

TREATMENT OF ADVANCED PANCREATIC CANCER

Conventional chemotherapies

For over a decade, gemcitabine-based therapy has been considered a first-line treatment for locally advanced and metastatic pancreatic cancer. Preference for gemcitabine over 5-fluorouracil (5-FU) was established in the mid-1990s, when a phase Ⅲ trial comparing gemcitabine monotherapy to 5-FU demonstrated clinical benefit in gemcitabine-treated patients with advanced pancreatic cancer[3]. The value of radiotherapy in the management of locally advanced pancreatic cancer remains unclear[4].

Numerous attempts have been made to improve the efficacy of standard gemcitabine monotherapy in patients with advanced pancreatic cancer, but little success has been achieved. Several phase Ⅲ trials were undertaken with gemcitabine in combination with a range of chemotherapy agents. However, the combinations of gemcitabine with 5-FU[5], as well as irinotecan, oxaliplatin, pemetrexed, exatecan and cisplatin[6-10], all failed to show superiority over gemcitabine monotherapy.

Capecitabine is an orally administered fluoropyrimidine that is metabolized in both liver and tumor cells into 5-FU, resulting in high intratumoral 5-FU concentrations. Phase Ⅲ trial results on capecitabine and gemcitabine combination appeared to be contradictory. In a recent phase Ⅲ trial, the combination significantly improved objective response rate and progression-free survival (PFS), but did not show superiority in overall survival (OS) in patients with advanced pancreatic cancer[11].

In addition, folfirinox (5-FU/leucovorin, irinotecan, and oxaliplatin) treatment of metastatic pancreatic cancer patients in a randomized phase Ⅱ trial of folfirinox versus gemcitabine, indicated that response rate was more than 30%. Furthermore, the median OS was 11.1 mo in the folfirinox group as compared with 6.8 mo in the gemcitabine group, indicating that folfirinox is an option for the treatment of patients with metastatic pancreatic cancer[12].

Targeted therapies

Targeted therapies have also been investigated for advanced pancreatic cancer. The matrix metalloproteinase inhibitors (MMPIs) marimastat and talomastat (BAY 12-9566) inhibit enzymes that play a key role in extracellular matrix (ECM) degradation, and angiogenesis. In phase Ⅲ trials, neither marimastat monotherapy nor marimastat with gemcitabine, improved OS compared with gemcitabine monotherapy[13].

The farnesyl transferase enzyme Kras regulator tipifarnib in combination with gemcitabine did not improve OS compared with gemcitabine monotherapy in a phase Ⅲ trial[14].

Erlotinib is a small-molecule tyrosine kinase inhibitor (TKI) of the human epidermal growth factor receptor (EGFR). A multicenter, randomized, double-blind, placebo-controlled phase Ⅲ clinical trial of erlotinib in combination with gemcitabine, in patients with locally advanced or metastatic pancreatic adenocarcinoma met its primary endpoint, with the combination regimen being the first gemcitabine combination to demonstrate a statistically significant survival advantage over gemcitabine monotherapy and the regimen was consequently approved for metastatic disease[15].

Cetuximab, an anti-EGFR monoclonal antibody, blocks the extracellular EGFR domain, preventing ligand-dependent or independent activation and downstream signaling. An open-label, randomized phase Ⅲ trial, unfortunately, recently failed to demonstrate a clinically significant advantage of the addition of cetuximab to gemcitabine for response and OS[16].

Bevacizumab is a recombinant, humanized IgG1 monoclonal antibody that selectively binds to vascular endothelial growth factor (VEGF), inhibiting its interaction with VEGF receptor-1 and -2, on the surface of endothelial cells. Despite recently reported negative results, a number of phase Ⅱ and Ⅲ studies are underway in advanced pancreatic cancer that include bevacizumab and cetuximab in combination with other agents[17].

Finally, a wide range of molecular-targeted agents that interact with crucial pathways for cell survival in pancreatic cancer are currently being explored. These include agents that target polyADP-ribose polymerase, histone deacetylase (HDAC), Src/Abl kinases, and mammalian target of rapamycin[18].

Given the positive data observed in phase Ⅲ trials, gemcitabine, erlotinib and capecitabine are likely to form the base for future treatment strategies for advanced pancreatic cancer.

Histone acetyltransferases and HDACs

The organization of chromatin is crucial for the regulation of gene expression. Accumulating evidence suggests that the acetylation and deacetylation of histones play significant roles in transcriptional regulation of eukaryotic cells. Histone acetylation and deacetylation are catalysed by specific enzyme families, Histone acetyltransferases (HATs) and HDACs, respectively[19,20]. There are at least four groups of proteins with intrinsic HAT activity, while 18 HDACs belonging to four distinct classes have been described in mammalian cells. Eleven of the HDACs are zinc dependent, classified on the basis of homology to yeast HDACs: Class I includes HDACs 1, 2, 3 and 8; Class ⅡA includes HDACs 4, 5, 7 and 9; Class Ⅱ B, HDACs 6 and 10; and Class IV, HDAC11. Class Ⅲ HDACs, sirtuins 1-7, have an absolute requirement for NAD^+, are not zinc dependent, and generally not inhib-

ited by compounds that inhibit zinc-dependent deacetylases[21]. Apart from deacetylating histones, HDACs have also been reported to interact with non-histone proteins, involved in numerous important cell pathways including control of gene expression, regulation of cell proliferation, differentiation, migration and death. The balance between acetylation and deacetylation is an important factor in regulating gene expression and disruption of HAT or HDAC activity is possibly associated with cancer development[22,23].

HDAC inhibitors

HDAC inhibitors (HDACIs) are divided into different classes based on their chemical properties, including hydroxamic acids, such as suberoylanilide hydroxamic acid (SAHA), trichostatin A (TSA), LBH589 (panobinostat) and PXD101 (belinostat); short chain fatty acids, such as sodium butyrate (NaBu), 4-phenylbutyrate (4-PB) and valproic acid; cyclic tetrapeptides, such as trapoxin, apicidin and depsipeptide-also known as FK228 or romidepsin; benzamides, such as MS-275, CI-994 and MGCD0103, and a variety of other chemical compounds and synthetic inhibitors. HDACIs have three common structural characteristics: a Zn-binding moiety, an opposite capping group, and a straight chain alkyl, vinyl or aryl linker connecting the two[24,25].

HDAC inhibition is able to promote a variety of different anticancer mechanisms including apoptosis, cell differentiation and cell cycle inhibition. HDACIs are a new class of antineoplastic agents currently being evaluated in clinical trials. While these agents have been studied extensively in the laboratory, only recently has their mechanism of action begun to be elucidated. Several HDACIs are now in various stages of development, including clinical trials as monotherapy and in combination with other anticancer drugs and radiation[26,27].

In the present review, the available so far data regarding the different classes of HDACIs used in clinical trials including patients with pancreatic cancer are presented in Table 1.

Hydroxamic acids

SAHA (N-hydroxy-N'-phenyl-octanediamide, vorinostat) is a synthetic hydroxamic acid, which is structurally related to the natural product, TSA (7-[4-(dimethylamino)phenyl]-*N*-hydroxyl-4,6-dimethyl-7-oxo-(2*E*,4*E*,6*R*)-2,4-heptadienamide), produced by selected strains of *Streptomyces platensis*, *Streptomyces hygroscopicus* Y-50 or *Streptomyces sioyaensis*. Hydroxamic acids have a high affinity to biometals, including Fe^{3+}, Ni^{2+} and Zn^{2+}. The synthesis of SAHA and its potency to induce differentiation of murine erythroleukemia (MEL) cells was first reported in 1996. SAHA and TSA comprise a hydroxamic-acid-based metal-binding domain that coordinates the catalytic Zn^{2+} in the HDAC active site, a 5 (TSA) or 6 (SAHA)-membered carbon-based linker that mimics the Cα functional group of lysine, and a hydrophobic motif that interacts with the periphery of the HDAC binding pocket[28].

SAHA: SAHA (vorinostat) is now undergoing several clinical trials. One current phase I trial is studying the side effects and best dose of SAHA given together with flavopiridol in treating patients with advanced solid tumors. Another trial is studying SAHA in patients with metastatic or unresectable solid tumors or lymphoma and liver dysfunction. Combination with doxorubicin is also under survey for solid tumors, as well as that with bortezomib, vinorelbine, gemcitabine and other agents like paclitaxel and carboplatin, fluorouracil/leucovorin and oxaliplatin. A phase I / II trial is studying the highest tolerable dose of SAHA that can be given in combination with radiotherapy to patients with locally advanced pancreatic cancer, as well the efficacy of combined therapy. Another phase I clinical trial is now examining the safety, pharmacokinetics, pharmacodynamics and efficacy of *iv* proteasome inhibitor NPI-0052 in combination with oral SAHA in patients with non-small cell lung cancer, pancreatic cancer, melanoma or lymphoma. A phase I/ II study of SAHA in combination with radiotherapy and infusional 5-FU in patients with locally advanced adenocarcinoma of the pancreas is under way. Finally, SAHA in combination with capecitabine plus radiotherapy is also being evaluated in patients with non-metastatic pancreatic cancer. The above studies are recruiting for participants and possible patients suffering from pancreatic cancer remain to determine the efficiency of these therapies[29].

Cyclic peptides

FK228 (FR901228, depsipeptide, romidepsin): Depsipeptide (1S,4S,7Z,10S,16E,21R)-7-ethylidene-4,21-bis (1-methyletheyl)-2-oxa-12,13-dithia-5,8-,20,23-tetraazabicyclo[8.7.6]tricos-16-ene-3,6,9,19,22-pentone, is a bicyclic peptide isolated from *Chromobacterium violaceum* and has demonstrated potent *in vitro* cytotoxic activity against human tumor cell lines and *in vivo* efficacy against human tumor xenografts. Upon entering cells, FK228 is reduced to an active compound, capable of preferentially interacting with the zinc in the active site of the HDAC class I enzymes, however, it is still generally classified as a broad-spectrum inhibitor as it does inhibit class II enzymes. It was approved by the United States Food and Drug Administration (FDA) for the treatment of cutaneous T-cell lymphoma (CTCL)[30].

Among other current clinical trials for patients with advanced solid tumors, FK228 is being studied in combination with gemcitabine in patients with pancreatic cancer. This phase I / II dose escalation trial is designed to determine the maximum tolerated dose for the combination, as well as evaluate toxicities and objective disease responses. Furthermore, another phase II trial is studying the effectiveness of FK228 in patients who have locally advanced or metastatic neuroendocrine tumors; among them pancreatic islet tumors[29].

Short-chain fatty acids

Valproic acid: Valproic acid (VPA) is now an established antiepileptic drug, by affecting the function of the neu-

Table 1 Clinical studies on histone deacetylase inhibitors for treatment of patients with pancreatic cancer

HDACI	Patients total number	Patients with PC number	Time schedule	Dosage schedule (HDACI)	MTD (HDACI)	Responses (for patients with pancreatic cancer treated with HDACI)			Main toxicities	Ref.
						PR	SD	PD		
VPA + epirubicin phase I	48	1	q21 d	15-160 mg/kg per day *iv*/*po* d1, d2	140 mg/kg per day	1	-	-	Neutropenia, thrombocyto-penia, hypocalcaemia, fatigue, QTc prolongation	[33]
4-PB phase I	21	1	q28 d	60-360 mg/kg *iv* 2 × daily d1-d5, d8-d12	300 mg/kg per day	-	-	1	Fatigue, headache, nausea, vomiting, confusion	[36]
MS-275 + 13-cis retinoic acid phase I	Not defined	1	q28 d	4-5 mg/m^2 *po*, once weekly	5 mg/m^2	-	1	-	Hyponatremia, neutropenia, anemia, fatigue	[39]
MS-275 phase I	27	1	a: q14 d b: q28 d c: q28 d	2-6 mg/m^2 *po* a: one dose 1st wk b: 2 × weekly for 3 wk c: 1 × weekly for 3 wk	a: 6 mg/m^2 b: not defined c: 4 mg/m^2	-	-	1	Hypophospha-temia, hyponatremia, nausea, asthenia, fatigue, anorexia[1]	[40]
CI-994 phase II	17	17	Continues	8 mg/m^2 per day *po*	-	-	2	N/D	Thrombocyto-penia, fatigue, anorexia, nausea, vomiting, bruising, hematuria	[42]
CI-994 phase I	53	1	a: acute q21 d b: chronic q10 wk	a: 10-15 mg/m^2 per day *po* d1-d14 b: 5-12.5 mg/m^2 per day *po* d1-d56	a: 15 mg/m^2 b: 8 mg/m^2	-	-	1	Thrombocyto-penia, neutropenia, nausea, vomiting, diarrhea, fatigue, hoarseness, paresthesias, alopecia	[43]
CI-994 + capecita-bine phase I	54	4 sch. c	a: q21 d b: q42 d c: q21 d	a: 4-10 mg/m^2 per day *po* d1-d14 b: 6 mg/m^2 per day *po* d1-d35 c: 4-8 mg/m^2 per day *po* d1-d14	a: 10 mg/m^2 b: (abandoned) c: 8 mg/m^2	-	-	4	Thrombocyto-penia, anemia, anorexia, diarrhea, nausea, vomiting, fatigue	[44]
CI-994 + carboplatin paclitaxel phase I	30	2	q21 d	4-6 mg/m^2 per day *po* d1-d14 or d1-d7	6 mg/m^2 (d1-d7)	-	-	2	Thrombocyto-penia, neutropenia, fatigue, alopecia, nausea	[45]
CI-994 + gemcita-bine *vs* gemcita-bine alone phase II	174	174 (86 + 88)	q28 d	6 mg/m^2 per day *po* d1-d21	-	8/68	-	60/68	Thrombocyto-penia, neutropenia, anemia, nausea, vomiting, anorexia, diarrhea	[46]
MGCD-0103 phase I	38	2	q21 d	12.5-56 mg/m^2 per day *po* d1, 3, 5, 8, 10, 12	56 mg/m^2 RPTD: 45 mg/m^2	-	-	2	Fatigue, anorexia, nausea, vomiting, diarrhea, abdominal pain, dehydration	[48]
LAQ824 phase I	39	3	q21 d	6-100 mg/m^2 per day *iv* d1-d3	72 mg/m^2 RPTD: < 72 mg/m^2	-	-	3	Thrombocyto-penia, fatigue, anorexia, nausea, vomiting, diarrhea, nonspecific ST-segment, hyperbiliru-binemia, transaminitis[2]	[50]

[1]The patient with pancreatic cancer experienced grade 3 hypophosphatemia as DLT and was finally excluded from study after cycle 2 due to PD; [2]At 100 mg/m^2 one patient with advanced pancreatic cancer, developed grade 4 hyperbilirubinemia associated with febrile neutropenia on day 3. The patient developed thrombocytopenia on day 8 of treatment and anemia, necessitating platelet and blood transfusions. This was followed by an episode of atrial fibrillation on day 13 and then acute renal failure. The patient finally died on d18. PC: Pancreatic cancer; PD: Progressive disease; PR: Partial response; RPTD: Recommended dose for phase II; SD: Stable disease.

rotransmitter GABA. The finding that VPA was an effective inhibitor of HDACs arose from the observations that VPA was able to relieve transcriptional repression of a peroxisomal proliferation and activation of a glucocorticoid receptor (GR)-PPARε hybrid receptor, suggesting that it acts on a common mechanism in gene regulation, such as histone deacetylation, rather than on individual transcription factors or receptors. Consistent with this finding, it was shown that VPA causes hyperacetylation of the N-terminal tails of histones H3 and H4 *in vitro* and *in vivo*, and was found to inhibit HDAC enzymatic activity at a concentration of 0.5 mmol/L[31]. VPA has shown potent antitumor effects in a variety of *in vitro* and *in vivo* systems, by modulating multiple pathways including cell cycle arrest, apoptosis, angiogenesis, metastasis, differentiation and senescence. Most preclinical and clinical data on the anticancer effects of VPA have been generated for malignant hematological diseases[32].

A phase I clinical trial investigated the safety, toxicity and maximum-tolerated dose of VPA and the topoisomerase Ⅱ inhibitor epirubicin in solid tumors. Forty-eight patients with different malignancies were enrolled; one with pancreatic cancer. The patient suffering from pancreatic cancer experienced a partial response at 100 mg/kg VPA and 100 mg/m^2 on day 3 of the cycle, while no dose-limiting toxicities occurred. All patients with a partial response had at least a twofold increase in histone acetylation[33].

Phase I clinical trials are currently testing VPA in combination with other agents such as erlotinib, 5-FU, cyclophosphamide, bevacizumab, and azacytidine, as well as epirubicin to determine safety, tolerability and effectiveness in treating patients with advanced solid tumors. In addition, another phase Ⅱ trial is studying VPA combined with the hypomethylating factor hydralazine. A phase I clinical trial is undertaking recruitment to determine maximum tolerated doses of VPA in combination with sunitinib, sorafenib, dasatinib, erlotinib, lapatinib, or lenalidomide for the treatment of patients with advanced solid tumors, as well as to estimate the safety and treatment response[29].

4-PB: 4-PB is a short-chain fatty acid known to inhibit reversibly class I and Ⅱ HDACs. It is considered as an HDAC inhibitor of the first generation, as the HDAC inhibitory effect is not specific. Working concentrations are rather high, in the millimolar range, and the effects are pleiotropic. 4-PB is known to exert multiple effects in the cell, including the modulation of protein isoprenylation, which importantly regulates the ras proto-oncoprotein, and activation of the nuclear steroid PPAR[34]. 4-PB exerts a potent antitumor effect *in vitro* and has been shown to cause growth inhibition and differentiation in various human cancer cell lines[35].

A phase I dose escalating trial to evaluate twice daily *iv* 4-PB infusion has been undertaken. 4-PB was administered for five consecutive days for a total of 20 doses over two consecutive weeks from 60 to 360 mg/kg per day. Twenty-one patients with different malignancies, including one with pancreatic carcinoma, participated in the trial. Dose limiting toxicities were fatigue and headache, while no significant myelosuppression was seen. Three patients with brain malignancies remained stable for an average of 6 mo[36].

A current phase I clinical trial is investigating oral phenylbutyrate three times daily in patients with refractory solid tumors. Combination with azacytidine is also being studied to determine effectiveness and maximum dose in patients with advanced or metastatic solid tumors[29].

NaBu: NaBu has multiple effects on cultured mammalian cells that include inhibition of proliferation, induction of differentiation and induction or repression of gene expression. Sodium butyrate inhibits most HDACs except class Ⅲ HDAC and class Ⅱ HDAC6 and-10. Promoters of butyrate-responsive genes have butyrate response elements, and the action of butyrate is often mediated through Sp1/Sp3 binding sites[37].

In a recruiting phase I clinical trial, the butyrate prodrug tributyrin is being studied in patients with various advanced solid tumors[29].

Benzamides

MS-275: This synthetic benzamide derivative (3-pyridylmethyl-*N*-{4-[(2-aminophenyl)carbamoyl]benzyl}carbamate) has been shown to inhibit HDACs, and has antitumor activity in many preclinical models. The first clinical trial with this agent in 2005 included patients with advanced solid tumors or lymphoma. At high concentrations of MS-275, there is a marked induction of reactive oxygen species, mitochondrial damage, caspase activation and apoptosis. Treatment of sensitive tumor cell lines with MS-275 induces gelsolin, a maturation marker, and produces a change in the cell cycle distribution with a decrease in S phase and an accumulation of cells in G_1. The *in vivo* therapeutic efficacy of MS-275 has been shown in a variety of human tumor xenograft models[38].

A phase I study determined the maximum tolerated dose, the dose limiting toxicity and the pharmacokinetic or pharmacodynamic profile of MS-275 in combination with 13-cis-retinoic acid. Patients with advanced solid tumors were treated with MS-275 orally, once weekly, and 13-cis-retinoic acid orally, 1 mg/kg twice daily, for 3 wk every 4 wk. One patient suffering from pancreatic cancer remained on treatment for 6 mo and a patient with renal cell carcinoma showed a partial response in the lungs. Side effects included hyponatremia, neutropenia, anemia and fatigue[39].

Another phase I study evaluated the toxicity and pharmacokinetic profiles of MS-275 in patients with refractory solid tumors and lymphomas, including one patient suffering from metastatic pancreatic cancer, on three different schedules. The patient with metastatic pancreatic cancer developed grade 3 hypophosphatemia, thus meeting criteria for dose limiting toxicity, and was

finally removed from study due to disease progression after cycle 2. Objective responses were only observed in patients receiving the every-other-week dosing schedule, but the numbers of patients enrolled were too small to determine whether this dosing regimen was truly more efficacious[40].

CI-994: CI-994 or N-acetyldinaline [4-(acetylamino)-N-(2-amino-phenyl) benzamide] is a novel oral compound with a wide spectrum of antitumor activity in preclinical models. The mechanism of action may involve inhibition of histone deacetylation and cell cycle arrest. CI-994 is currently undergoing clinical trials. Although several changes in cellular metabolism induced by the drug have been characterized, the primary molecular mechanism of its antitumor activity remains unknown[41].

A phase Ⅱ trial of CI-994 in patients with advanced pancreatic cancer evaluated the antitumor activity and safety of CI-994. CI-994 was administered orally at 8 mg/m^2 per day. Seventeen patients were enrolled, including 15 with metastatic disease. Among patients evaluable for response, stable disease for 8 wk occurred in two patients (12%). Overall, median time to progressive disease was 6 wk and median survival was 10 wk, with one patient alive at 41 wk. Grade 3 thrombocytopenia occurred in eight patients but grade 4 in none. Most common non-hematological toxicities were generally grade 1 or 2 and included fatigue, anorexia, nausea, vomiting and bruising. According to this study, CI-994 was well tolerated but resulted in no objective responses in patients with advanced pancreatic cancer[42].

A phase 1 study in patients with solid tumors was carried out to determine the maximum tolerated daily oral dose for CI-994 administered on a chronic basis. Fifty-three patients, most of them suffering from colorectal, lung and renal malignancies, including one with pancreatic cancer, received CI-994 daily for treatment durations ranging from 2 to 10 wk. Antitumor effects were documented in four patients, including a durable partial response in one pretreated non-small cell lung cancer patient and stable disease in three patients with non-small cell lung, colon and renal cancers[43].

Another study investigated the toxicity profile, maximum tolerated dose and pharmacokinetics of CI-994 in combination with capecitabine. Fifty-four patients were treated according to three different dosing schemes in which the capecitabine dose was fixed and the CI-994 dose was escalated. In schedule C, 22 patients, including four with pancreatic cancer, were treated with capecitabine 2000 mg/m^2 per day and CI-994 for 2 of 3 wk. One partial response was achieved at the 4 mg/m^2 dose level of schedule A in a patient with colorectal cancer. Disease stabilization was seen in adenocarcinoma of unknown primary origin, appendiceal cancer, breast cancer, colorectal cancer and mesothelioma[44].

A phase I study of oral CI-994 in combination with carboplatin and paclitaxel in patients with advanced solid tumors was carried out. A total of 30 patients were entered into five treatment cohorts, including two suffering from pancreatic cancer. Five patients achieved a partial response (non-small cell lung, colon, unknown primary origin) and two patients achieved a complete response (esophageal and bladder cancer). It was observed that patients whose histone H3 acetylation in peripheral blood lymphocytes was at least 1.5-fold greater after treatment had an objective clinical response or stable disease[45].

A randomized, double-blind, placebo-controlled, multicenter study compared whether CI-994 plus gemcitabine improved OS, duration of response, time to treatment failure, and quality of life compared to gemcitabine alone. Patients had diagnosis of advanced or metastatic adenocarcinoma of the exocrine pancreas and were not considered surgical candidates. A total of 174 patients received CI-994 6 mg/m^2 per d orally on days 1-21 plus gemcitabine 1000 mg/m^2 on days 1, 8 and 15 or placebo plus gemcitabine 1000 mg/m^2 on days 1, 8 and 15 of each 28-d cycle. There was no observed difference in survival time between the two cases. The estimated median survival was 194 and 214 d and objective response rates based on investigator assessments were 12% and 14%, respectively. In addition, pain responses did not differ significantly. Treatment with CI-994 was associated with more cases of grade 3/4 thrombocytopenia, anemia and leukopenia than with placebo, while non-hematological toxicities such as nausea, vomiting, anorexia and diarrhea were identical with both treatments. Consequently, in this study, CI-994 in combination with gemcitabine did not appear to offer any benefit compared to gemcitabine as a single agent for the treatment of pancreatic cancer[46].

A randomized phase Ⅱ trial comparing the effectiveness of gemcitabine with or without CI-994 in patients with advanced pancreatic cancer is still ongoing[29].

MGCD0103: MGCD0103 is an isotype-specific aminophenylbenzamide that inhibits HDAC classes I and Ⅳ, with almost no class Ⅱ effect. MGCD0103 is well tolerated and exhibits favorable pharmacokinetic and pharmacodynamic profiles, demonstrating target inhibition and clinical responses. It induces cell death and autophagy, synergizes with proteasomal inhibitors and affects non-histone targets, such as microtubules[47].

In a phase I study MGCD0103 was given three times weekly orally to patients with advanced solid tumors to determine safety, tolerability and pharmacokinetics. Thirty-eight patients were enrolled and completed a total of 99 cycles of MGCD0103, including two with pancreatic cancer. No objective tumor responses were observed. Five patients with previously progressive colorectal, renal cell and lung cancers had stable disease for four or more cycles. Furthermore, MGCD0103 exerted dose-dependent HDAC inhibitory activity and was able to induce histone acetylation in peripheral leukocytes[48].

Oral MGCD0103 three times weekly is currently being studied in combination with gemcitabine, in patients with advanced solid tumors. In this phase Ⅰ/Ⅱ study, patients with locally advanced or metastatic pancreatic

cancer can participate. Maximum tolerated dose of MGCD0103 and objective response of patients remain to be determined[29,49].

Other HDACIs

A phase I study of LAQ824 has determined the safety, maximum tolerated dose, and pharmacokinetic-pharmacodynamic profile in patients with advanced solid tumors. Thirty-nine patients were recruited and were eligible for assessment of toxicity, including three with pancreatic cancer. At 100 mg/m^2, one patient who had advanced pancreatic cancer, developed grade 4 hyperbilirubinemia associated with febrile neutropenia on day 3. The patient finally died after an episode of atrial fibrillation and acute renal failure 18 d after the first infusion. All patients treated with LAQ824 at 12 mg/m^2 or above showed increase in histone acetylation. No objective responses were documented. One patient with hepatocellular carcinoma and two with fibrosarcoma and papillary carcinoma of the thyroid showed disease stabilization[50].

A phase I study is investigating safety, pharmacodynamic, antitumor activity, and pharmacokinetics of PXD101 (belinostat) alone and in combination with 5-FU in patients with advanced solid tumors or lymphoma[51]. Another recruiting phase I trial is studying the safety, tolerability and pharmacokinetics of orally administered PXD101 in combination with carboplatin and/or paclitaxel in patients with advanced solid tumors or lymphoma[52].

A recruiting phase I study is evaluating the pharmacokinetics and safety of oral LBH589 (panobinostat) in patients with advanced solid tumors and varying degrees of renal function. Another phase Ⅱ trial will determine tumor response, toxicity and tolerability of LBH589 in patients with gastrointestinal neuroendocrine tumors. A phase I A, dose-escalating study of *iv* LBH589 in adult patients with advanced solid tumors is ongoing. Another phase I study will evaluate the safety and tolerability of the combination of LBH589 and paclitaxel/carboplatin in patients with metastatic or locally advanced solid tumors. Finally, a phase I dose escalation trial of LBH589 and gemcitabine in patients with solid malignancies has been temporarily suspended[53,54].

Other HDACIs, such as CHR-3996, CRA-024781, SB939 and R306465, are under phase I trials for the treatment of patients with advanced solid tumors[29].

CONCLUSION

The experimental results of HDACIs led to their use in clinical trials, especially in pretreated and multiply relapsed patients at an advanced cancer stage. Additionally, HDACIs constitute a promising treatment for cancer due to their low toxicity. The first HDACIs tested in clinical trials have shown encouraging antitumor effects, at doses well tolerated by patients. Vorinostat (SAHA) was the first HDACI to be approved by the FDA for clinical use in patients with hematological malignancy (CTCL). HDACIs alone and in combination with a variety of cytotoxic or other targeted anticancer agents are now being tested. To date, at least 10 different HDACIs, including SAHA, VPA, NaBu, MS-275, CI-994, FK228, PXD101, LAQ824 and others, are in phase Ⅱ or Ⅲ clinical trials for the treatment of hematological and solid tumors.

Despite efforts in recent decades, conventional treatments such as surgery, radiation and chemotherapy, have slightly affected the course of pancreatic cancer. The development of effective systemic treatments, capable of reversing the biology of this aggressive disease, remains a critical requirement. Unfortunately, clinical data for HDACIs on patients with pancreatic cancer are inadequate (Table 1). So far, only a few studies have included patients suffering from this type of neoplasm. Additionally, the number of pancreatic cancer patients that entered HDACIs phase Ⅱ/Ⅲ trials, among others with advanced solid tumors, is very limited. Although HDACIs are recognized as some of the most promising agents, more studies recruiting candidates with pancreatic cancer remain to determine the efficacy of these therapies.

REFERENCES

1 **Raimondi S**, Maisonneuve P, Lowenfels AB. Epidemiology of pancreatic cancer: an overview. *Nat Rev Gastroenterol Hepatol* 2009; **6**: 699-708 [PMID: 19806144 DOI: 10.1038/nrgastro.2009.17]

2 **Hidalgo M**. Pancreatic cancer. *N Engl J Med* 2010; **362**: 1605-1617 [PMID: 20427809 DOI: 10.1056/NEJMra0901557]

3 **Burris HA**, Moore MJ, Andersen J, Green MR, Rothenberg ML, Modiano MR, Cripps MC, Portenoy RK, Storniolo AM, Tarassoff P, Nelson R, Dorr FA, Stephens CD, Von Hoff DD. Improvements in survival and clinical benefit with gemcitabine as first-line therapy for patients with advanced pancreas cancer: a randomized trial. *J Clin Oncol* 1997; **15**: 2403-2413 [PMID: 9196156]

4 **Micke O**, Hesselmann S, Bruns F, Horst E, Devries A, Schüller P, Willich N, Schäfer U. Results and follow-up of locally advanced cancer of the exocrine pancreas treated with radiochemotherapy. *Anticancer Res* 2005; **25**: 1523-1530 [PMID: 16033054]

5 **Riess H**, Helm A, Niedergethmann M, Schmidt-Wolf I, Moik M, Hammer C, Zippel K. A randomised, prospective, multicenter, phase iii trial of gemcitabine, 5-fluorouracil (5-fu), folinic acid vs. gemcitabine alone in patients with advanced pancreatic cancer. *J Clin Oncol* 2005; **23**: 4009

6 **Rocha Lima CM**, Green MR, Rotche R, Miller WH, Jeffrey GM, Cisar LA, Morganti A, Orlando N, Gruia G, Miller LL. Irinotecan plus gemcitabine results in no survival advantage compared with gemcitabine monotherapy in patients with locally advanced or metastatic pancreatic cancer despite increased tumor response rate. *J Clin Oncol* 2004; **22**: 3776-3783 [PMID: 15365074 DOI: 10.1200/JCO.2004.12.082]

7 **Poplin E**, Feng Y, Berlin J, Rothenberg ML, Hochster H, Mitchell E, Alberts S, O'Dwyer P, Haller D, Catalano P, Cella D, Benson AB. Phase III, randomized study of gemcitabine and oxaliplatin versus gemcitabine (fixed-dose rate infusion) compared with gemcitabine (30-minute infusion) in patients with pancreatic carcinoma E6201: a trial of the Eastern Cooperative Oncology Group. *J Clin Oncol* 2009; **27**: 3778-3785 [PMID: 19581537 DOI: 10.1200/JCO.2008.20.9007]

8 **Oettle H**, Richards D, Ramanathan RK, van Laethem JL, Peeters M, Fuchs M, Zimmermann A, John W, Von Hoff D, Arning M, Kindler HL. A phase III trial of pemetrexed plus

gemcitabine versus gemcitabine in patients with unresectable or metastatic pancreatic cancer. *Ann Oncol* 2005; **16**: 1639-1645 [PMID: 16087696 DOI: 10.1093/annonc/mdi309]
9 **Abou-Alfa GK**, Letourneau R, Harker G, Modiano M, Hurwitz H, Tchekmedyian NS, Feit K, Ackerman J, De Jager RL, Eckhardt SG, O'Reilly EM. Randomized phase III study of exatecan and gemcitabine compared with gemcitabine alone in untreated advanced pancreatic cancer. *J Clin Oncol* 2006; **24**: 4441-4447 [PMID: 16983112 DOI: 10.1200/JCO.2006.07.0201]
10 **Heinemann V**, Quietzsch D, Gieseler F, Gonnermann M, Schönekäs H, Rost A, Neuhaus H, Haag C, Clemens M, Heinrich B, Vehling-Kaiser U, Fuchs M, Fleckenstein D, Gesierich W, Uthgenannt D, Einsele H, Holstege A, Hinke A, Schalhorn A, Wilkowski R. Randomized phase III trial of gemcitabine plus cisplatin compared with gemcitabine alone in advanced pancreatic cancer. *J Clin Oncol* 2006; **24**: 3946-3952 [PMID: 16921047 DOI: 10.1200/JCO.2005.05.1490]
11 **Cunningham D**, Chau I, Stocken DD, Valle JW, Smith D, Steward W, Harper PG, Dunn J, Tudur-Smith C, West J, Falk S, Crellin A, Adab F, Thompson J, Leonard P, Ostrowski J, Eatock M, Scheithauer W, Herrmann R, Neoptolemos JP. Phase III randomized comparison of gemcitabine versus gemcitabine plus capecitabine in patients with advanced pancreatic cancer. *J Clin Oncol* 2009; **27**: 5513-5518 [PMID: 19858379 DOI: 10.1200/JCO.2009.24.2446]
12 **Conroy T**, Desseigne F, Ychou M, Bouché O, Guimbaud R, Bécouarn Y, Adenis A, Raoul JL, Gourgou-Bourgade S, de la Fouchardière C, Bennouna J, Bachet JB, Khemissa-Akouz F, Péré-Vergé D, Delbaldo C, Assenat E, Chauffert B, Michel P, Montoto-Grillot C, Ducreux M. FOLFIRINOX versus gemcitabine for metastatic pancreatic cancer. *N Engl J Med* 2011; **364**: 1817-1825 [PMID: 21561347 DOI: 10.1056/NEJMoa1011923]
13 **Bramhall SR**, Schulz J, Nemunaitis J, Brown PD, Baillet M, Buckels JA. A double-blind placebo-controlled, randomised study comparing gemcitabine and marimastat with gemcitabine and placebo as first line therapy in patients with advanced pancreatic cancer. *Br J Cancer* 2002; **87**: 161-167 [PMID: 12107836 DOI: 10.1038/sj.bjc.6600446]
14 **Van Cutsem E**, van de Velde H, Karasek P, Oettle H, Vervenne WL, Szawlowski A, Schoffski P, Post S, Verslype C, Neumann H, Safran H, Humblet Y, Perez Ruixo J, Ma Y, Von Hoff D. Phase III trial of gemcitabine plus tipifarnib compared with gemcitabine plus placebo in advanced pancreatic cancer. *J Clin Oncol* 2004; **22**: 1430-1438 [PMID: 15084616 DOI: 10.1200/JCO.2004.10.112]
15 **Moore MJ**, Goldstein D, Hamm J, Figer A, Hecht JR, Gallinger S, Au HJ, Murawa P, Walde D, Wolff RA, Campos D, Lim R, Ding K, Clark G, Voskoglou-Nomikos T, Ptasynski M, Parulekar W. Erlotinib plus gemcitabine compared with gemcitabine alone in patients with advanced pancreatic cancer: a phase III trial of the National Cancer Institute of Canada Clinical Trials Group. *J Clin Oncol* 2007; **25**: 1960-1966 [PMID: 17452677 DOI: 10.1200/JCO.2006.07.9525]
16 **Philip PA**, Benedetti J, Fenoglio-Preiser C, Zalupski M, Lenz H, O'Reilly E, Wong R, Atkins J, Abruzzese J, Blanke C. Phase III study of gemcitabine [G] plus cetuximab [C] versus gemcitabine in patients [pts] with locally advanced or metastatic pancreatic adenocarcinoma [PC]: SWOG S0205 study. *J Clin Oncol* 2007; **25**: 199s
17 **Kindler HL**, Niedzwiecki D, Hollis D, Oraefo E, Schrag D, Hurwitz H, McLeod HL, Mulcahy MF, Schilsky RL, Goldberg RM. Cancer and Leukemia Group B. A double-blind, placebo-controlled, randomized phase III trial of gemcitabine (G) plus bevacizumab (B) versus gemcitabine plus placebo (P) in patients (pts) with advanced pancreatic cancer (PC): a preliminary analysis of Cancer and Leukemia Group B (CALGB). *J Clin Oncol* 2007; **25**: 199s
18 **Rocha-Lima CM**. New directions in the management of advanced pancreatic cancer: a review. *Anticancer Drugs* 2008; **19**: 435-446 [PMID: 18418211]
19 **Bernstein BE**, Meissner A, Lander ES. The mammalian epigenome. *Cell* 2007; **128**: 669-681 [PMID: 17320505 DOI: 10.1016/j.cell.2007.01.033]
20 **Kouraklis G**, Theocharis S. Histone acetylation and cancer. *Acta Oncol* 2003; **42**: 792 [PMID: 14690169]
21 **Gregoretti IV**, Lee YM, Goodson HV. Molecular evolution of the histone deacetylase family: functional implications of phylogenetic analysis. *J Mol Biol* 2004; **338**: 17-31 [PMID: 15050820 DOI: 10.1016/j.jmb.2004.02.006]
22 **Kristensen LS**, Nielsen HM, Hansen LL. Epigenetics and cancer treatment. *Eur J Pharmacol* 2009; **625**: 131-142 [PMID: 19836388 DOI: 10.1016/j.ejphar.2009.10.011]
23 **Marks PA**, Xu WS. Histone deacetylase inhibitors: Potential in cancer therapy. *J Cell Biochem* 2009; **107**: 600-608 [PMID: 19459166 DOI: 10.1002/jcb.22185]
24 **Mai A**, Altucci L. Epi-drugs to fight cancer: from chemistry to cancer treatment, the road ahead. *Int J Biochem Cell Biol* 2009; **41**: 199-213 [PMID: 18790076 DOI: 10.1016/j.biocel.2008.08.020]
25 **Xu WS**, Parmigiani RB, Marks PA. Histone deacetylase inhibitors: molecular mechanisms of action. *Oncogene* 2007; **26**: 5541-5552 [PMID: 17694093 DOI: 10.1038/sj.onc.1210620]
26 **Kouraklis G**, Theocharis S. Histone deacetylase inhibitors: a novel target of anticancer therapy (review). *Oncol Rep* 2006; **15**: 489-494 [PMID: 16391874]
27 **Kouraklis G**, Theocharis S. Histone deacetylase inhibitors and anticancer therapy. *Curr Med Chem Anticancer Agents* 2002; **2**: 477-484 [PMID: 12678732]
28 **Codd R**, Braich N, Liu J, Soe CZ, Pakchung AA. Zn(II)-dependent histone deacetylase inhibitors: suberoylanilide hydroxamic acid and trichostatin A. *Int J Biochem Cell Biol* 2009; **41**: 736-739 [PMID: 18725319 DOI: 10.1016/j.biocel.2008.05.026]
29 National Institutes of Health (NIH), Clinical Trials. Available from: URL: http://www.clinicaltrials.gov
30 **Grant C**, Rahman F, Piekarz R, Peer C, Frye R, Robey RW, Gardner ER, Figg WD, Bates SE. Romidepsin: a new therapy for cutaneous T-cell lymphoma and a potential therapy for solid tumors. *Expert Rev Anticancer Ther* 2010; **10**: 997-1008 [PMID: 20645688 DOI: 10.1586/era.10.88]
31 **Göttlicher M**, Minucci S, Zhu P, Krämer OH, Schimpf A, Giavara S, Sleeman JP, Lo Coco F, Nervi C, Pelicci PG, Heinzel T. Valproic acid defines a novel class of HDAC inhibitors inducing differentiation of transformed cells. *EMBO J* 2001; **20**: 6969-6978 [PMID: 11742974 DOI: 10.1093/emboj/20.24.6969]
32 **Duenas-Gonzalez A**, Candelaria M, Perez-Plascencia C, Perez-Cardenas E, de la Cruz-Hernandez E, Herrera LA. Valproic acid as epigenetic cancer drug: preclinical, clinical and transcriptional effects on solid tumors. *Cancer Treat Rev* 2008; **34**: 206-222 [PMID: 18226465 DOI: 10.1016/j.ctrv.2007.11.003]
33 **Münster P**, Marchion D, Bicaku E, Schmitt M, Lee JH, DeConti R, Simon G, Fishman M, Minton S, Garrett C, Chiappori A, Lush R, Sullivan D, Daud A. Phase I trial of histone deacetylase inhibition by valproic acid followed by the topoisomerase II inhibitor epirubicin in advanced solid tumors: a clinical and translational study. *J Clin Oncol* 2007; **25**: 1979-1985 [PMID: 17513804 DOI: 10.1200/JCO.2006.08.6165]
34 **Pineau T**, Hudgins WR, Liu L, Chen LC, Sher T, Gonzalez FJ, Samid D. Activation of a human peroxisome proliferator-activated receptor by the antitumor agent phenylacetate and its analogs. *Biochem Pharmacol* 1996; **52**: 659-667 [PMID: 8759039]
35 **Svechnikova I**, Almqvist PM, Ekström TJ. HDAC inhibitors effectively induce cell type-specific differentiation in human glioblastoma cell lines of different origin. *Int J Oncol* 2008; **32**: 821-827 [PMID: 18360709]
36 **Camacho LH**, Olson J, Tong WP, Young CW, Spriggs DR, Malkin MG. Phase I dose escalation clinical trial of phenyl-

butyrate sodium administered twice daily to patients with advanced solid tumors. *Invest New Drugs* 2007; **25**: 131-138 [PMID: 17053987 DOI: 10.1007/s10637-006-9017-4]
37 **Davie JR**. Inhibition of histone deacetylase activity by butyrate. *J Nutr* 2003; **133**: 2485S-2493S [PMID: 12840228]
38 **Hess-Stumpp H**, Bracker TU, Henderson D, Politz O. MS-275, a potent orally available inhibitor of histone deacetylases--the development of an anticancer agent. *Int J Biochem Cell Biol* 2007; **39**: 1388-1405 [PMID: 17383217 DOI: 10.1016/j.biocel.2007.02.009]
39 **Pili R**, Dudek M, Altiok S. Phase I pharmacokinetic and pharmacodynamic study of the histone deacetylase inhibitor MS-275 in combination with 13-cis-retinoic acid in patients with advanced solid tumors. *J Clin Oncol* 2006; **24**: 3055
40 **Gore L**, Rothenberg ML, O'Bryant CL, Schultz MK, Sandler AB, Coffin D, McCoy C, Schott A, Scholz C, Eckhardt SG. A phase I and pharmacokinetic study of the oral histone deacetylase inhibitor, MS-275, in patients with refractory solid tumors and lymphomas. *Clin Cancer Res* 2008; **14**: 4517-4525 [PMID: 18579665 DOI: 10.1158/1078-0432.CCR-07-1461]
41 **Kraker AJ**, Mizzen CA, Hartl BG, Miin J, Allis CD, Merriman RL. Modulation of histone acetylation by [4-(acetylamino)-N-(2-amino-phenyl) benzamide] in HCT-8 colon carcinoma. *Mol Cancer Ther* 2003; **2**: 401-408 [PMID: 12700284]
42 **Zalpuski M**, O'Shaughnessy J, Vulkeja A, Shields A, Diener K, Grove W. Phase II trial of CI-994 in patients with advanced pancreatic cancer. *Proc Am Soc Clin Oncol* 2000; **19**: 285a
43 **Prakash S**, Foster BJ, Meyer M, Wozniak A, Heilbrun LK, Flaherty L, Zalupski M, Radulovic L, Valdivieso M, LoRusso PM. Chronic oral administration of CI-994: a phase 1 study. *Invest New Drugs* 2001; **19**: 1-11 [PMID: 11291827]
44 **Undevia SD**, Kindler HL, Janisch L, Olson SC, Schilsky RL, Vogelzang NJ, Kimmel KA, Macek TA, Ratain MJ. A phase I study of the oral combination of CI-994, a putative histone deacetylase inhibitor, and capecitabine. *Ann Oncol* 2004; **15**: 1705-1711 [PMID: 15520075 DOI: 10.1093/annonc/mdh438]
45 **Pauer LR**, Olivares J, Cunningham C, Williams A, Grove W, Kraker A, Olson S, Nemunaitis J. Phase I study of oral CI-994 in combination with carboplatin and paclitaxel in the treatment of patients with advanced solid tumors. *Cancer Invest* 2004; **22**: 886-896 [PMID: 15641487]
46 **Richards DA**, Boehm KA, Waterhouse DM, Wagener DJ, Krishnamurthi SS, Rosemurgy A, Grove W, Macdonald K, Gulyas S, Clark M, Dasse KD. Gemcitabine plus CI-994 offers no advantage over gemcitabine alone in the treatment of patients with advanced pancreatic cancer: results of a phase II randomized, double-blind, placebo-controlled, multicenter study. *Ann Oncol* 2006; **17**: 1096-1102 [PMID: 16641168 DOI: 10.1093/annonc/mdl081]
47 **Boumber Y**, Younes A, Garcia-Manero G. Mocetinostat (MGCD0103): a review of an isotype-specific histone deacetylase inhibitor. *Expert Opin Investig Drugs* 2011; **20**: 823-829 [PMID: 21554162 DOI: 10.1517/13543784.2011.577737]
48 **Siu LL**, Pili R, Duran I, Messersmith WA, Chen EX, Sullivan R, MacLean M, King S, Brown S, Reid GK, Li Z, Kalita AM, Laille EJ, Besterman JM, Martell RE, Carducci MA. Phase I study of MGCD0103 given as a three-times-per-week oral dose in patients with advanced solid tumors. *J Clin Oncol* 2008; **26**: 1940-1947 [PMID: 18421048 DOI: 10.1200/JCO.2007.14.5730]
49 **Kell J**. Drug evaluation: MGCD-0103, a histone deacetylase inhibitor for the treatment of cancer. *Curr Opin Investig Drugs* 2007; **8**: 485-492 [PMID: 17621879]
50 **de Bono JS**, Kristeleit R, Tolcher A, Fong P, Pacey S, Karavasilis V, Mita M, Shaw H, Workman P, Kaye S, Rowinsky EK, Aherne W, Atadja P, Scott JW, Patnaik A. Phase I pharmacokinetic and pharmacodynamic study of LAQ824, a hydroxamate histone deacetylase inhibitor with a heat shock protein-90 inhibitory profile, in patients with advanced solid tumors. *Clin Cancer Res* 2008; **14**: 6663-6673 [PMID: 18927309 DOI: 10.1158/1078-0432.CCR-08-0376]
51 **Gimsing P**. Belinostat: a new broad acting antineoplastic histone deacetylase inhibitor. *Expert Opin Investig Drugs* 2009; **18**: 501-508 [PMID: 19335278 DOI: 10.1517/13543780902852560]
52 **Lassen U**, Molife LR, Sorensen M, Engelholm SA, Vidal L, Sinha R, Penson RT, Buhl-Jensen P, Crowley E, Tjornelund J, Knoblauch P, de Bono JS. A phase I study of the safety and pharmacokinetics of the histone deacetylase inhibitor belinostat administered in combination with carboplatin and/or paclitaxel in patients with solid tumours. *Br J Cancer* 2010; **103**: 12-17 [PMID: 20588278 DOI: 10.1038/sj.bjc.6605726]
53 **Tan J**, Cang S, Ma Y, Petrillo RL, Liu D. Novel histone deacetylase inhibitors in clinical trials as anti-cancer agents. *J Hematol Oncol* 2010; **3**: 5 [PMID: 20132536 DOI: 10.1186/1756-8722-3-5]
54 **Tomillero A**, Moral MA. Gateways to clinical trials. *Methods Find Exp Clin Pharmacol* 2009; **31**: 661-700 [PMID: 20140276]

P- Reviewer Michl P **S- Editor** Cheng JX **L- Editor** A **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i8.1182

World J Gastroenterol 2013 February 28; 19(8): 1182-1192
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

REVIEW

DNA and histone methylation in gastric carcinogenesis

Danielle Queiroz Calcagno, Carolina Oliveira Gigek, Elizabeth Suchi Chen, Rommel Rodriguez Burbano, Marília de Arruda Cardoso Smith

Danielle Queiroz Calcagno, Carolina Oliveira Gigek, Elizabeth Suchi Chen, Marília de Arruda Cardoso Smith, Department of Morphology and Genetics, Federal University of Sao Paulo, Sao Paulo, SP 04023-900, Brazil
Rommel Rodriguez Burbano, Laboratory of Human Cytogenetics, Institute of Biological Sciences, Federal University of Pará, Belém, PA 66075-110, Brazil
Author contributions: Calcagno DQ, Gigek CO, Chen ES, Burbano RR and Smith MAC contributed to the review design and wrote the manuscript.
Supported by Sao Paulo State Research Foundation (FAPESP), No. 2009/07145-9 and 2010/11174-1; National Counsel of Technological and Scientific Development (CNPq); Coordination for the Improvement of Higher Level Personnel (CAPES)
Correspondence to: Danielle Queiroz Calcagno, PhD, Division of Genetics, Department of Morphology and Genetics, Federal University of Sao Paulo, Rua Botucatu 740, Sao Paulo, SP 04023-900, Brazil. danicalcagno@gmail.com
Telephone: +55-11-55764260 Fax: +55-11-55764264
Received: February 29, 2012 Revised: June 13, 2012
Accepted: June 28, 2012
Published online: February 28, 2013

Abstract

Epigenetic alterations contribute significantly to the development and progression of gastric cancer, one of the leading causes of cancer death worldwide. Epigenetics refers to the number of modifications of the chromatin structure that affect gene expression without altering the primary sequence of DNA, and these changes lead to transcriptional activation or silencing of the gene. Over the years, the study of epigenetic processes has increased, and novel therapeutic approaches that target DNA methylation and histone modifications have emerged. A greater understanding of epigenetics and the therapeutic potential of manipulating these processes is necessary for gastric cancer treatment. Here, we review recent research on the effects of aberrant DNA and histone methylation on the onset and progression of gastric tumors and the development of compounds that target enzymes that regulate the epigenome.

© 2013 Baishideng. All rights reserved.

Key words: Epigenetic; DNA methylation; Histone methylation; Gastric cancer; Gastric carcinogenesis

Calcagno DQ, Gigek CO, Chen ES, Burbano RR, Smith MAC. DNA and histone methylation in gastric carcinogenesis. *World J Gastroenterol* 2013; 19(8): 1182-1192 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i8/1182.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i8.1182

INTRODUCTION

Gastric cancer (GC) is the fourth most frequent cancer and is the second leading cause of cancer-related death worldwide[1]. Histologically, gastric tumors are divided into intestinal and diffuse types according to the Lauren classification[2]. The intestinal type of GC mostly progresses through the successive steps of normal gastric mucosa, leading to acute and chronic gastritis, atrophic gastritis, intestinal metaplasia, dysplasia, and finally a gastric tumor[3]. In contrast, the sequence of events in the development of diffuse type GC is poorly understood, although a subset of diffuse type GC appears to develop independently of atrophic gastritis or intestinal metaplasia[4,5]. Differences in the clinicopathological characteristics between these two histological types indicate that development occurs through distinct molecular pathways[6-10]. Each histological type is a consequence of a progressive accumulation of different genetic and epigenetic alterations.

Epigenetics refers to a number of modifications in the chromatin structure that affect gene expression without altering the primary DNA sequence, and these changes lead to transcriptional activation or silencing of the gene. Interestingly, epigenetic modifications of DNA can also increases mutagenesis and influence the interactions between DNA and carcinogens and ultraviolet light[11]. Epigenetic modifications play a central role in gastric car-

cinogenesis[12]. Recent reports indicate that infection with *Helicobacter pylori* (*H. pylori*) or Epstein-Barr virus (EBV), pathogens with a substantial role in development of GC, are associated with elevated levels of aberrant DNA methylation in GC[13-16]. The study of epigenetic processes has increased in recent years, and novel therapeutic approaches that target DNA methylation and histone modifications have emerged. A greater understanding of epigenetics and the therapeutic potential of intervention into these processes is necessary to help GC treatment.

In this review, after a brief introduction to the methylation machinery, we focus on the roles that aberrant DNA and histone methylation play in the onset and progression of gastric tumors, and the development of compounds that target enzymes that regulate the epigenome.

METHYLATION MACHINERY

DNA methylation refers to the addition or subtraction of a methyl moiety at the 5 position of the cytosine ring within CpG dinucleotides that are usually located in CpG-rich regions or CpG islands and around the gene promoter. DNA methylation in gene promoter regions represses transcription of their downstream genes associated with the suppression of gene expression[17]. However, methylation in gene bodies does not block transcription and is sometimes associated with active transcription[18]. Methylation status is controlled by DNA methyltransferases (DNMT1, DNMT3A, and DNMT3B)[19]. DNMT1 maintains the existing methylation patterns following DNA replication, whereas DNMT3A and DNMT3B target unmethylated CpGs to initiate methylation and are highly expressed during embryogenesis and minimally expressed in adult tissues[20]. Another DNA methyltransferase family member, DNMT3L, interacts with DNMT3A and DNMT3B to facilitate methylation of retrotransposons[21]. Many studies have shown that overexpression of DNA methyltransferases is closely related to tumorigenesis, although the role of DNMT3L in cancer is still unclear (Table 1). In addition, *H. pylori* infection may increase DNA methyltransferase activity through upregulation of the epidermal growth factor and its receptor or via the release of inflammatory mediators, such as nitric oxide[22]. In particular, *DNMT1* overexpression has been associated with EBV infection in GC[23-25].

DNA methylation has also been implicated in the regulation of higher order chromatin structure, the maintenance of genome integrity, and stable patterns of gene expression. These biological effects of DNA methylation are, at least in part, mediated by proteins that preferentially bind to methylated DNA[26]. Methylated DNA is specifically recognized by a set of proteins called methyl-CpG-binding proteins (MBPs), which belong to three different structural families: methyl-CpG binding domain proteins (MBDs), Kaiso domain proteins, and SET and RING finger-associated domain (SRA) domain proteins[27,28]. MBD family proteins (MeCP2, MBD1, MBD2, MBD3 and MBD4) bind methylated CpG (5mCpG) through a conserved protein motif called the methyl-CpG binding domain[29,30]. Over the last decade, proteins that utilize different structures to recognize and bind DNA or its components have been identified. In 2001, Prokhortchouk *et al*[31] identified Kaiso proteins, which bind methylated DNA through a zinc finger motif. Other MBPs including UHRF1 and UHRF2 were identified, and these proteins use the SRA to bind 5mCpG[32,33].

In cancer, the roles of MBPs are related to their functions as transcriptional repressors or chromatin remodelers (Table 1)[34-36]. However, a few studies have reported MBPs in GC (Table 1). Mutations in *MBD4* have been found in gastric tumors in association with microsatellite instability[37,38]. *MBD4* encodes a protein that interacts with the mismatch repair protein hMLH1. Therefore, it has been postulated that mutations in *MBD4* may result in mismatch repair deficiency[30].

The processes of DNA methylation and histone modification often involve dynamic interactions that either reinforce or inhibit epigenetic changes. Thus, histone modification can also alter chromatin remodeling, and this is a possible mechanism for decreased gene expression[39-41].

The nature of the interaction between DNA and histones, which are composed of pairs of the four core proteins H2A, H2B, H3, and H4, alters the accessibility of DNA transcription sites to RNA polymerase Ⅱ and other transcription factors. The interaction between histones and DNA is thought to be under epigenetic control, because specific amino acid residues on specific histone core proteins are subjected to post-translational modifications, such as acetylation, methylation, phosphorylation, ubiquitination, sumoylation, proline isomerization, and ADP ribosylation[42,43]. Histone acetylation and methylation are the only modifications that have been clinically associated with pathological epigenetic disruption in cancer cells[44]. In this review, we focus on histone methylation modifications.

Histones can be mono-, di-, or trimethylated at lysine and arginine residues by histone methyltransferases (HMTs) or demethylated by histone demethylases (HDTs). Depending on the residue and the level of methylation, the chromatin may be transcriptionally active or inactive. In general, trimethylation at H3K4 and H3K36 or monomethylation at H3K27, H3K9, H4K20, H3K79, and H2BK5 is associated with transcriptional activation. In contrast, trimethylation at H3K27, H3K9, and H4K20 or monomethylation at H3K27, H3K9, H4K20, H3K79, and H2BK5 is associated with transcriptional repression[44].

A growing number of studies have analyzed the HMTs and HDMs in tumor cells, whereas few genes involved in histone methylation activity have been described for GC (Table 1). EZH2, an HMT that plays a role in trimethylation of H3K27 and leads to silencing of important genes in carcinogenesis, is overexpressed in several types of cancer, including GC[45,46]. Cai *et al*[47] reported that EZH2 plays an important role in the multistep process of intestinal-type GC. In addition, Fujii *et al*[48] demonstrated that silencing of *EZH2* by siRNA resulted in a lower H3K27me3 protein level in GC cells.

Table 1 Methylation machinery in gastric cancer

Gene	Function	Alteration in cancer	Ref.
DNMT1	Maintenance of methylation Repression of transcription	Upregulation Mutation	Kanai *et al*[93] Fang *et al*[94] Ding *et al*[95] Yang *et al*[96] Mutze *et al*[97]
DNMT3A	*De novo* methylation during embryogenesis Imprint establishment Repression	Upregulation Mutation	Ding *et al*[95] Fan *et al*[98] Yang *et al*[96]
DNMT3B	*De novo* methylation during embryogenesis Repeat methylation Repression	Upregulation Mutation	Ding *et al*[95] Su *et al*[99] Hu *et al*[100] Yang *et al*[96]
MeCP2	Transcription repression	Upregulation Mutation	Wada *et al*[101]
MBD1	Transcription repression	Upregulation Mutation	-
MBD2	Transcription repression DNA demethylase	Downregulation Mutation	Kanai *et al*[102]
MBD3	Transcription repression, but requires MBD2 to recruit it to methylated DNA	Upregulation Mutation	-
MBD4	Transcription repression DNA repair Glycosylase domain, repair of deaminated 5-methyl C	Downregulation Mutation	Pinto *et al*[38] D'Errico *et al*[37]
Kaiso	Transcription repression	Upregulation	Ogden *et al*[103]
G9a	Histone methyltransferase	Gene Repression	Lee *et al*[104]
RIZ1	Histone methyltransferase	Underexpression	Oshimo *et al*[105]
PRDM2		Mutation	Pan *et al*[106]
SUZ12	Histone methyltransferase	Upregulation	Yoo *et al*[107]
BMI1	Histone methyltransferase	Upreguletion	Liu *et al*[108] Xiao *et al*[109] Lu *et al*[110] Zhang *et al*[111] Li *et al*[112]
EVI1	Histone methyltransferase	Chromosomal rearrangement	Takahata *et al*[113]
EZH2	Histone methyltransferase	Amplification Upregulation Mutation	Mattioli *et al*[114] Varambally *et al*[115] Fujii *et al*[48] Cai *et al*[47] Choi *et al*[46] Zhou *et al*[116]
NSD2/MMMSET	Histone methyltransferase	Upregulation Translocation	Hudlebusch *et al*[117]
SUV39H1 -2	Histone methyltransferase	Polymorphism	Li *et al*[84]
LSD1/BHC110	Histone demethylase	Downregulation	Magerl *et al*[118]
JARID1A-D	Histone demethylase	Upregulation Inactivation	Zeng *et al*[51]
JMJD2A *JHDM3A*	Histone demethylase	Mutation Upregulation	Li *et al*[119]
JMJD1A-C	Histone demethylase	Downregulation	Katoh *et al*[120]

DNMT: DNA methyltransferase; *EVH1*: Domain containing 1; *EZH2*: Enhancer of zest homolog2; *JARID*: Jumonji, AT-rich interactive-domain; *JHDM*: JmjC domain-containing histone demethylase 1; *JMJD*: Jumonji domain containing 2; *LSD1*: Lysine specific demethylase; *MBD*: Methyl-CpG-binding domain; *NSD2*: Nuclear receptor-binding SET-domain protein 2; *PRMT*: Protein arginine methyltransferase 1; *RIZ1*: Retinoblastoma protein-interacting zinc finger 1; *SUV39H*: Suppressor of variation 3-9 homolog.

Among the HDTs, RBP2 is a newly identified member of the JARID family of proteins, and RBP2 specifically targets tri- and dimethylated H3K4 for demethylation in cancer[49,50]. Zeng *et al*[51] reported that *RBP2* is overexpressed in GC and suggested that HDT inhibition by targeting RBP2 may be an anticancer strategy.

DNA METHYLATION

DNA methylation contributes to cancer mainly through DNA hypo- or hypermethylation. DNA hypomethylation, which refers to the loss of DNA methylation, affects chromosomal stability and increases aneuploidy[52]. DNA hypermethylation, which refers to the gain of methylation at a locus originally unmethylated, usually results in stable transcriptional silencing, which functions in regulating gene expression[53,54].

Global DNA hypomethylation is usually considered one of the hallmarks of cancer cells, because aberrant hypermethylation-vulnerable genes are overlapped by

Table 2 Aberrant DNA methylation in gastric cancer

Gene	Role	Aberrant methylation	Ref.
ABCB1	Multidrug resistance	Hyper	Poplawski *et al*[121], Tahara *et al*[122], Lee *et al*[123]
ADAM23	Tissue cell invasion and metastasis	Hyper	Takada *et al*[124], Watanabe *et al*[125], Kim *et al*[126]
ALDH2	Oxidative pathway of alcohol metabolism	Hypo	Balassiano *et al*[127]
APC	Tissue cell invasion and metastasis Signal transduction	Hyper	Bernal *et al*[128], Ksiaa *et al*[63], Shin *et al*[69], Geddert *et al*[129]
ARPC1B (*p41ARC*)	Cell morphology	Hyper	Maekita *et al*[130], Shin *et al*[69]
BNIP3	Apoptosis	Hyper	Murai *et al*[131], Hiraki *et al*[132], Sugita *et al*[133]
BRCA1	DNA repair	Hyper	Bernal *et al*[128], Ryan *et al*[134]
CAV1	Tissue cell invasion and metastasis	Hyper	Yamashida *et al*[135]
CDH1	Tissue invasion and metastasis	Hyper	Leal *et al*[136], Bernal *et al*[136], Borges *et al*[61], Tahara *et al*[122], Al-Moundhri *et al*[137], Balassiano *et al*[127]
CHFR	Cell cycle regulation	Hyper	Oki *et al*[138], Hiraki *et al*[139], Hu *et al*[140]
DAPK	Apoptosis	Hyper	Bernal *et al*[128], Zou *et al*[74], Hu *et al*[140], Tahara *et al*[122], Sugita *et al*[133]
FHIT	Apoptosis	Hyper	Leal *et al*[136], Bernal *et al*[128]
FLNC	Cell morphology	Hyper	Kim *et al*[126], Shi *et al*[141]
GATA4/5	Transcriptional factor	Hyper	Akiyama *et al*[142], Wen *et al*[143],
HAND1	Cell differentiation	Hyper	Maekita *et al*[130], Shin *et al*[69], Shi *et al*[141]
HRAS	Signal transduction	Hypo	Fang *et al*[144], Luo *et al*[145]
IGFBP3	Cell cycle regulation	Hyper	Gigek *et al*[146], Ryan *et al*[134], Chen *et al*[147]
LOX	Tissue cell invasion and adhesion	Hyper	Maekita *et al*[130], Shin *et al*[69], Tamura *et al*[148]
MGMT	DNA repair	Hyper	Bernal *et al*[128], Hibi *et al*[149], Ksiaa *et al*[63]; Zou *et al*[74], Schneider *et al*[14], Hiraki *et al*[139], Balassiano *et al*[127], Shi *et al*[141]
MLF1	Cell differentiation	Hyper	Watanabe *et al*[125], Shi *et al*[141], Yamashita *et al*[135]
MLH1	DNA repair	Hyper	Bernal *et al*[128], Poplawski *et al*[121], Hiraki *et al*[139], Kim *et al*[150], Shin *et al*[58]
MOS	Cell cycle regulation	Hypo	Shin *et al*[58]
MTHFR	DNA synthesis DNA repair DNA methylation	Hypo	Balassiano *et al*[127]
MYC	Cell cycle regulation	Hypo	Fang *et al*[144], Luo *et al*[145]
P14ARF	Cell cycle regulation Apoptosis Cell differentiation	Hyper	Balassiano *et al*[127], Geddert *et al*[129]
P16	Cell cycle regulation	Hyper	Ksiaa *et al*[63], Dong *et al*[151], Zou *et al*[74], Shin *et al*[69], Hu *et al*[140], Ryan *et al*[134], Geddert *et al*[129], Balassiano *et al*[124], Al-Moundhri *et al*[137], Shin *et al*[58]
PRDM5	Cell differentiation	Hyper	Watanabe *et al*[125], Shu *et al*[152]
RAR-beta 2	DNA binding Activation transcription	Hyper	Bernal *et al*[128], Ksiaa *et al*[63]
RASSF1A/RASSF2	DNA repair Cell cycle regulation	Hyper	Zou *et al*[74], Guo *et al*[153], Shin *et al*[58]
RORA	Cell differentiation	Hyper	Watanabe *et al*[125], Yamashida *et al*[131]
RPRM	Cell cycle regulation	Hyper	Bernal *et al*[128], Schneider *et al*[14]
RUNX3	Signal transduction	Hyper	Bernal *et al*[128], Sakakura *et al*[154], Lee *et al*[104], Zou *et al*[74], Hiraki *et al*[139], Tamura *et al*[148], Hu *et al*[140], Fan *et al*[155], Al-Moundhri *et al*[137]
SHP1	Signal transduction	Hyper	Bernal *et al*[128], Ksiaa *et al*[63],
TERT	Cell senescence	Hyper	Kang *et al*[67], Wang *et al*[75], Gigek *et al*[77]
TFF1	Repair gene	Hyper	Carvalho *et al*[156], Ryan *et al*[134]
THBD	Inflammation response	Hyper	Maekita *et al*[130]; Shin *et al*[69]
TWIST1	Cell differentiation	Hyper	Kang *et al*[67], Schneider *et al*[14]

ABCB1: ATP-binding cassette, sub-family B (MDR/TAP), member 1; *ADAM23*: ADAM metallopeptidase domain 23; *ALDH2*: Aldehyde dehydrogenase 2 family (mitochondrial); *APC*: Adenomatous polyposis coli; *ARPC1B* (*p41ARC*): Actin related protein 2/3 complex, subunit 1B, 41kDa; *BNIP3*: Adenovirus E1B 19kDa interacting protein 3; *BRCA1*: Breast cancer 1 gene; *CAV1*: Caviolin 1; *CDH1*: Cadherin 1; *CHFR*: Checkpoint with forkhead and ring finger domains; *DAPK*: Dapk death associated protein kinase; *FHIT*: Fragile histidine triad gene; *FLNC*: Filamin C, gamma; GATA4/5: GATA binding protein 4/5; GSTP1: Glutathione S-transferase pi 1; *HAND1*: Heart and neural crest derivatives expressed 1; *HRAS*: v-Ha-ras Harvey rat sarcoma viral oncogene homolog; IGFBP3: Insulin-like growth factor; binding protein 3; *LOX*: Lysyl oxidase; *MGMT*: O-6-methylguanine-DNA methyltransferase; *MLF1*: Myeloid leukemia factor 1; *MLH1*: MutL homolog 1; *MOS*: Moloney murine sarcoma viral oncogene homolog; *MTHFR*: Methylenetetrahydrofolate reductase (NADPH); *MYC*: v-myc myelocytomatosis viral oncogene homolog (avian); *P14ARF*: Cyclin-dependent kinase inhibitor 2A; *P16*: Cyclin-dependent kinase inhibitor 2A; *PRDM5*: PR domain containing 5; *RAR-beta 2*: Retinoic acid receptor β 2 gene; *RASSF1A/RASSF2*: Ras association (RalGDS/AF-6) domain family member 1/member 2; *RORA*: RAR-related orphan receptor A; *RPRM*: TP53 dependent G_2 arrest mediator candidate; *RUNX3*: Runt-related transcription factor 3; *SHP1*: Hematopoietic cell-specific protein-tyrosine phosphatase; *TERT*: Telomerase reverse transcriptase; *TFF1*: Trefoil factor 1; *TFPI2*: Tissue factor pathway inhibitor 2; *THBD*: Thrombomodulin; *TWIST1*: Twist homolog 1.

genes targeted by hypomethylation[55,56]. Compare *et al*[57] suggested that global DNA hypomethylation may be implicated in GC associated with *H. pylori* infection at an early stage. At the individual gene level, DNA hypomethylation is often associated with activation of proto-oncogenes.

In GC, few studies have shown promoter hypomethylation associated with the activation of proto-oncogenes (Table 2). In particular, Shin *et al*[58] reported that the hypomethylation of the *MOS* promoter in GC was associated with tumor invasion, lymph node metastasis, and the diffuse type. A number of genes involved in cell cycle regulation, tumor cell invasion, DNA repair, chromatin remodeling, cell signaling, transcription, and apoptosis are known to be silenced by hypermethylation in GC (Table 2).

Multiple reports have been published regarding gene hypermethylation in both intestinal and diffuse types of GC. Interestingly, the methylation profile differs between the intestinal and diffuse types of GC[54].

The epithelial cadherin gene *CDH1*, which is a well-studied gene involved in cancer, is downregulated in gastric tumors and is hypermethylated more frequently in the diffuse type than in the intestinal type of GC. Loss of *CDH1* during tumor progression has led to the notion that this is a tumor suppressor gene[59,60]. In addition, mapping of the *CDH1* promoter has revealed a positive association between hypermethylation and older age, as well as a significant correlation between DNA hypermethylation and the A allele of the -160 C→A polymorphism. The A allele has been described to increase the risk of developing GC in association with the methylation status[61]. Unlike the *CDH1* gene, the *P16* gene is hypermethylated mainly in the intestinal type of GC[54,62,63]. This epigenetic mark was recently associated with tumor location and *H. pylori* infection in GC[64].

Other studies have also described a number of genes that are silenced by hypermethylation in association with *H. pylori* or EBV infection: *APC*, *SHP1*, *p14*, and *CDH1*[63,65-67]. According to Chan *et al*[68], the eradication of *H. pylori* infection significantly reduces the methylation index of the *CDH1* promoter. In contrast, it has been shown that a portion of the aberrant DNA methylation induced by *H. pylori* infection may persist even after the infection has disappeared[69,70]. Shin *et al*[58] observed that the methylation levels in *MOS* remained significantly increased in patients with previous *H. pylori* infection compared with *H. pylori*-negative subjects.

Moreover, hypermethylation of several gene promoters has also been observed in the premalignant stages of GC, suggesting that aberrant methylation occurs early during gastric carcinogenesis[59,71-74]. For example, the methylation levels of the catalytic subunit of the telomerase gene (*hTERT*) promoter are increased during gastric carcinogenesis. Wang *et al*[75] reported that the *hTERT* promoter was more methylated in GC than in precancerous lesions and non-neoplastic gastric tissues. Therefore, it has been suggested that the degree of methylation of the *hTERT* promoter may be useful in the early diagnosis of GC and/or may have an impact on the anti-telomerase strategy for cancer therapy. Other studies, however, showed that methylation of the *hTERT* promoter and resultant gene expression were opposite to the general model of regulation by DNA methylation, which is usually dependent on the CpG islands studied[76,77].

Recently, aberrant hypermethylation of the newly associated metastatic suppressor gene *RECK* was found to be associated with GC development and may also be useful for early diagnosis and treatment[78]. These abovementioned findings lead to the possibilities that epigenetic alterations may also occur at different stages of gastric tumorigenesis.

HISTONE METHYLATION

Histone modifications leading to gene expression alterations have been described in several cancer types, but the methylation status of chromatin is still unclear for GC. Using the ChIP-on-chip technique, Zhang *et al*[79] identified candidate genes with significant differences in H3K27me3 in GC samples compared to adjacent non-neoplastic gastric tissues. These genes included oncogenes, tumor suppressor genes, cell cycle regulators, and genes involved in cell adhesion. Moreover, these investigators demonstrated that higher levels of H3K27me3 produce gene expression changes in *MMP15*, *UNC5B,* and *SHH*.

In 2011, Kwon *et al*[80] showed that *LAMB3* and *LAMC2* were overexpressed in GC samples in comparison with non-neoplastic adjacent tissue samples. Furthermore, these researchers demonstrated that overexpression of these genes was a result of the enrichment of H3K4me3 in the gene promoter. Using immunohistochemistry, Park *et al*[81] showed that higher levels of H3K9me3, which is a repressive mark, was associated with higher T stage, lymphovascular invasion, and recurrence in gastric tumors. They also observed that the level of H3K9me3 was correlated with patient survival, because stronger methylation corresponded to a worse prognosis and intermediate methylation to an intermediate prognosis.

Taken together with results from previous studies, these results have suggested that histone methylation results in a worse prognosis by inactivating certain tumor suppressor genes[82,83]. Moreover, Li *et al*[84] used GC cell lines to demonstrate that the PRC1 member CBX7 initiated trimethylation of H3K9 at the *P16* locus through recruitment and/or activation of the HMT SUV39H2 to the target locus. This finding links two repressive epigenetic landmarks, H3K9me3 formation and PRC1 binding within the silenced domains in euchromatin, and builds up a full pathway for epigenetic inactivation of *P16* by histone modifications.

Recently, Angrisano *et al*[85] reported that *H. pylori* infection is followed by activation of *iNOS* gene expression, chromatin changes at the *iNOS* promoter (including decreased H3K9 methylation and increased H3K4 methylation), and selective release of MBD2 from the *iNOS*

promoter in a GC cell line.

METHYLATION INHIBITOR DRUGS

The silencing of cancer-related genes by DNA methylation and chromatin modification are reversible and may represent a viable epigenetic therapeutic target. In the last decade, drugs that modify chromatin or DNA methylation status have been used alone or in combination in order to affect therapeutic outcomes[86]. Specially, cytosine analogs (5-azacytidine and 5-aza-2'- deoxycytidine) are powerful mechanism-based inhibitors of DNA cytosine methylation. These cytosine analogs are incorporated into the DNA of replicating cells after the drugs have been metabolized to the appropriate dNTP. After incorporation into the DNA, the analogs interact with DNA methyltransferases to form covalent intermediates, and this interaction inhibits DNA methylation in subsequent rounds of DNA synthesis[87]. Both drugs have been approved by the US Food and Drug Administration for use in hematological malignancy treatment[88].

In GC, surgery remains the primary curative treatment for gastric tumors. Currently, adjuvant and neoadjuvant therapies are accepted[89]; however, so-called epigenetic therapy has not yet been used in treatment of GC patients.

In the past few years, epigenetic screening techniques using treatment with a demethylating agent have been developed to identify genes with epigenetic aberrations in GC cell lines. Zheng *et al*[90] treated a GC cell line with 5-aza-2'-deoxycytidine and performed DNA methylation array analysis of these cells with six normal mucosal samples from healthy patients. These results revealed 82 hypermethylated gene promoters. These authors investigated 15 candidate genes by methylation-specific PCR and confirmed five highly methylated promoters: *BX141696*, *WT1*, *CYP26B1*, *KCNA4,* and *FAM84A*. All of these, except *FAM84A*, also showed DNA hypermethylation in serum of GC patients, suggesting that serum DNA offers a readily accessible bioresource for methylation analysis.

A similar study conducted by Jee *et al*[91] described 11 selected genes and validated the genes in three GC cell lines and in non-neoplastic gastric tissue by bisulfate sequencing. Differential DNA hypermethylation was observed in *GPX1*, *IGFBP6*, *IRF7*, *GPX3*, *TFPI2,* and *DMRT1* promoter regions in GC cells but not in non-neoplastic tissues. Moreover, a poor survival rate was observed in those individuals with higher methylation status at the *TFPI2* gene. *TFPI2* is a serine protease inhibitor, which negatively regulates the enzymatic activities of trypsin, plasmin, and a tissue factor complex. Therefore, it has been proposed that this gene inactivation may be implicated in human carcinogenesis and metastasis[92].

CONCLUSION

In summary, aberrant DNA methylation and histone modification play a crucial role in gastric carcinogenesis. Thus, the recognition of the methylation machinery, genes with aberrant methylation status, and histone methylation levels in gastric carcinogenesis exemplified in this review allow us to contemplate the possibility of dealing with the aforementioned oncological issue in a new way that may have a significant impact on the therapy and management of GC.

REFERENCES

1 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]

2 **Lauren P**. The two histological main types of gastric carcinoma: diffuse and so-called intestinal-type carcinoma. an attempt at a histo-clinical classification. *Acta Pathol Microbiol Scand* 1965; **64**: 31-49 [PMID: 14320675]

3 **Correa P**, Haenszel W, Cuello C, Tannenbaum S, Archer M. A model for gastric cancer epidemiology. *Lancet* 1975; **2**: 58-60 [PMID: 49653]

4 **Humar B**, Guilford P. Hereditary diffuse gastric cancer: a manifestation of lost cell polarity. *Cancer Sci* 2009; **100**: 1151-1157 [PMID: 19432899 DOI: 10.1111/j.1349-7006.2009.01163.x]

5 **Carneiro F**, Huntsman DG, Smyrk TC, Owen DA, Seruca R, Pharoah P, Caldas C, Sobrinho-Simões M. Model of the early development of diffuse gastric cancer in E-cadherin mutation carriers and its implications for patient screening. *J Pathol* 2004; **203**: 681-687 [PMID: 15141383 DOI: 10.1002/path.1564]

6 **Tahara E**. Genetic pathways of two types of gastric cancer. *IARC Sci Publ* 2004; (**157**): 327-349 [PMID: 15055305]

7 **Calcagno DQ**, Guimarães AC, Leal MF, Seabra AD, Khayat AS, Pontes TB, Assumpção PP, De Arruda Cardoso Smith M, Burbano RR. MYC insertions in diffuse-type gastric adenocarcinoma. *Anticancer Res* 2009; **29**: 2479-2483 [PMID: 19596917]

8 **Calcagno DQ**, Leal MF, Assumpcao PP, Smith MA, Burbano RR. MYC and gastric adenocarcinoma carcinogenesis. *World J Gastroenterol* 2008; **14**: 5962-5968 [PMID: 18932273]

9 **Calcagno DQ**, Leal MF, Seabra AD, Khayat AS, Chen ES, Demachki S, Assumpção PP, Faria MH, Rabenhorst SH, Ferreira MV, de Arruda Cardoso Smith M, Burbano RR. Interrelationship between chromosome 8 aneuploidy, C-MYC amplification and increased expression in individuals from northern Brazil with gastric adenocarcinoma. *World J Gastroenterol* 2006; **12**: 6207-6211 [PMID: 17036397]

10 **Calcagno DQ**, Leal MF, Taken SS, Assumpção PP, Demachki S, Smith Mde A, Burbano RR. Aneuploidy of chromosome 8 and C-MYC amplification in individuals from northern Brazil with gastric adenocarcinoma. *Anticancer Res* 2005; **25**: 4069-4074 [PMID: 16309200]

11 **Pfeifer GP**, Tang M, Denissenko MF. Mutation hotspots and DNA methylation. *Curr Top Microbiol Immunol* 2000; **249**: 1-19 [PMID: 10802935]

12 **Gigek CO**, Chen ES, Calcagno DQ, Wisnieski F, Burbano RR, Smith MA. Epigenetic mechanisms in gastric cancer. *Epigenomics* 2012; **4**: 279-294 [PMID: 22690664]

13 **Ferrasi AC**, Pinheiro NA, Rabenhorst SH, Caballero OL, Rodrigues MA, de Carvalho F, Leite CV, Ferreira MV, Barros MA, Pardini MI. Helicobacter pylori and EBV in gastric carcinomas: methylation status and microsatellite instability. *World J Gastroenterol* 2010; **16**: 312-319 [PMID: 20082476]

14 **Schneider BG**, Peng DF, Camargo MC, Piazuelo MB, Sicinschi LA, Mera R, Romero-Gallo J, Delgado AG, Bravo LE, Wilson KT, Peek RM, Correa P, El-Rifai W. Promoter DNA hypermethylation in gastric biopsies from subjects at high and low risk for gastric cancer. *Int J Cancer* 2010; **127**: 2588-2597 [PMID: 20178103 DOI: 10.1002/ijc.25274]

15 **Shin CM**, Kim N, Jung Y, Park JH, Kang GH, Park WY, Kim JS, Jung HC, Song IS. Genome-wide DNA methylation profiles in noncancerous gastric mucosae with regard to Helicobacter pylori infection and the presence of gastric cancer. *Helicobacter* 2011; **16**: 179-188 [PMID: 21585603 DOI: 10.1111/j.1523-5378.2011.00838.x]

16 **Matsusaka K**, Kaneda A, Nagae G, Ushiku T, Kikuchi Y, Hino R, Uozaki H, Seto Y, Takada K, Aburatani H, Fukayama M. Classification of Epstein-Barr virus-positive gastric cancers by definition of DNA methylation epigenotypes. *Cancer Res* 2011; **71**: 7187-7197 [PMID: 21990320 DOI: 10.1158/0008-5472.CAN-11-1349]

17 **Jones PA**, Takai D. The role of DNA methylation in mammalian epigenetics. *Science* 2001; **293**: 1068-1070 [PMID: 11498573 DOI: 10.1126/science.1063852]

18 **Baylin SB**, Ohm JE. Epigenetic gene silencing in cancer - a mechanism for early oncogenic pathway addiction? *Nat Rev Cancer* 2006; **6**: 107-116 [PMID: 16491070 DOI: 10.1038/nrc1799]

19 **Kim H**, Park J, Jung Y, Song SH, Han SW, Oh DY, Im SA, Bang YJ, Kim TY. DNA methyltransferase 3-like affects promoter methylation of thymine DNA glycosylase independently of DNMT1 and DNMT3B in cancer cells. *Int J Oncol* 2010; **36**: 1563-1572 [PMID: 20428781]

20 **Okano M**, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* 1999; **99**: 247-257 [PMID: 10555141]

21 **Wienholz BL**, Kareta MS, Moarefi AH, Gordon CA, Ginno PA, Chédin F. DNMT3L modulates significant and distinct flanking sequence preference for DNA methylation by DNMT3A and DNMT3B in vivo. *PLoS Genet* 2010; **6**: [PMID: 20838592 DOI: 10.1371/journal.pgen.1001106]

22 **Wallasch C**, Crabtree JE, Bevec D, Robinson PA, Wagner H, Ullrich A. Helicobacter pylori-stimulated EGF receptor transactivation requires metalloprotease cleavage of HB-EGF. *Biochem Biophys Res Commun* 2002; **295**: 695-701 [PMID: 12099696]

23 **Etoh T**, Kanai Y, Ushijima S, Nakagawa T, Nakanishi Y, Sasako M, Kitano S, Hirohashi S. Increased DNA methyltransferase 1 (DNMT1) protein expression correlates significantly with poorer tumor differentiation and frequent DNA hypermethylation of multiple CpG islands in gastric cancers. *Am J Pathol* 2004; **164**: 689-699 [PMID: 14742272 DOI: 10.1016/S0002-9440(10)63156-2]

24 **Hino R**, Uozaki H, Murakami N, Ushiku T, Shinozaki A, Ishikawa S, Morikawa T, Nakaya T, Sakatani T, Takada K, Fukayama M. Activation of DNA methyltransferase 1 by EBV latent membrane protein 2A leads to promoter hypermethylation of PTEN gene in gastric carcinoma. *Cancer Res* 2009; **69**: 2766-2774 [PMID: 19339266 DOI: 10.1158/0008-5472.CAN-08-3070]

25 **Fukayama M**. Epstein-Barr virus and gastric carcinoma. *Pathol Int* 2010; **60**: 337-350 [PMID: 20518883 DOI: 10.1111/j.1440-1827.2010.02533.x]

26 **Bogdanović O**, Veenstra GJ. DNA methylation and methyl-CpG binding proteins: developmental requirements and function. *Chromosoma* 2009; **118**: 549-565 [PMID: 19506892 DOI: 10.1007/s00412-009-0221-9]

27 **Fournier A**, Sasai N, Nakao M, Defossez PA. The role of methyl-binding proteins in chromatin organization and epigenome maintenance. *Brief Funct Genomics* 2012; **11**: 251-264 [PMID: 22184333 DOI: 10.1093/bfgp/elr040]

28 **Defossez PA**, Stancheva I. Biological functions of methyl-CpG-binding proteins. *Prog Mol Biol Transl Sci* 2011; **101**: 377-398 [PMID: 21507359 DOI: 10.1016/B978-0-12-387685-0.00012-3]

29 **Hendrich B**, Bird A. Identification and characterization of a family of mammalian methyl-CpG binding proteins. *Mol Cell Biol* 1998; **18**: 6538-6547 [PMID: 9774669]

30 **Bellacosa A**. Role of MED1 (MBD4) Gene in DNA repair and human cancer. *J Cell Physiol* 2001; **187**: 137-144 [PMID: 11267993 DOI: 10.1002/jcp.1064]

31 **Prokhortchouk A**, Hendrich B, Jørgensen H, Ruzov A, Wilm M, Georgiev G, Bird A, Prokhortchouk E. The p120 catenin partner Kaiso is a DNA methylation-dependent transcriptional repressor. *Genes Dev* 2001; **15**: 1613-1618 [PMID: 11445535 DOI: 10.1101/gad.198501]

32 **Unoki M**, Nishidate T, Nakamura Y. ICBP90, an E2F-1 target, recruits HDAC1 and binds to methyl-CpG through its SRA domain. *Oncogene* 2004; **23**: 7601-7610 [PMID: 15361834 DOI: 10.1038/sj.onc.1208053]

33 **Hashimoto H**, Horton JR, Zhang X, Cheng X. UHRF1, a modular multi-domain protein, regulates replication-coupled crosstalk between DNA methylation and histone modifications. *Epigenetics* 2009; **4**: 8-14 [PMID: 19077538]

34 **Sansom OJ**, Maddison K, Clarke AR. Mechanisms of disease: methyl-binding domain proteins as potential therapeutic targets in cancer. *Nat Clin Pract Oncol* 2007; **4**: 305-315 [PMID: 17464338 DOI: 10.1038/ncponc0812]

35 **Lopez-Serra L**, Ballestar E, Ropero S, Setien F, Billard LM, Fraga MF, Lopez-Nieva P, Alaminos M, Guerrero D, Dante R, Esteller M. Unmasking of epigenetically silenced candidate tumor suppressor genes by removal of methyl-CpG-binding domain proteins. *Oncogene* 2008; **27**: 3556-3566 [PMID: 18223687 DOI: 10.1038/sj.onc.1211022]

36 **Parry L**, Clarke AR. The Roles of the Methyl-CpG Binding Proteins in Cancer. *Genes Cancer* 2011; **2**: 618-630 [PMID: 21941618 DOI: 10.1177/1947601911418499]

37 **D'Errico M**, de Rinaldis E, Blasi MF, Viti V, Falchetti M, Calcagnile A, Sera F, Saieva C, Ottini L, Palli D, Palombo F, Giuliani A, Dogliotti E. Genome-wide expression profile of sporadic gastric cancers with microsatellite instability. *Eur J Cancer* 2009; **45**: 461-469 [PMID: 19081245 DOI: 10.1016/j.ejca.2008.10.032]

38 **Pinto M**, Wu Y, Suriano G, Mensink RG, Duval A, Oliveira C, Carvalho B, Hamelin R, Seruca R, Hofstra RM. MBD4 mutations are rare in gastric carcinomas with microsatellite instability. *Cancer Genet Cytogenet* 2003; **145**: 103-107 [PMID: 12935920]

39 **Shilatifard A**. Chromatin modifications by methylation and ubiquitination: implications in the regulation of gene expression. *Annu Rev Biochem* 2006; **75**: 243-269 [PMID: 16756492 DOI: 10.1146/annurev.biochem.75.103004.142422]

40 **Weisbrod S**. Active chromatin. *Nature* 1982; **297**: 289-295 [PMID: 6210847]

41 **Richards EJ**, Elgin SC. Epigenetic codes for heterochromatin formation and silencing: rounding up the usual suspects. *Cell* 2002; **108**: 489-500 [PMID: 11909520]

42 **Zhang Y**, Reinberg D. Transcription regulation by histone methylation: interplay between different covalent modifications of the core histone tails. *Genes Dev* 2001; **15**: 2343-2360 [PMID: 11562345 DOI: 10.1101/gad.927301]

43 **Sawan C**, Herceg Z. Histone modifications and cancer. *Adv Genet* 2010; **70**: 57-85 [PMID: 20920745 DOI: 10.1016/B978-0-12-380866-0.60003-4]

44 **Ellis L**, Atadja PW, Johnstone RW. Epigenetics in cancer: targeting chromatin modifications. *Mol Cancer Ther* 2009; **8**: 1409-1420 [PMID: 19509247 DOI: 10.1158/1535-7163.MCT-08-0860]

45 **Matsukawa Y**, Semba S, Kato H, Ito A, Yanagihara K, Yokozaki H. Expression of the enhancer of zeste homolog 2 is correlated with poor prognosis in human gastric cancer. *Cancer Sci* 2006; **97**: 484-491 [PMID: 16734726 DOI: 10.1111/j.1349-7006.2006.00203.x]

46 **Choi JH**, Li Y, Guo J, Pei L, Rauch TA, Kramer RS, Macmil SL, Wiley GB, Bennett LB, Schnabel JL, Taylor KH, Kim S, Xu D, Sreekumar A, Pfeifer GP, Roe BA, Caldwell CW, Bhalla KN, Shi H. Genome-wide DNA methylation maps in follicular lymphoma cells determined by methylation-enriched bisulfite sequencing. *PLoS One* 2010; **5**: [PMID: 20927367 DOI: 10.1371/journal.pone.0013020]

47 **Cai GH**, Wang K, Miao Q, Peng YS, Chen XY. Expression of polycomb protein EZH2 in multi-stage tissues of gastric carcinogenesis. *J Dig Dis* 2010; **11**: 88-93 [PMID: 20402834 DOI: 10.1111/j.1751-2980.2010.00420.x]

48 **Fujii S**, Ito K, Ito Y, Ochiai A. Enhancer of zeste homologue 2 (EZH2) down-regulates RUNX3 by increasing histone H3 methylation. *J Biol Chem* 2008; **283**: 17324-17332 [PMID: 18430739 DOI: 10.1074/jbc.M800224200]

49 **Christensen J**, Agger K, Cloos PA, Pasini D, Rose S, Sennels L, Rappsilber J, Hansen KH, Salcini AE, Helin K. RBP2 belongs to a family of demethylases, specific for tri-and dimethylated lysine 4 on histone 3. *Cell* 2007; **128**: 1063-1076 [PMID: 17320161 DOI: 10.1016/j.cell.2007.02.003]

50 **Lopez-Bigas N**, Kisiel TA, Dewaal DC, Holmes KB, Volkert TL, Gupta S, Love J, Murray HL, Young RA, Benevolenskaya EV. Genome-wide analysis of the H3K4 histone demethylase RBP2 reveals a transcriptional program controlling differentiation. *Mol Cell* 2008; **31**: 520-530 [PMID: 18722178 DOI: 10.1016/j.molcel.2008.08.004]

51 **Zeng J**, Ge Z, Wang L, Li Q, Wang N, Björkholm M, Jia J, Xu D. The histone demethylase RBP2 Is overexpressed in gastric cancer and its inhibition triggers senescence of cancer cells. *Gastroenterology* 2010; **138**: 981-992 [PMID: 19850045]

52 **Gaudet F**, Hodgson JG, Eden A, Jackson-Grusby L, Dausman J, Gray JW, Leonhardt H, Jaenisch R. Induction of tumors in mice by genomic hypomethylation. *Science* 2003; **300**: 489-492 [PMID: 12702876 DOI: 10.1126/science.1083558]

53 **Esteller M**, Corn PG, Baylin SB, Herman JG. A gene hypermethylation profile of human cancer. *Cancer Res* 2001; **61**: 3225-3229 [PMID: 11309270]

54 **Selaru FM**, David S, Meltzer SJ, Hamilton JP. Epigenetic events in gastrointestinal cancer. *Am J Gastroenterol* 2009; **104**: 1910-1912 [PMID: 19661933 DOI: 10.1038/ajg.2008.145]

55 **Yoshida T**, Yamashita S, Takamura-Enya T, Niwa T, Ando T, Enomoto S, Maekita T, Nakazawa K, Tatematsu M, Ichinose M, Ushijima T. Alu and Satα hypomethylation in Helicobacter pylori-infected gastric mucosae. *Int J Cancer* 2011; **128**: 33-39 [PMID: 20602342 DOI: 10.1002/ijc.25534]

56 **Najjar Sadeghi R**, Zojaji H, Mohebbi SR, Chiani M, Vahedi M, Mirsattari D, Molaei M, Mashayekhi R, Zali MR. Evaluation of global genome methylation in gastritis lesion and its correlation with clinicopatological findings. *Oncol Res* 2009; **17**: 549-558 [PMID: 19806785]

57 **Compare D**, Rocco A, Liguori E, D'Armiento FP, Persico G, Masone S, Coppola-Bottazzi E, Suriani R, Romano M, Nardone G. Global DNA hypomethylation is an early event in Helicobacter pylori-related gastric carcinogenesis. *J Clin Pathol* 2011; **64**: 677-682 [PMID: 21617174 DOI: 10.1136/jcp.2010.087858]

58 **Shin CM**, Kim N, Park JH, Kang GH, Kim JS, Jung HC, Song IS. Prediction of the risk for gastric cancer using candidate methylation markers in the non-neoplastic gastric mucosae. *J Pathol* 2012; **226**: 654-665 [PMID: 22252584 DOI: 10.1002/path.2990]

59 **Yamamoto E**, Suzuki H, Takamaru H, Yamamoto H, Toyota M, Shinomura Y. Role of DNA methylation in the development of diffuse-type gastric cancer. *Digestion* 2011; **83**: 241-249 [PMID: 21273772 DOI: 10.1159/000320453]

60 **Cavallaro U**, Christofori G. Multitasking in tumor progression: signaling functions of cell adhesion molecules. *Ann N Y Acad Sci* 2004; **1014**: 58-66 [PMID: 15153420]

61 **Borges Bdo N**, Santos Eda S, Bastos CE, Pinto LC, Anselmo NP, Quaresma JA, Calcagno DQ, Burbano RM, Harada ML. Promoter polymorphisms and methylation of E-cadherin (CDH1) and KIT in gastric cancer patients from northern Brazil. *Anticancer Res* 2010; **30**: 2225-2233 [PMID: 20651373]

62 **Lima EM**, Leal MF, Burbano RR, Khayat AS, Assumpção PP, Bello MJ, Rey JA, Smith MA, Casartelli C. Methylation status of ANAPC1, CDKN2A and TP53 promoter genes in individuals with gastric cancer. *Braz J Med Biol Res* 2008; **41**: 539-543 [PMID: 18622497]

63 **Ksiaa F**, Ziadi S, Amara K, Korbi S, Trimeche M. Biological significance of promoter hypermethylation of tumor-related genes in patients with gastric carcinoma. *Clin Chim Acta* 2009; **404**: 128-133 [PMID: 19336228 DOI: 10.1016/j.cca.2009.03.044]

64 **Alves MK**, Lima VP, Ferrasi AC, Rodrigues MA, De Moura Campos Pardini MI, Rabenhorst SH. CDKN2A promoter methylation is related to the tumor location and histological subtype and associated with Helicobacter pylori flaA(+) strains in gastric adenocarcinomas. *APMIS* 2010; **118**: 297-307 [PMID: 20402675 DOI: 10.1111/j.1600-0463.2010.02591.x]

65 **Ding SZ**, Fischer W, Kaparakis-Liaskos M, Liechti G, Merrell DS, Grant PA, Ferrero RL, Crowe SE, Haas R, Hatakeyama M, Goldberg JB. Helicobacter pylori-induced histone modification, associated gene expression in gastric epithelial cells, and its implication in pathogenesis. *PLoS One* 2010; **5**: e9875 [PMID: 20368982 DOI: 10.1371/journal.pone.0009875]

66 **Hu XT**, He C. Recent progress in the study of methylated tumor suppressor genes in gastric cancer. *Chin J Cancer* 2013; **32**: 31-41 [PMID: 22059906 DOI: 10.5732/cjc.011.10175]

67 **Kang GH**, Lee S, Cho NY, Gandamihardja T, Long TI, Weisenberger DJ, Campan M, Laird PW. DNA methylation profiles of gastric carcinoma characterized by quantitative DNA methylation analysis. *Lab Invest* 2008; **88**: 161-170 [PMID: 18158559 DOI: 10.1038/labinvest.3700707]

68 **Chan AO**. E-cadherin in gastric cancer. *World J Gastroenterol* 2006; **12**: 199-203 [PMID: 16482618]

69 **Shin CM**, Kim N, Yang HJ, Cho SI, Lee HS, Kim JS, Jung HC, Song IS. Stomach cancer risk in gastric cancer relatives: interaction between Helicobacter pylori infection and family history of gastric cancer for the risk of stomach cancer. *J Clin Gastroenterol* 2010; **44**: e34-e39 [PMID: 19561529 DOI: 10.1097/MCG.0b013e3181a159c4]

70 **Nakajima T**, Enomoto S, Yamashita S, Ando T, Nakanishi Y, Nakazawa K, Oda I, Gotoda T, Ushijima T. Persistence of a component of DNA methylation in gastric mucosae after Helicobacter pylori eradication. *J Gastroenterol* 2010; **45**: 37-44 [PMID: 19821005 DOI: 10.1007/s00535-009-0142-7]

71 **Kang GH**, Shim YH, Jung HY, Kim WH, Ro JY, Rhyu MG. CpG island methylation in premalignant stages of gastric carcinoma. *Cancer Res* 2001; **61**: 2847-2851 [PMID: 11306456]

72 **Kang GH**, Lee S, Kim JS, Jung HY. Profile of aberrant CpG island methylation along the multistep pathway of gastric carcinogenesis. *Lab Invest* 2003; **83**: 635-641 [PMID: 12746473]

73 **Jang BG**, Kim WH. Molecular pathology of gastric carcinoma. *Pathobiology* 2011; **78**: 302-310 [PMID: 22104201 DOI: 10.1159/000321703]

74 **Zou XP**, Zhang B, Zhang XQ, Chen M, Cao J, Liu WJ. Promoter hypermethylation of multiple genes in early gastric adenocarcinoma and precancerous lesions. *Hum Pathol* 2009; **40**: 1534-1542 [PMID: 19695681 DOI: 10.1016/j.humpath.2009.01.029]

75 **Wang YC**, Xu JH, Geng X, Zhang WM. [Preliminary study on the alternative splicing pattern of human telomerase reverse transcriptase gene during gastric carcinogenesis]. *Zhonghua Yi Xue Yi Chuan Xue Zazhi* 2009; **26**: 151-155 [PMID: 19350505 DOI: 10.3760/cma.j.issn.1003-9406.2009.02.007]

76 **Guilleret I**, Yan P, Grange F, Braunschweig R, Bosman FT, Benhattar J. Hypermethylation of the human telomerase catalytic subunit (hTERT) gene correlates with telomerase activity. *Int J Cancer* 2002; **101**: 335-341 [PMID: 12209957 DOI: 10.1002/ijc.10593]

77 **Gigek CO**, Leal MF, Silva PN, Lisboa LC, Lima EM, Calcagno DQ, Assumpção PP, Burbano RR, Smith Mde A. hTERT methylation and expression in gastric cancer. *Biomarkers* 2009; **14**: 630-636 [PMID: 20001710 DOI: 10.3109/13547500903225912]

78 **Du YY**, Dai DQ, Yang Z. Role of RECK methylation in gastric cancer and its clinical significance. *World J Gastroenterol* 2010; **16**: 904-908 [PMID: 20143471]

79 **Zhang L**, Zhong K, Dai Y, Zhou H. Genome-wide analysis of histone H3 lysine 27 trimethylation by ChIP-chip in gas-

tric cancer patients. *J Gastroenterol* 2009; **44**: 305-312 [PMID: 19267258 DOI: 10.1007/s00535-009-0027-9]
80 **Kwon OH**, Park JL, Kim M, Kim JH, Lee HC, Kim HJ, Noh SM, Song KS, Yoo HS, Paik SG, Kim SY, Kim YS. Aberrant up-regulation of LAMB3 and LAMC2 by promoter demethylation in gastric cancer. *Biochem Biophys Res Commun* 2011; **406**: 539-545 [PMID: 21345334 DOI: 10.1016/j.bbrc.2011.02.082]
81 **Park YS**, Jin MY, Kim YJ, Yook JH, Kim BS, Jang SJ. The global histone modification pattern correlates with cancer recurrence and overall survival in gastric adenocarcinoma. *Ann Surg Oncol* 2008; **15**: 1968-1976 [PMID: 18470569 DOI: 10.1245/s10434-008-9927-9]
82 **Kondo Y**, Shen L, Issa JP. Critical role of histone methylation in tumor suppressor gene silencing in colorectal cancer. *Mol Cell Biol* 2003; **23**: 206-215 [PMID: 12482974]
83 **Watanabe Y**, Toyota M, Kondo Y, Suzuki H, Imai T, Ohe-Toyota M, Maruyama R, Nojima M, Sasaki Y, Sekido Y, Hiratsuka H, Shinomura Y, Imai K, Itoh F, Tokino T. PRDM5 identified as a target of epigenetic silencing in colorectal and gastric cancer. *Clin Cancer Res* 2007; **13**: 4786-4794 [PMID: 17699856 DOI: 10.1158/1078-0432.CCR-07-0305]
84 **Li Q**, Wang X, Lu Z, Zhang B, Guan Z, Liu Z, Zhong Q, Gu L, Zhou J, Zhu B, Ji J, Deng D. Polycomb CBX7 directly controls trimethylation of histone H3 at lysine 9 at the p16 locus. *PLoS One* 2010; **5**: e13732 [PMID: 21060834 DOI: 10.1371/journal.pone.0013732]
85 **Angrisano T**, Lembo F, Peluso S, Keller S, Chiariotti L, Pero R. Helicobacter pylori regulates iNOS promoter by histone modifications in human gastric epithelial cells. *Med Microbiol Immunol* 2012; **201**: 249-257 [PMID: 22215089 DOI: 10.1007/s00430-011-0227-9]
86 **Egger G**, Liang G, Aparicio A, Jones PA. Epigenetics in human disease and prospects for epigenetic therapy. *Nature* 2004; **429**: 457-463 [PMID: 15164071 DOI: 10.1038/nature02625]
87 **Jones PA**, Taylor SM. Cellular differentiation, cytidine analogs and DNA methylation. *Cell* 1980; **20**: 85-93 [PMID: 6156004]
88 **Gal-Yam EN**, Saito Y, Egger G, Jones PA. Cancer epigenetics: modifications, screening, and therapy. *Annu Rev Med* 2008; **59**: 267-280 [PMID: 17937590 DOI: 10.1146/annurev.med.59.061606.095816]
89 **Dikken JL**, van Sandick JW, Maurits Swellengrebel HA, Lind PA, Putter H, Jansen EP, Boot H, van Grieken NC, van de Velde CJ, Verheij M, Cats A. Neo-adjuvant chemotherapy followed by surgery and chemotherapy or by surgery and chemoradiotherapy for patients with resectable gastric cancer (CRITICS). *BMC Cancer* 2011; **11**: 329 [PMID: 21810227 DOI: 10.1186/1471-2407-11-329]
90 **Zheng Y**, Zhang Y, Huang X, Chen L. Analysis of the RUNX3 gene methylation in serum DNA from esophagus squamous cell carcinoma, gastric and colorectal adenocarcinoma patients. *Hepatogastroenterology* 2011; **58**: 2007-2011 [PMID: 22234069 DOI: 10.5754/hge10016]
91 **Jee CD**, Kim MA, Jung EJ, Kim J, Kim WH. Identification of genes epigenetically silenced by CpG methylation in human gastric carcinoma. *Eur J Cancer* 2009; **45**: 1282-1293 [PMID: 19195878 DOI: 10.1016/j.ejca.2008.12.027]
92 **Hibi K**, Goto T, Shirahata A, Saito M, Kigawa G, Nemoto H, Sanada Y. Detection of TFPI2 methylation in the serum of gastric cancer patients. *Anticancer Res* 2011; **31**: 3835-3838 [PMID: 22110206]
93 **Kanai Y**, Ushijima S, Kondo Y, Nakanishi Y, Hirohashi S. DNA methyltransferase expression and DNA methylation of CPG islands and peri-centromeric satellite regions in human colorectal and stomach cancers. *Int J Cancer* 2001; **91**: 205-212 [PMID: 11146446]
94 **Fang JY**, Cheng ZH, Chen YX, Lu R, Yang L, Zhu HY, Lu LG. Expression of Dnmt1, demethylase, MeCP2 and methylation of tumor-related genes in human gastric cancer. *World J Gastroenterol* 2004; **10**: 3394-3398 [PMID: 15526354]
95 **Ding WJ**, Fang JY, Chen XY, Peng YS. The expression and clinical significance of DNA methyltransferase proteins in human gastric cancer. *Dig Dis Sci* 2008; **53**: 2083-2089 [PMID: 18253830 DOI: 10.1007/s10620-007-0145-2]
96 **Yang J**, Wei X, Wu Q, Xu Z, Gu D, Jin Y, Shen Y, Huang H, Fan H, Chen J. Clinical significance of the expression of DNA methyltransferase proteins in gastric cancer. *Mol Med Report* 2011; **4**: 1139-1143 [PMID: 21887466 DOI: 10.3892/mmr.2011.578]
97 **Mutze K**, Langer R, Schumacher F, Becker K, Ott K, Novotny A, Hapfelmeier A, Höfler H, Keller G. DNA methyltransferase 1 as a predictive biomarker and potential therapeutic target for chemotherapy in gastric cancer. *Eur J Cancer* 2011; **47**: 1817-1825 [PMID: 21458988 DOI: 10.1016/j.ejca.2011.02.024]
98 **Fan H**, Liu D, Qiu X, Qiao F, Wu Q, Su X, Zhang F, Song Y, Zhao Z, Xie W. A functional polymorphism in the DNA methyltransferase-3A promoter modifies the susceptibility in gastric cancer but not in esophageal carcinoma. *BMC Med* 2010; **8**: 12 [PMID: 20128888 DOI: 10.1186/1741-7015-8-12]
99 **Su X**, Lv C, Qiao F, Qiu X, Huang W, Wu Q, Zhao Z, Fan H. Expression pattern and clinical significance of DNA methyltransferase 3B variants in gastric carcinoma. *Oncol Rep* 2010; **23**: 819-826 [PMID: 20127025]
100 **Hu J**, Fan H, Liu D, Zhang S, Zhang F, Xu H. DNMT3B promoter polymorphism and risk of gastric cancer. *Dig Dis Sci* 2010; **55**: 1011-1016 [PMID: 19517237 DOI: 10.1007/s10620-009-0831-3]
101 **Wada R**, Akiyama Y, Hashimoto Y, Fukamachi H, Yuasa Y. miR-212 is downregulated and suppresses methyl-CpG-binding protein MeCP2 in human gastric cancer. *Int J Cancer* 2010; **127**: 1106-1114 [PMID: 20020497 DOI: 10.1002/ijc.25126]
102 **Kanai Y**, Ushijima S, Nakanishi Y, Hirohashi S. Reduced mRNA expression of the DNA demethylase, MBD2, in human colorectal and stomach cancers. *Biochem Biophys Res Commun* 1999; **264**: 962-966 [PMID: 10544038 DOI: 10.1006/bbrc.1999.1613]
103 **Ogden SR**, Wroblewski LE, Weydig C, Romero-Gallo J, O'Brien DP, Israel DA, Krishna US, Fingleton B, Reynolds AB, Wessler S, Peek RM. p120 and Kaiso regulate Helicobacter pylori-induced expression of matrix metalloproteinase-7. *Mol Biol Cell* 2008; **19**: 4110-4121 [PMID: 18653469 DOI: 10.1091/mbc.E08-03-0283]
104 **Lee SH**, Kim J, Kim WH, Lee YM. Hypoxic silencing of tumor suppressor RUNX3 by histone modification in gastric cancer cells. *Oncogene* 2009; **28**: 184-194 [PMID: 18850007 DOI: 10.1038/onc.2008.377]
105 **Oshimo Y**, Oue N, Mitani Y, Nakayama H, Kitadai Y, Yoshida K, Chayama K, Yasui W. Frequent epigenetic inactivation of RIZ1 by promoter hypermethylation in human gastric carcinoma. *Int J Cancer* 2004; **110**: 212-218 [PMID: 15069684 DOI: 10.1002/ijc.20090]
106 **Pan KF**, Lu YY, Liu WG, Zhang L, You WC. Detection of frameshift mutations of RIZ in gastric cancers with microsatellite instability. *World J Gastroenterol* 2004; **10**: 2719-2722 [PMID: 15309726]
107 **Yoo EJ**, Park SY, Cho NY, Kim N, Lee HS, Kang GH. Helicobacter pylori-infection-associated CpG island hypermethylation in the stomach and its possible association with polycomb repressive marks. *Virchows Arch* 2008; **452**: 515-524 [PMID: 18335237 DOI: 10.1007/s00428-008-0596-7]
108 **Liu JH**, Song LB, Zhang X, Guo BH, Feng Y, Li XX, Liao WT, Zeng MS, Huang KH. Bmi-1 expression predicts prognosis for patients with gastric carcinoma. *J Surg Oncol* 2008; **97**: 267-272 [PMID: 18041745 DOI: 10.1002/jso.20934]
109 **Xiao J**, Deng C. Knockdown of Bmi-1 impairs growth and invasiveness of human gastric carcinoma cells. *Oncol Res* 2009; **17**: 613-620 [PMID: 19806792]
110 **Lu YW**, Li J, Guo WJ. Expression and clinicopathological significance of Mel-18 and Bmi-1 mRNA in gastric carcinoma.

J Exp Clin Cancer Res 2010; **29**: 143 [PMID: 21059209 DOI: 10.1186/1756-9966-29-143]
111 **Zhang XW**, Sheng YP, Li Q, Qin W, Lu YW, Cheng YF, Liu BY, Zhang FC, Li J, Dimri GP, Guo WJ. BMI1 and Mel-18 oppositely regulate carcinogenesis and progression of gastric cancer. *Mol Cancer* 2010; **9**: 40 [PMID: 20170541 DOI: 10.1186/1476-4598-9-40]
112 **Li W**, Li Y, Tan Y, Ma K, Cui J. Bmi-1 is critical for the proliferation and invasiveness of gastric carcinoma cells. *J Gastroenterol Hepatol* 2010; **25**: 568-575 [PMID: 19968751 DOI: 10.1111/j.1440-1746.2009.06045.x]
113 **Takahata M**, Inoue Y, Tsuda H, Imoto I, Koinuma D, Hayashi M, Ichikura T, Yamori T, Nagasaki K, Yoshida M, Matsuoka M, Morishita K, Yuki K, Hanyu A, Miyazawa K, Inazawa J, Miyazono K, Imamura T. SKI and MEL1 cooperate to inhibit transforming growth factor-beta signal in gastric cancer cells. *J Biol Chem* 2009; **284**: 3334-3344 [PMID: 19049980 DOI: 10.1074/jbc.M808989200]
114 **Mattioli E**, Vogiatzi P, Sun A, Abbadessa G, Angeloni G, D'Ugo D, Trani D, Gaughan JP, Vecchio FM, Cevenini G, Persiani R, Giordano A, Claudio PP. Immunohistochemical analysis of pRb2/p130, VEGF, EZH2, p53, p16(INK4A), p27(KIP1), p21(WAF1), Ki-67 expression patterns in gastric cancer. *J Cell Physiol* 2007; **210**: 183-191 [PMID: 16998811 DOI: 10.1002/jcp.20833]
115 **Varambally S**, Cao Q, Mani RS, Shankar S, Wang X, Ateeq B, Laxman B, Cao X, Jing X, Ramnarayanan K, Brenner JC, Yu J, Kim JH, Han B, Tan P, Kumar-Sinha C, Lonigro RJ, Palanisamy N, Maher CA, Chinnaiyan AM. Genomic loss of microRNA-101 leads to overexpression of histone methyltransferase EZH2 in cancer. *Science* 2008; **322**: 1695-1699 [PMID: 19008416 DOI: 10.1126/science.1165395]
116 **Zhou Y**, Du WD, Wu Q, Liu Y, Chen G, Ruan J, Xu S, Yang F, Zhou FS, Tang XF, Tang HY, Zuo XB, Zhang FY, Sun LD, Zhang XJ. EZH2 genetic variants affect risk of gastric cancer in the Chinese Han population. *Mol Carcinog* 2012; Epub ahead of print [PMID: 22228224 DOI: 10.1002/mc.21871]
117 **Hudlebusch HR**, Santoni-Rugiu E, Simon R, Ralfkiær E, Rossing HH, Johansen JV, Jørgensen M, Sauter G, Helin K. The histone methyltransferase and putative oncoprotein MMSET is overexpressed in a large variety of human tumors. *Clin Cancer Res* 2011; **17**: 2919-2933 [PMID: 21385930 DOI: 10.1158/1078-0432.CCR-10-1302]
118 **Magerl C**, Ellinger J, Braunschweig T, Kremmer E, Koch LK, Höller T, Büttner R, Lüscher B, Gütgemann I. H3K4 dimethylation in hepatocellular carcinoma is rare compared with other hepatobiliary and gastrointestinal carcinomas and correlates with expression of the methylase Ash2 and the demethylase LSD1. *Hum Pathol* 2010; **41**: 181-189 [PMID: 19896696 DOI: 10.1016/j.humpath.2009.08.007]
119 **Li W**, Zhao L, Zang W, Liu Z, Chen L, Liu T, Xu D, Jia J. Histone demethylase JMJD2B is required for tumor cell proliferation and survival and is overexpressed in gastric cancer. *Biochem Biophys Res Commun* 2011; **416**: 372-378 [PMID: 22133676 DOI: 10.1016/j.bbrc.2011.11.045]
120 **Katoh M**, Katoh M. Comparative integromics on JMJD1C gene encoding histone demethylase: conserved POU5F1 binding site elucidating mechanism of JMJD1C expression in undifferentiated ES cells and diffuse-type gastric cancer. *Int J Oncol* 2007; **31**: 219-223 [PMID: 17549425]
121 **Poplawski T**, Tomaszewska K, Galicki M, Morawiec Z, Blasiak J. Promoter methylation of cancer-related genes in gastric carcinoma. *Exp Oncol* 2008; **30**: 112-116 [PMID: 18566573]
122 **Tahara T**, Shibata T, Nakamura M, Yamashita H, Yoshioka D, Okubo M, Yonemura J, Maeda Y, Maruyama N, Kamano T, Kamiya Y, Fujita H, Nakagawa Y, Nagasaka M, Iwata M, Hirata I, Arisawa T. Increased number of CpG island hypermethylation in tumor suppressor genes of non-neoplastic gastric mucosa correlates with higher risk of gastric cancer. *Digestion* 2010; **82**: 27-36 [PMID: 20150736 DOI: 10.1159/000252766]
123 **Lee TB**, Park JH, Min YD, Kim KJ, Choi CH. Epigenetic mechanisms involved in differential MDR1 mRNA expression between gastric and colon cancer cell lines and rationales for clinical chemotherapy. *BMC Gastroenterol* 2008; **8**: 33 [PMID: 18673531 DOI: 10.1186/1471-230X-8-33]
124 **Takada H**, Imoto I, Tsuda H, Nakanishi Y, Ichikura T, Mochizuki H, Mitsufuji S, Hosoda F, Hirohashi S, Ohki M, Inazawa J. ADAM23, a possible tumor suppressor gene, is frequently silenced in gastric cancers by homozygous deletion or aberrant promoter hypermethylation. *Oncogene* 2005; **24**: 8051-8060 [PMID: 16103878 DOI: 10.1038/sj.onc.1208952]
125 **Watanabe Y**, Kim HS, Castoro RJ, Chung W, Estecio MR, Kondo K, Guo Y, Ahmed SS, Toyota M, Itoh F, Suk KT, Cho MY, Shen L, Jelinek J, Issa JP. Sensitive and specific detection of early gastric cancer with DNA methylation analysis of gastric washes. *Gastroenterology* 2009; **136**: 2149-2158 [PMID: 19375421 DOI: 10.1053/j.gastro.2009.02.085]
126 **Kim JH**, Jung EJ, Lee HS, Kim MA, Kim WH. Comparative analysis of DNA methylation between primary and metastatic gastric carcinoma. *Oncol Rep* 2009; **21**: 1251-1259 [PMID: 19360301]
127 **Balassiano K**, Lima S, Jenab M, Overvad K, Tjonneland A, Boutron-Ruault MC, Clavel-Chapelon F, Canzian F, Kaaks R, Boeing H, Meidtner K, Trichopoulou A, Laglou P, Vineis P, Panico S, Palli D, Grioni S, Tumino R, Lund E, Bueno-de-Mesquita HB, Numans ME, Peeters PH, Ramon Quirós J, Sánchez MJ, Navarro C, Ardanaz E, Dorronsoro M, Hallmans G, Stenling R, Ehrnström R, Regner S, Allen NE, Travis RC, Khaw KT, Offerhaus GJ, Sala N, Riboli E, Hainaut P, Scoazec JY, Sylla BS, Gonzalez CA, Herceg Z. Aberrant DNA methylation of cancer-associated genes in gastric cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST). *Cancer Lett* 2011; **311**: 85-95 [PMID: 21831520 DOI: 10.1016/j.canlet.2011.06.038]
128 **Bernal C**, Aguayo F, Villarroel C, Vargas M, Díaz I, Ossandon FJ, Santibáñez E, Palma M, Aravena E, Barrientos C, Corvalan AH. Reprimo as a potential biomarker for early detection in gastric cancer. *Clin Cancer Res* 2008; **14**: 6264-6269 [PMID: 18829507 DOI: 10.1158/1078-0432.CCR-07-4522]
129 **Geddert H**, zur Hausen A, Gabbert HE, Sarbia M. EBV-infection in cardiac and non-cardiac gastric adenocarcinomas is associated with promoter methylation of p16, p14 and APC, but not hMLH1. *Cell Oncol (Dordr)* 2011; **34**: 209-214 [PMID: 21538028 DOI: 10.1007/s13402-011-0028-6]
130 **Maekita T**, Nakazawa K, Mihara M, Nakajima T, Yanaoka K, Iguchi M, Arii K, Kaneda A, Tsukamoto T, Tatematsu M, Tamura G, Saito D, Sugimura T, Ichinose M, Ushijima T. High levels of aberrant DNA methylation in Helicobacter pylori-infected gastric mucosae and its possible association with gastric cancer risk. *Clin Cancer Res* 2006; **12**: 989-995 [PMID: 16467114 DOI: 10.1158/1078-0432.CCR-05-2096]
131 **Murai M**, Toyota M, Suzuki H, Satoh A, Sasaki Y, Akino K, Ueno M, Takahashi F, Kusano M, Mita H, Yanagihara K, Endo T, Hinoda Y, Tokino T, Imai K. Aberrant methylation and silencing of the BNIP3 gene in colorectal and gastric cancer. *Clin Cancer Res* 2005; **11**: 1021-1027 [PMID: 15709167]
132 **Hiraki M**, Kitajima Y, Koga Y, Tanaka T, Nakamura J, Hashiguchi K, Noshiro H, Miyazaki K. Aberrant gene methylation is a biomarker for the detection of cancer cells in peritoneal wash samples from advanced gastric cancer patients. *Ann Surg Oncol* 2011; **18**: 3013-3019 [PMID: 21409489 DOI: 10.1245/s10434-011-1636-0]
133 **Sugita H**, Iida S, Inokuchi M, Kato K, Ishiguro M, Ishikawa T, Takagi Y, Enjoji M, Yamada H, Uetake H, Kojima K, Sugihara K. Methylation of BNIP3 and DAPK indicates lower response to chemotherapy and poor prognosis in gastric cancer. *Oncol Rep* 2011; **25**: 513-518 [PMID: 21152877 DOI: 10.3892/or.2010.1085]
134 **Ryan JL**, Jones RJ, Kenney SC, Rivenbark AG, Tang W, Knight ER, Coleman WB, Gulley ML. Epstein-Barr virus-specific methylation of human genes in gastric cancer

cells. *Infect Agent Cancer* 2010; **5**: 27 [PMID: 21194482 DOI: 10.1186/1750-9378-5-27]
135 **Yamashita S**, Tsujino Y, Moriguchi K, Tatematsu M, Ushijima T. Chemical genomic screening for methylation-silenced genes in gastric cancer cell lines using 5-aza-2′-deoxycytidine treatment and oligonucleotide microarray. *Cancer Sci* 2006; **97**: 64-71 [PMID: 16367923 DOI: 10.1111/j.1349-7006.2006.00136.x]
136 **Leal M**, Lima E, Silva P, Assumpção P, Calcagno D, Payão S, Burbano RR, Smith M. Promoter hypermethylation of CDH1, FHIT, MTAP and PLAGL1 in gastric adenocarcinoma in individuals from Northern Brazil. *World J Gastroenterol* 2007; **13**: 2568-2574 [PMID: 17552003]
137 **Al-Moundhri MS**, Al-Nabhani M, Tarantini L, Baccarelli A, Rusiecki JA. The prognostic significance of whole blood global and specific DNA methylation levels in gastric adenocarcinoma. *PLoS One* 2010; **5**: e15585 [PMID: 21203466 DOI: 10.1371/journal.pone.0015585]
138 **Oki E**, Zhao Y, Yoshida R, Masuda T, Ando K, Sugiyama M, Tokunaga E, Morita M, Kakeji Y, Maehara Y. Checkpoint with forkhead-associated and ring finger promoter hypermethylation correlates with microsatellite instability in gastric cancer. *World J Gastroenterol* 2009; **15**: 2520-2525 [PMID: 19469003]
139 **Hiraki M**, Kitajima Y, Sato S, Mitsuno M, Koga Y, Nakamura J, Hashiguchi K, Noshiro H, Miyazaki K. Aberrant gene methylation in the lymph nodes provides a possible marker for diagnosing micrometastasis in gastric cancer. *Ann Surg Oncol* 2010; **17**: 1177-1186 [PMID: 19957042 DOI: 10.1245/s10434-009-0815-8]
140 **Hu SL**, Kong XY, Cheng ZD, Sun YB, Shen G, Xu WP, Wu L, Xu XC, Jiang XD, Huang DB. Promoter methylation of p16, Runx3, DAPK and CHFR genes is frequent in gastric carcinoma. *Tumori* 2010; **96**: 726-733 [PMID: 21302620]
141 **Shi J**, Zhang G, Yao D, Liu W, Wang N, Ji M, He N, Shi B, Hou P. Prognostic significance of aberrant gene methylation in gastric cancer. *Am J Cancer Res* 2012; **2**: 116-129 [PMID: 22206050]
142 **Akiyama Y**, Watkins N, Suzuki H, Jair KW, van Engeland M, Esteller M, Sakai H, Ren CY, Yuasa Y, Herman JG, Baylin SB. GATA-4 and GATA-5 transcription factor genes and potential downstream antitumor target genes are epigenetically silenced in colorectal and gastric cancer. *Mol Cell Biol* 2003; **23**: 8429-8439 [PMID: 14612389 DOI: 10.1128/MCB.23.23.8429-8439.2003]
143 **Wen XZ**, Akiyama Y, Pan KF, Liu ZJ, Lu ZM, Zhou J, Gu LK, Dong CX, Zhu BD, Ji JF, You WC, Deng DJ. Methylation of GATA-4 and GATA-5 and development of sporadic gastric carcinomas. *World J Gastroenterol* 2010; **16**: 1201-1208 [PMID: 20222162 DOI: 10.3748/wjg.v16.i10.1201]
144 **Fang JY**, Zhu SS, Xiao SD, Jiang SJ, Shi Y, Chen XY, Zhou XM, Qian LF. Studies on the hypomethylation of c-myc, c-Ha-ras oncogenes and histopathological changes in human gastric carcinoma. *J Gastroenterol Hepatol* 1996; **11**: 1079-1082 [PMID: 8985834]
145 **Luo J**, Li YN, Wang F, Zhang WM, Geng X. S-adenosylmethionine inhibits the growth of cancer cells by reversing the hypomethylation status of c-myc and H-ras in human gastric cancer and colon cancer. *Int J Biol Sci* 2010; **6**: 784-795 [PMID: 21152119]
146 **Gigek CO**, Leal MF, Lisboa LC, Silva PN, Chen ES, Lima EM, Calcagno DQ, Assumpção PP, Burbano RR, Smith Mde A. Insulin-like growth factor binding protein-3 gene methylation and protein expression in gastric adenocarcinoma. *Growth Horm IGF Res* 2010; **20**: 234-238 [PMID: 20219400 DOI: 10.1016/j.ghir.2010.02.005]
147 **Chen HY**, Zhu BH, Zhang CH, Yang DJ, Peng JJ, Chen JH, Liu FK, He YL. High CpG island methylator phenotype is associated with lymph node metastasis and prognosis in gastric cancer. *Cancer Sci* 2012; **103**: 73-79 [PMID: 22017425 DOI: 10.1111/j.1349-7006.2011.02129.x]
148 **Tamura G**, So K, Miyoshi H, Honda T, Nishizuka S, Motoyama T. Quantitative assessment of gene methylation in neoplastic and non-neoplastic gastric epithelia using methylation-specific DNA microarray. *Pathol Int* 2009; **59**: 895-899 [PMID: 20021617 DOI: 10.1111/j.1440-1827.2009.02458.x]
149 **Hibi K**, Sakata M, Yokomizo K, Kitamura YH, Sakuraba K, Shirahata A, Goto T, Mizukami H, Saito M, Ishibashi K, Kigawa G, Nemoto H, Sanada Y. Methylation of the MGMT gene is frequently detected in advanced gastric carcinoma. *Anticancer Res* 2009; **29**: 5053-5055 [PMID: 20044616]
150 **Kim HG**, Lee S, Kim DY, Ryu SY, Joo JK, Kim JC, Lee KH, Lee JH. Aberrant methylation of DNA mismatch repair genes in elderly patients with sporadic gastric carcinoma: A comparison with younger patients. *J Surg Oncol* 2010; **101**: 28-35 [PMID: 19894224 DOI: 10.1002/jso.21432]
151 **Dong CX**, Deng DJ, Pan KF, Zhang L, Zhang Y, Zhou J, You WC. Promoter methylation of p16 associated with Helicobacter pylori infection in precancerous gastric lesions: a population-based study. *Int J Cancer* 2009; **124**: 434-439 [PMID: 18821580 DOI: 10.1002/ijc.23891]
152 **Shu XS**, Geng H, Li L, Ying J, Ma C, Wang Y, Poon FF, Wang X, Ying Y, Yeo W, Srivastava G, Tsao SW, Yu J, Sung JJ, Huang S, Chan AT, Tao Q. The epigenetic modifier PRDM5 functions as a tumor suppressor through modulating WNT/β-catenin signaling and is frequently silenced in multiple tumors. *PLoS One* 2011; **6**: e27346 [PMID: 22087297 DOI: 10.1371/journal.pone.0027346]
153 **Guo W**, Dong Z, Chen Z, Yang Z, Wen D, Kuang G, Guo Y, Shan B. Aberrant CpG island hypermethylation of RASSF1A in gastric cardia adenocarcinoma. *Cancer Invest* 2009; **27**: 459-465 [PMID: 19160099 DOI: 10.1080/07357900802620828]
154 **Sakakura C**, Hamada T, Miyagawa K, Nishio M, Miyashita A, Nagata H, Ida H, Yazumi S, Otsuji E, Chiba T, Ito K, Ito Y. Quantitative analysis of tumor-derived methylated RUNX3 sequences in the serum of gastric cancer patients. *Anticancer Res* 2009; **29**: 2619-2625 [PMID: 19596937]
155 **Fan XY**, Hu XL, Han TM, Wang NN, Zhu YM, Hu W, Ma ZH, Zhang CJ, Xu X, Ye ZY, Han CM, Pan WS. Association between RUNX3 promoter methylation and gastric cancer: a meta-analysis. *BMC Gastroenterol* 2011; **11**: 92 [PMID: 21867527 DOI: 10.1186/1471-230X-11-92]
156 **Carvalho R**, Kayademir T, Soares P, Canedo P, Sousa S, Oliveira C, Leistenschneider P, Seruca R, Gött P, Blin N, Carneiro F, Machado JC. Loss of heterozygosity and promoter methylation, but not mutation, may underlie loss of TFF1 in gastric carcinoma. *Lab Invest* 2002; **82**: 1319-1326 [PMID: 12379766]

P- Reviewers Feo F, Shibata T **S- Editor** Gou SX
L- Editor Kerr C **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i8.1193

World J Gastroenterol 2013 February 28; 19(8): 1193-1199
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

Management of small hepatocellular carcinoma in cirrhosis: Focus on portal hypertension

Virginia Hernandez-Gea, Fanny Turon, Annalisa Berzigotti, Augusto Villanueva

Virginia Hernandez-Gea, Augusto Villanueva, Hepatocellular Carcinoma Translational Research Laboratory, Barcelona-Clinic Liver Cancer Group, Institut d'Investigacions Biomediques August Pi i Sunyer, Liver Unit, Hospital Clinic, 08036 Barcelona, Spain
Fanny Turon, Annalisa Berzigotti, Hepatic Hemodynamic Laboratory, Liver Unit, Hospital Clinic, University of Barcelona, 08036 Barcelona, Spain
Annalisa Berzigotti, Augusto Villanueva, Centro de Investigación Biomédica en Red de Enfermedades Hepáticas y Digestivas, Instituto Carlos Ⅲ, 08036 Barcelona, Spain
Author contributions: All the authors contributed equally to this manuscript.
Correspondence to: Augusto Villanueva, MD, Hepatocellular Carcinoma Translational Laboratory, Barcelona-Clinic Liver Cancer Group, Institut d'Investigacions Biomediques August Pi i Sunyer, Liver Unit, Hospital Clínic, Villarroel, 170, 08036 Barcelona, Spain. augusto.villanueva@ciberehd.org
Telephone: +34-932-279155 Fax: +34-932-275792
Received: December 10, 2012 Revised: January 17, 2013
Accepted: February 5, 2013
Published online: February 28, 2013

Abstract

The incidence of hepatocellular carcinoma (HCC) is rising worldwide being currently the fifth most common cancer and third cause of cancer-related mortality. Early detection of HCC through surveillance programs have enabled the identification of small nodules with higher frequency, and nowadays account for 10%-15% of patients diagnosed in the West and almost 30% in Japan. Patients with small HCC can be candidates for potential curative treatments: liver transplantation, surgical resection and percutaneous ablation, depending on the presence of portal hypertension and co-morbidities. This review will analyze recent advancements in the clinical management of these individuals, focusing on issues related to the role of portal hypertension, the debate between resection and ablative therapies and the future impact of molecular technologies.

© 2013 Baishideng. All rights reserved.

Key words: Portal hypertension; Hepatic venous pressure gradient; Clinically significant portal hypertension; Liver stiffness; Liver cancer; Hepatocellular carcinoma; Resection; Radiofrequency ablation; Percutaneous ethanol injection

Hernandez-Gea V, Turon F, Berzigotti A, Villanueva A. Management of small hepatocellular carcinoma in cirrhosis: Focus on portal hypertension. *World J Gastroenterol* 2013; 19(8): 1193-1199 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i8/1193.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i8.1193

INTRODUCTION

The incidence of hepatocellular carcinoma (HCC) is rising worldwide being currently the fifth most common cancer and third cause of cancer-related mortality[1]. HCC accounts for more than 90% of primary liver cancers, and it usually arises in the setting of long-term underlying liver disease. In fact, HCC is now the first cause of death among cirrhotic patients[2]. According to the Barcelona Clinic Liver Cancer (BCLC) algorithm, HCC can be classified into 5 stages when considering variables related to tumor burden, liver function and health status. Additionally, this algorithm links tumor stage with a specific treatment strategy[3]. Despite recent advancements in HCC management such as the introduction of the molecular targeted agent sorafenib for advanced stages[4], the mainstream potential curative therapies in HCC are still resection, transplantation and percutaneous ablation.

HCC surveillance programs for cirrhotic patients have enabled the identification of small nodules with higher frequency[5], and nowadays account for 10%-15% of patients diagnosed in the West and almost 30% in Japan[6]. "Small HCC" is a term frequently used to define tumors less than 2 cm in diameter. This cut-off is based

on the outstanding outcomes of patients with these tumors treated with surgical resection when compared to those with larger ones[7]. However, small HCC can be two different entities in pathology: vaguely and distinctly nodular[7]. Vaguely nodular tumors are well-differentiated without local invasiveness and around 12 mm in size; whereas distinctly nodular are frequently larger (16 mm) and often show local invasiveness features such as microvascular invasion[8]. European Association for the Study of the Liver (EASL) and American Association for the Society of Liver Diseases (AASLD) guideless for HCC management enables an accurate diagnosis of tumors larger than 1 cm in cirrhotic livers when a dynamic imaging technique [computed tomographic (CT) or magnetic resonance (MR)] show the so-called "hallmark" features of HCC: uptake in the arterial phase with early wash-out in the portal phase[9]. For nodules between 1-2 cm, this criterion is still maintained particularly in referral centers with a dense experience in HCC management. In case these imaging features are absent, diagnosis still requires a biopsy. According to the BCLC algorithm, patients with small HCC can be candidates for potential curative treatments, depending on portal hypertension and co-morbidities. This short review will analyze recent advancements in the clinical management of these individuals, focusing on issues related to the role of portal hypertension, the debate between resection and ablative therapies and the future impact of molecular technologies.

TREATMENT STRATEGY OF SMALL HCC AND IMPACT OF PORTAL HYPERTENSION ON POSTOPERATIVE OUTCOME

The EASL[9] and AASLD[10] guidelines recommend three alternatives for the treatment of patients with small HCC, depending on the presence of portal hypertension and co-morbidities: liver transplantation, surgical resection and percutaneous ablation. Overall, these therapies achieve a 5-year survival rate between 60%-75% in early HCC (BCLC 0/A), even though in small HCC survival rates can even be higher. About 80% of HCC develop in patients with underlying chronic liver disease, where liver failure and portal hypertension may be present. Since different treatment options are available for patients with small HCC, allocation to a given option should rely on evidence-based criteria, considering expected outcomes rather than merely treatment feasibility[11].

As for surgical resection, peri-operative mortality has decreased to 2%-3% with current surgical techniques. On the other hand a pioneer study by our group in Barcelona[12] showed that in a series of 29 Child A cirrhotic patients and HCC less than 5 cm undergoing liver resection surgery, hepatic venous pressure gradient (HVPG) $\geqslant$ 10 mmHg was independently associated with unresolved decompensation 3 mo following surgery. In a larger series published later, post-operative survival of patients with Child A cirrhosis without clinically significant portal hypertension (CSPH) was 70% at 5 years, while it dropped to 25%-50% in patients with CSPH[3]. In addition, even small increases in bilirubin, above the 1 mg/dL cutoff, were also independently associated with increased mortality. The impact of PH-related variables on the risk of post-operative liver failure and mortality has been confirmed in many Western and in Eastern series[3,12-24] (Table 1). In these studies 3-year survival was in mean 59% (range: 45%-71%) in patients with clinical signs of portal hypertension, while it increased to 72% (62%-81%) in patients without any clinical sign of the syndrome. A similar difference is observed analyzing post-opertative liver decompensation and 5-year survival, and similar results were obtained in a Japanese cohort of resected HCC[25]. Overall these data show that even if the presence of CSPH is not an absolute contraindication for performing liver resection for HCC, it clearly increases the risk of post-operative complications and death, suggesting that other available options such as liver transplantation (see below) should be considered in this subgroup of patients. Hence, the best candidates for surgical resection are patients with solitary tumors, preserved liver function and absence of CSPH. Consequently, the European and American Associations for the study of the Liver (EASL[9] and AASLD[10]) guidelines for HCC management recommend surgical resection as the first-line option in patients with small HCC without CSPH or liver dysfunction.

A main drawback of liver resection is the risk to develop a tumor recurrence, that accounts for 70% at 5 years[26] either because of true metastasis or *de novo* HCC.

By definition, patients with small HCC are within Milan criteria for liver transplantation[27], and besides treating the tumor liver transplantation provides a solution for the underlying liver disease. However, the scarcity of donors limits its feasibility and increases time in the waiting list. Even though there are no robust data, the risk of dropout from the waiting list seems lower in patients listed with small HCC. Hence, liver transplantation is the best option for patients in BCLC stage 0/A, CSPH and absence of other medical conditions that contraindicate this procedure.

Local ablation

Local ablation is considered as a competitive alternative to resection or transplantation in patients with small HCC[28] not suitable for surgery. Both radiofrequency ablation (RFA) and percutaneous ethanol injection (PEI) are standard of care techniques for tumor ablation, being used worldwide. They are both able to induce necrosis through different mechanisms. PEI presents a higher post-treatment rate of recurrence that can reach 40% at two years in larger lesions[29,30]. RFA has shown its ability to better control the disease with a lower local recurrence rate (2%-18% at 2 years) when compared with PEI (11%-45%) in randomized control trials (RCT)[31-35]. However, differences in survival were not described. Therefore, current guidelines recommend the use of PEI in cases where RFA is not feasible for technical reasons[9]. Five-year

Table 1 Relevant reported studies describing the impact of hepatic venous pressure gradient or portal-hypertension related variables in patients with potentially resectable or resected hepatocellular carcinoma

Ref.	Patients included (*n*)	Portal hypertension-related variables studied	Outcome
Llovet *et al*[3]	43	HVPG	CSPH independently associated with 5-yr post-operative mortality
Bruix *et al*[12]	29	HVPG	CSPH independently associated with PLF at 3-mo
Berzigotti *et al*[13]	63	Spleen size; platelet count; platelet count/spleen diameter; liver stiffness; LSPS PH risk score	Best single predictor of CSPH: liver stiffness; combination with spleen size and platelet count improved the results (AUROC LSPS 0.852; PH risk score 0.884)
Boleslawski *et al*[14]	43	HVPG Platelet count; spleen size; esophageal varices = indirect signs of PH	CSPH independently associated with increased PLF and 90-d mortality. Indirect signs of PH showed no discriminative ability
Capussotti *et al*[15]	217	Platelet count; spleen size; esophageal varices	PH associated with lower 3-yr and 5-yr survival
Cescon *et al*[16]	90	Liver stiffness; platelet count; spleen size; esophageal varices	LS (but not other signs) independently associated with the risk of PLF
Chen *et al*[17]	190	Intraoperative measurement of PVP	PVP independently associated with PLF on multivariate analysis
Cucchetti *et al*[18]	241	Platelet count; spleen size; esophageal varices	PH associated with lower 3-yr and 5-yr survival, but not after adjusting for MELD, albumin and extent of resection no
Figueras *et al*[19]	39	HVPG	CSPH associated with increased risk of morbidity
Giuliante *et al*[20]	588	Platelet count; spleen size; esophageal varices	PH independently associated with increased mortality
Imamura *et al*[21]	532	Varices, hypersplenism or hepatofugal portal flow	PH associated with a higher risk of post-operative ascites
Ishizawa *et al*[22]	203	Platelet count	Platelet count < 100 × 10^3/mL independently associated with PLF
Kim *et al*[23]	72	Liver stiffness	LS predicted PLF with good accuracy; LS better than ICG15
Llop *et al*[24]	79	Liver stiffness	CSPH predicted with good accuracy
Ishizawa *et al*[25]	434	Platelet count; spleen size; esophageal varices	PH associated with lower 3-yr and 5-yr survival

HVPG: Hepatic venous pressure gradient; PH: Portal hypertension; LS: Liver stiffness; PVP: Portal vein pressure; PLF: Post-operative liver failure; CSPH: Clinically significant PH (HVPG ⩾ 10 mmHg); AUROC: Area under receiver operating characteristic curve; MELD: Model for end-stage liver disease.

survival with RFA reaches 70%[36,37], with the best results in patients with good liver function (Child-Pugh A) and small tumors[38]. Although resection remains the first-line option for patients without CSPH, well-preserved liver function and solitary tumors less than 2 cm, there is an increasing debate as to whether ablative therapies (particularly RFA) could be a competitive option in these patients. Theoretically, in these solitary small tumors RFA could be as effective as surgery in terms of oncological results, and avoid all the possible complications related to the surgical procedure. However, available evidence is still limited as the few RCT available evaluating face-to-face both therapeutic options show controversial results (Table 2). In addition, there are several methodological issues that preclude reaching any robust conclusion from these trials (*e.g.*, treatment allocation, patient selection criteria, trial implementation, short follow-up, *etc.*). In fact, none of them was specifically design to only include patients with HCC less than 2 cm. Replacement of resection by RFA as first line therapy in patients with early HCC cannot be recommended at this point. Further evidence will be needed to appropriately address this issue.

Tumor recurrence is the main drawback in patients treated with resection or ablation, since the risk factor for HCC development (*i.e.*, cirrhosis) is still present[39]. Early recurrence usually occurs within the 2 years after surgery and it is due to true metastatic spread. However, late recurrence appears later on, usually beyond 2 years, and it is consider as *de novo* tumor developed as a consequence of the carcinogenic effect of underlying cirrhosis (field effect)[21]. Besides clinical differences[21], these two patterns of recurrence also differ in their molecular profile[40]. A large Japanese study found that non-anatomical resection, high AFP serum levels and presence of microscopic vascular invasion were risk factors for early recurrence, whereas grade of hepatitis activity, multiplicity and gross tumor classification impacted mostly late recurrence[21].

PORTAL HYPERTENSION ASSESSMENT IN PATIENTS WITH CIRRHOSIS AND SMALL HCC

The above-mentioned data underscore the need of an accurate discrimination of the presence of portal hypertension in patients with small HCC. Portal hypertension (PH) is a clinical syndrome hemodynamically defined as an increase in the pressure gradient across the liver (between portal pressure and inferior vena cava pressure) above the normal value of 5 mmHg[41]. In patients with cirrhosis, this gradient can be estimated by its clinical equivalent-the HVPG-which is assessed at hepatic vein catheterization and avoids the need to directly puncture the portal vein[41]. An elevated HVPG between 6 to 9 mmHg defines subclinical portal hypertension, whereas an HVPG ⩾ 10 mmHg defines clinically CSPH, since all the potential complications of the syndrome (*e.g.*, varices, ascites, *etc.*) can appear above this threshold[41]. The gold standard for the diagnosis and assessment of PH is the measurement of the HVPG, which is obtained as

Table 2 Randomized controlled trials comparing surgical resection and percutaneous ablation in patients with early hepatocellular carcinoma

Ref.	Treatment allocation	Sample size (*n*)	Serum bilirubin	Tumor median size	Nodules	Median follow-up	Recurrence rate	1/3/5 yr survival
Chen *et al*[17]	Multidisciplinary team of doctors	90 (resection)	> 2 mg/dL (33%)	< 3 cm (52%)	Single (100%)	NR	NR	94%/68%/NR
		71 (ablation)	> 2 mg/dL (26%)	< 3 cm (46%)	Single (100%)	NR	NR	93%/73%/NR
Huang *et al*[66]	Consecutive enrolment	38 (ablation)	NR	≤ 2 cm (55%)/2-3 cm (45%)	Single (79%)	37.7 ± 14.5	47%	100%/95%/92%
		38 (resection)	NR	≤ 2 cm (63%)/2-3 cm (37%)	Single (89%)	38.4 ± 16.4	39%	97%/89%/87%
Huang *et al*[67]	Consecutive enrolment	115 (ablation)	15.3 ± 4.6 μmol/L	≤ 3 cm (49%)	Single (73%)	NR	63%	86.9%/69.6%/54.78%
		115 (resection)	16.4 ± 5.3 μmol/L	≤ 3 cm (39%)	Single (84%)	NR	41%	98.26%/92.17%/75.6%
Feng *et al*[68]	Consecutive enrolment	84 (ablation)	17.2 μmol/L	≤ 2 cm (37%)/> 2 cm and < 4 cm (63%)	Single (57%)	NR	42%	96%/87.6%/NR
		84 (resection)	15.1 μmol/L	≤ 2 cm (30%)/> 2 cm and < 4 cm (46%)	Single (62%)	NR	32%	93.1%/83.1%/NR

NR: Not reported.

the difference between "wedged" (occluded) and "free" hepatic venous pressures. This method is safe, objective, reproducible, and accurate; and it provides prognostic information independent of liver function. It is currently the best marker to predict clinical events in patients with liver diseases within research protocols[42].

Since development of gastroesophageal varices is a direct consequence of CSPH, the presence of esophageal varices on endoscopy is a 100% specific sign of CSPH. However, approximately 50% of well compensated patients without any evident sign of portal hypertension (*e.g.*, without esophageal varices) already show CSPH on hemodynamic assessment[43]. In the specific setting of well-compensated patients without varices, CSPH independently increases the risk of developing esophageal varices, clinical decompensation and HCC during follow-up[43-45], resulting in a higher mortality. Hence, CSPH should be always assessed in patients with cirrhosis for prognostic stratification[41].

HVPG measurement has some limitations such as cost, invasiveness (even if minor) and the need for a specific training for its performance and interpretation. Since it is not available in all centers, noninvasive surrogate methods to diagnose portal hypertension are needed, and have been widely evaluated. Non-invasive markers include laboratory tests, ultrasonography and liver stiffness. Regarding routine laboratory tests, the objective components of Child-Pugh score (albumin, bilirubin, INR) correlate with HVPG[46-48] and with the prevalence and grade of esophageal varices in cirrhotic patients. Interestingly this correlation is also observed in patients with compensated cirrhosis[49] suggesting that there is a close correlation between liver structural damage and the onset of portal hypertension and hepatocellular dysfunction. Low platelet count, either alone or in combination with spleen size[50-52] is associated with CSPH and esophageal varices, and is the single most commonly reported non-invasive sign of portal hypertension. Nonetheless, there is not any established cut-off for platelet count able to accurately diagnose or exclude CSPH in patients with cirrhosis[13].

Ultrasound (US) is the first-line imaging technique used in patients with suspected cirrhosis and portal hypertension, since it is cheap, repeatable, and allows direct visualization of the anatomical changes induced by PH[53,54]. US signs of PH in cirrhosis are multiple[55,56]; overall, the sensitivity of the technique is moderate, but its specificity is above 90%. Hence, the presence of one or more US signs of PH allows to diagnose CSPH, but the absence of signs do not exclude CSPH, suggesting that it should be investigated by more sensitive techniques (*e.g.*, HVPG measurement). Splenomegaly, defined as an increase in spleen diameter above 12 cm, is among the most commonly reported signs. It is sensitive but poorly specific, while porto-collateral vessels have a low sensitivity but 100% specificity for the diagnosis of CSPH. This last sign can be therefore considered a reliable surrogate of PH if detected either on US or on CT/MR imaging.

Liver stiffness (LS) measured by transient elastography (Fibroscan®) is a well-accepted objective non-invasive method to reliably estimate liver fibrosis[57]. It has been shown that LS and HVPG show a good correlation in patients with compensated cirrhosis[13,58]. LS cut-off for the detection of CSPH varies across studies, but it is widely accepted that values above 21 kPa have a high specificity for CSPH. This has been recently confirmed by a longitudinal study using clinical endpoints that showed that HVPG ≥ 10 mmHg and LS > 21 kPa equally predict clinical decompensation in patients with compensated cirrhosis at baseline[59], making LS the most accurate method so far for predicting CSPH. Preliminary data suggests that spleen stiffness has a better correlation with HVPG than LS, but data are still limited[60].

The combination of different non-invasive methods offers potential benefits by integrating complementary information. In a recent study by our group where HVPG was used as the gold standard for diagnosing CSPH, the combination of LS, platelet count and spleen size either as LSPS[23] or as a newly calculated PH risk score improved the accuracy of LS alone for detecting CSPH, and allowed a correct diagnosis in 86% of cases[13].

The EASL[9] and AASLD[10] practice guidelines for HCC management recommend evaluation of portal hypertension prior treatment decision in HCC patients with a single tumour. For this purpose, HVPG measurement is the best available option and should be considered the standard-of-care. However, it cannot be routinely performed in all hospitals. Platelet count below 100×10^3/mL is often used to identify CSPH, especially when associated with splenomegaly[13]. In this regard, it should be underscored that both platelet count and spleen size are inaccurate for diagnosing CSPH in this specific setting[13]. Specifically, while in patients with both signs CSPH is highly probable, the absence of thrombocytopenia and/or splenomegaly cannot exclude CSPH. This has been recently confirmed in a prospective study in patients undergoing surgery for HCC including HVPG measurement[14] and reinforces the notion that more objective and accurate methods are still needed to accurately diagnose or exclude CSPH in patients with potentially resectable HCC.

Given that liver stiffness by transient elastography is currently considered the single most reliable non-invasive surrogate marker of PH, researchers from Eastern and Western countries evaluated its accuracy to predict CSPH and PH-related complication after surgery for HCC. A study including 72 patients found that LS had a good accuracy (area under receiver operating characteristic curve 0.824) in discriminating which patients would decompensate after surgery, being superior than the test usually used in Eastern countries (indocyanine green clearance at 15 min)[61]. Our group tested LS accuracy in the diagnosis of CSPH versus the gold-standard measurement of HVPG in a series of 90 patients with Child A cirrhosis and potentially resectable HCC[24]. In the 79 patients in whom LS was feasible (88% of applicability), the correlation with HVPG was lower than previously published in patients without HCC, suggesting that factors related to tumor location might interfere with LS measurements[24]. Nonetheless, results demonstrated that a cut-off of 13.6 kPa was 90% sensitive to detect CSPH, while the 21.1 kPa cutoff was highly specific. In other words, CSPH could be reasonably excluded in patients with LS < 13.6 kPa, and reliably diagnosed in those with LS ≥ 21.1 kPa. This simple rule could eventually decrease the need for HVPG measurement by half, being confined to those patients with intermediate LS levels (between 13.6-21.1 kPa) and those with unreliable measurements (*e.g.*, obesity, *etc.*). Furthermore, combination of LS with platelet count and spleen diameter has the potential of reducing the number of patients in this "grey zone", allowing to correctly diagnose CSPH in 85% of patients, although these findings need validation in independent series.

MOLECULAR PROGNOSTIC BIOMARKERS IN SMALL HCC

There is a pressing need to incorporate prognostic and predictive biomarkers in HCC management. Genome-wide expression studies have been applied in the HCC field trying to provide physicians with better tools to characterize early lesions and even to optimize patient selection for personalize therapies.

Different studies indicate that HCC can be broadly classified according to its molecular features in two major subclasses[62]. One is characterized by molecular signals of proliferation and cell cycle, usually enriched in TP53 inactivation; whereas the second subclass is characterized by *CTNNB1* mutations and enrichment in WNT target genes *GLUL*, *LGR5*, and *LECT2*. The subclass related to proliferation can be been further divided according to the activation of other cascades such as transforming growth factor-beta signaling, insulin-like growth factor[63], Notch[64], *etc.*

Besides molecular classification, a number of studies have reported gene signatures able to predict prognosis in HCC (thoroughly reviewed elsewhere[62]), not only generated from the tumor but also from the adjacent non-tumoral cirrhotic tissue. Recently an integrated prognostic model that combines genomic information from both the tumor and the adjacent tissue together with clinical-pathologic data was able to accurately predict outcome in patients with a single nodule early HCC[65]. Despite all these data from molecular profiling studies, gene signatures have not been yet incorporated clinical practice guidelines[62].

REFERENCES

1 **Jemal A**, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. *CA Cancer J Clin* 2009; **59**: 225-249 [PMID: 19474385]

2 **Seitz HK**, Stickel F. Risk factors and mechanisms of hepatocarcinogenesis with special emphasis on alcohol and oxidative stress. *Biol Chem* 2006; **387**: 349-360 [PMID: 16606331]

3 **Llovet JM**, Fuster J, Bruix J. Intention-to-treat analysis of surgical treatment for early hepatocellular carcinoma: resection versus transplantation. *Hepatology* 1999; **30**: 1434-1440 [PMID: 10573522]

4 **Llovet JM**, Ricci S, Mazzaferro V, Hilgard P, Gane E, Blanc JF, de Oliveira AC, Santoro A, Raoul JL, Forner A, Schwartz M, Porta C, Zeuzem S, Bolondi L, Greten TF, Galle PR, Seitz JF, Borbath I, Häussinger D, Giannaris T, Shan M, Moscovici M, Voliotis D, Bruix J. Sorafenib in advanced hepatocellular carcinoma. *N Engl J Med* 2008; **359**: 378-390 [PMID: 18650514]

5 **El-Serag HB**. Hepatocellular carcinoma. *N Engl J Med* 2011; **365**: 1118-1127 [PMID: 21992124]

6 **Kudo M**. Review of 4th Single Topic Conference on HCC. Hepatocellular carcinoma: International consensus and controversies. *Hepatol Res* 2007; **37** Suppl 2: S83-S87 [PMID: 17877501]

7 **Roskams T**. Anatomic pathology of hepatocellular carcinoma: impact on prognosis and response to therapy. *Clin Liver Dis* 2011; **15**: 245-259, vii-x [PMID: 21689611]

8 **Kojiro M**, Roskams T. Early hepatocellular carcinoma and dysplastic nodules. *Semin Liver Dis* 2005; **25**: 133-142 [PMID: 15918142]

9 European Association For The Study Of The Liver, European Organisation For Research And Treatment Of Cancer. EASL-EORTC clinical practice guidelines: management of hepatocellular carcinoma. *J Hepatol* 2012; **56**: 908-943 [PMID: 22424438]

10 **Bruix J**, Sherman M. Management of hepatocellular carcinoma: an update. *Hepatology* 2011; **53**: 1020-1022 [PMID: 21374666]

11 **Forner A**, Bruix J. East meets the West--portal pressure predicts outcome of surgical resection for hepatocellular carcinoma. *Nat Clin Pract Gastroenterol Hepatol* 2009; **6**: 14-15 [PMID: 18981995]
12 **Bruix J**, Castells A, Bosch J, Feu F, Fuster J, Garcia-Pagan JC, Visa J, Bru C, Rodés J. Surgical resection of hepatocellular carcinoma in cirrhotic patients: prognostic value of preoperative portal pressure. *Gastroenterology* 1996; **111**: 1018-1022 [PMID: 8831597]
13 **Berzigotti A**, Seijo S, Arena U, Abraldes JG, Vizzutti F, García-Pagán JC, Pinzani M, Bosch J. Elastography, spleen size, and platelet count identify portal hypertension in patients with compensated cirrhosis. *Gastroenterology* 2013; **144**: 102-111.e1 [PMID: 23058320]
14 **Boleslawski E**, Petrovai G, Truant S, Dharancy S, Duhamel A, Salleron J, Deltenre P, Lebuffe G, Mathurin P, Pruvot FR. Hepatic venous pressure gradient in the assessment of portal hypertension before liver resection in patients with cirrhosis. *Br J Surg* 2012; **99**: 855-863 [PMID: 22508371]
15 **Capussotti L**, Ferrero A, Viganò L, Muratore A, Polastri R, Bouzari H. Portal hypertension: contraindication to liver surgery? *World J Surg* 2006; **30**: 992-999 [PMID: 16736327]
16 **Cescon M**, Colecchia A, Cucchetti A, Peri E, Montrone L, Ercolani G, Festi D, Pinna AD. Value of transient elastography measured with FibroScan in predicting the outcome of hepatic resection for hepatocellular carcinoma. *Ann Surg* 2012; **256**: 706-712; discussion 712-713 [PMID: 23095613]
17 **Chen X**, Zhai J, Cai X, Zhang Y, Wei L, Shi L, Wu D, Shen F, Lau WY, Wu M. Severity of portal hypertension and prediction of postoperative liver failure after liver resection in patients with Child-Pugh grade A cirrhosis. *Br J Surg* 2012; **99**: 1701-1710 [PMID: 23132418]
18 **Cucchetti A**, Ercolani G, Vivarelli M, Cescon M, Ravaioli M, Ramacciato G, Grazi GL, Pinna AD. Is portal hypertension a contraindication to hepatic resection? *Ann Surg* 2009; **250**: 922-928 [PMID: 19855258]
19 **Figueras J**, Llado L, Ruiz D, Ramos E, Busquets J, Rafecas A, Torras J, Fabregat J. Complete versus selective portal triad clamping for minor liver resections: a prospective randomized trial. *Ann Surg* 2005; **241**: 582-590 [PMID: 15798459]
20 **Giuliante F**, Ardito F, Pinna AD, Sarno G, Giulini SM, Ercolani G, Portolani N, Torzilli G, Donadon M, Aldrighetti L, Pulitanò C, Guglielmi A, Ruzzenente A, Capussotti L, Ferrero A, Calise F, Scuderi V, Federico B, Nuzzo G. Liver resection for hepatocellular carcinoma ≤3 cm: results of an Italian multicenter study on 588 patients. *J Am Coll Surg* 2012; **215**: 244-254 [PMID: 22634119]
21 **Imamura H**, Matsuyama Y, Tanaka E, Ohkubo T, Hasegawa K, Miyagawa S, Sugawara Y, Minagawa M, Takayama T, Kawasaki S, Makuuchi M. Risk factors contributing to early and late phase intrahepatic recurrence of hepatocellular carcinoma after hepatectomy. *J Hepatol* 2003; **38**: 200-207 [PMID: 12547409]
22 **Ishizawa T**, Hasegawa K, Kokudo N, Sano K, Imamura H, Beck Y, Sugawara Y, Makuuchi M. Risk factors and management of ascites after liver resection to treat hepatocellular carcinoma. *Arch Surg* 2009; **144**: 46-51 [PMID: 19153324]
23 **Kim BK**, Han KH, Park JY, Ahn SH, Kim JK, Paik YH, Lee KS, Chon CY, Kim do Y. A liver stiffness measurement-based, noninvasive prediction model for high-risk esophageal varices in B-viral liver cirrhosis. *Am J Gastroenterol* 2010; **105**: 1382-1390 [PMID: 20087336]
24 **Llop E**, Berzigotti A, Reig M, Erice E, Reverter E, Seijo S, Abraldes JG, Bruix J, Bosch J, García-Pagan JC. Assessment of portal hypertension by transient elastography in patients with compensated cirrhosis and potentially resectable liver tumors. *J Hepatol* 2012; **56**: 103-108 [PMID: 21827733]
25 **Ishizawa T**, Hasegawa K, Aoki T, Takahashi M, Inoue Y, Sano K, Imamura H, Sugawara Y, Kokudo N, Makuuchi M. Neither multiple tumors nor portal hypertension are surgical contraindications for hepatocellular carcinoma. *Gastroenterology* 2008; **134**: 1908-1916 [PMID: 18549877]
26 **Llovet JM**, Burroughs A, Bruix J. Hepatocellular carcinoma. *Lancet* 2003; **362**: 1907-1917 [PMID: 14667750]
27 **Mazzaferro V**, Regalia E, Doci R, Andreola S, Pulvirenti A, Bozzetti F, Montalto F, Ammatuna M, Morabito A, Gennari L. Liver transplantation for the treatment of small hepatocellular carcinomas in patients with cirrhosis. *N Engl J Med* 1996; **334**: 693-699 [PMID: 8594428]
28 **Livraghi T**, Meloni F, Di Stasi M, Rolle E, Solbiati L, Tinelli C, Rossi S. Sustained complete response and complications rates after radiofrequency ablation of very early hepatocellular carcinoma in cirrhosis: Is resection still the treatment of choice? *Hepatology* 2008; **47**: 82-89 [PMID: 18008357]
29 **Castells A**, Bruix J, Bru C, Fuster J, Vilana R, Navasa M, Ayuso C, Boix L, Visa J, Rodés J. Treatment of small hepatocellular carcinoma in cirrhotic patients: a cohort study comparing surgical resection and percutaneous ethanol injection. *Hepatology* 1993; **18**: 1121-1126 [PMID: 8225217]
30 **Livraghi T**, Bolondi L, Lazzaroni S, Marin G, Morabito A, Rapaccini GL, Salmi A, Torzilli G. Percutaneous ethanol injection in the treatment of hepatocellular carcinoma in cirrhosis. A study on 207 patients. *Cancer* 1992; **69**: 925-929 [PMID: 1310435]
31 **Lin SM**, Lin CJ, Lin CC, Hsu CW, Chen YC. Randomised controlled trial comparing percutaneous radiofrequency thermal ablation, percutaneous ethanol injection, and percutaneous acetic acid injection to treat hepatocellular carcinoma of 3 cm or less. *Gut* 2005; **54**: 1151-1156 [PMID: 16009687]
32 **Lin SM**, Lin CJ, Lin CC, Hsu CW, Chen YC. Radiofrequency ablation improves prognosis compared with ethanol injection for hepatocellular carcinoma & lt; or =4 cm. *Gastroenterology* 2004; **127**: 1714-1723 [PMID: 15578509]
33 **Lencioni RA**, Allgaier HP, Cioni D, Olschewski M, Deibert P, Crocetti L, Frings H, Laubenberger J, Zuber I, Blum HE, Bartolozzi C. Small hepatocellular carcinoma in cirrhosis: randomized comparison of radio-frequency thermal ablation versus percutaneous ethanol injection. *Radiology* 2003; **228**: 235-240 [PMID: 12759473]
34 **Brunello F**, Veltri A, Carucci P, Pagano E, Ciccone G, Moretto P, Sacchetto P, Gandini G, Rizzetto M. Radiofrequency ablation versus ethanol injection for early hepatocellular carcinoma: A randomized controlled trial. *Scand J Gastroenterol* 2008; **43**: 727-735 [PMID: 18569991]
35 **Shiina S**, Teratani T, Obi S, Sato S, Tateishi R, Fujishima T, Ishikawa T, Koike Y, Yoshida H, Kawabe T, Omata M. A randomized controlled trial of radiofrequency ablation with ethanol injection for small hepatocellular carcinoma. *Gastroenterology* 2005; **129**: 122-130 [PMID: 16012942]
36 **Lencioni R**, Cioni D, Crocetti L, Franchini C, Pina CD, Lera J, Bartolozzi C. Early-stage hepatocellular carcinoma in patients with cirrhosis: long-term results of percutaneous image-guided radiofrequency ablation. *Radiology* 2005; **234**: 961-967 [PMID: 15665226]
37 **Omata M**, Tateishi R, Yoshida H, Shiina S. Treatment of hepatocellular carcinoma by percutaneous tumor ablation methods: Ethanol injection therapy and radiofrequency ablation. *Gastroenterology* 2004; **127**: S159-S166 [PMID: 15508080]
38 **Sala M**, Llovet JM, Vilana R, Bianchi L, Solé M, Ayuso C, Brú C, Bruix J. Initial response to percutaneous ablation predicts survival in patients with hepatocellular carcinoma. *Hepatology* 2004; **40**: 1352-1360 [PMID: 15565564]
39 **Lau WY**, Leung TW, Yu SC, Ho SK. Percutaneous local ablative therapy for hepatocellular carcinoma: a review and look into the future. *Ann Surg* 2003; **237**: 171-179 [PMID: 12560774]
40 **Finkelstein SD**, Marsh W, Demetris AJ, Swalsky PA, Sasatomi E, Bonham A, Subotin M, Dvorchik I. Microdissection-based allelotyping discriminates de novo tumor from intrahepatic spread in hepatocellular carcinoma. *Hepatology* 2003; **37**: 871-879 [PMID: 12668980]
41 **Bosch J**, Abraldes JG, Berzigotti A, García-Pagan JC. The clinical use of HVPG measurements in chronic liver disease. *Nat Rev Gastroenterol Hepatol* 2009; **6**: 573-582 [PMID:

19724251]
42 **Gluud C**, Brok J, Gong Y, Koretz RL. Hepatology may have problems with putative surrogate outcome measures. *J Hepatol* 2007; **46**: 734-742 [PMID: 17316871]
43 **Groszmann RJ**, Garcia-Tsao G, Bosch J, Grace ND, Burroughs AK, Planas R, Escorsell A, Garcia-Pagan JC, Patch D, Matloff DS, Gao H, Makuch R. Beta-blockers to prevent gastroesophageal varices in patients with cirrhosis. *N Engl J Med* 2005; **353**: 2254-2261 [PMID: 16306522]
44 **Ripoll C**, Groszmann R, Garcia-Tsao G, Grace N, Burroughs A, Planas R, Escorsell A, Garcia-Pagan JC, Makuch R, Patch D, Matloff DS, Bosch J. Hepatic venous pressure gradient predicts clinical decompensation in patients with compensated cirrhosis. *Gastroenterology* 2007; **133**: 481-488 [PMID: 17681169]
45 **Ripoll C**, Groszmann RJ, Garcia-Tsao G, Bosch J, Grace N, Burroughs A, Planas R, Escorsell A, Garcia-Pagan JC, Makuch R, Patch D, Matloff DS. Hepatic venous pressure gradient predicts development of hepatocellular carcinoma independently of severity of cirrhosis. *J Hepatol* 2009; **50**: 923-928 [PMID: 19303163]
46 **Gluud C**, Henriksen JH, Nielsen G. Prognostic indicators in alcoholic cirrhotic men. *Hepatology* 1988; **8**: 222-227 [PMID: 3258578]
47 **Stanley AJ**, Robinson I, Forrest EH, Jones AL, Hayes PC. Haemodynamic parameters predicting variceal haemorrhage and survival in alcoholic cirrhosis. *QJM* 1998; **91**: 19-25 [PMID: 9519209]
48 **Braillon A**, Cales P, Valla D, Gaudy D, Geoffroy P, Lebrec D. Influence of the degree of liver failure on systemic and splanchnic haemodynamics and on response to propranolol in patients with cirrhosis. *Gut* 1986; **27**: 1204-1209 [PMID: 3781335]
49 **Berzigotti A**, Gilabert R, Abraldes JG, Nicolau C, Bru C, Bosch J, García-Pagan JC. Noninvasive prediction of clinically significant portal hypertension and esophageal varices in patients with compensated liver cirrhosis. *Am J Gastroenterol* 2008; **103**: 1159-1167 [PMID: 18477345]
50 **Giannini E**, Botta F, Borro P, Risso D, Romagnoli P, Fasoli A, Mele MR, Testa E, Mansi C, Savarino V, Testa R. Platelet count/spleen diameter ratio: proposal and validation of a non-invasive parameter to predict the presence of oesophageal varices in patients with liver cirrhosis. *Gut* 2003; **52**: 1200-1205 [PMID: 12865282]
51 **Schepis F**, Cammà C, Niceforo D, Magnano A, Pallio S, Cinquegrani M, D'amico G, Pasta L, Craxì A, Saitta A, Raimondo G. Which patients with cirrhosis should undergo endoscopic screening for esophageal varices detection? *Hepatology* 2001; **33**: 333-338 [PMID: 11172334]
52 **Zaman A**, Hapke R, Flora K, Rosen HR, Benner K. Factors predicting the presence of esophageal or gastric varices in patients with advanced liver disease. *Am J Gastroenterol* 1999; **94**: 3292-3296 [PMID: 10566732]
53 **Berzigotti A**, Zoli M, Bru C, Gilabert R, de Franchis R. Ultrasound in the non-invasive evaluation of fibrosis and portal hypertension. In: Portal Hypertension V: Proceedings of the Fifth Baveno International Consensus Workshop. Wiley-Blackwell, 2010: 28-36
54 **Berzigotti A**. Transient elastography and prognosis of cirrhosis. *Hepatology* 2012; **55**: 1629-1631 [PMID: 22517548]
55 **Berzigotti A**, Piscaglia F. Ultrasound in portal hypertension--part 1. *Ultraschall Med* 2011; **32**: 548-568; quiz 569-571 [PMID: 22161554]
56 **Berzigotti A**, Piscaglia F. Ultrasound in portal hypertension--part 2--and EFSUMB recommendations for the performance and reporting of ultrasound examinations in portal hypertension. *Ultraschall Med* 2012; **33**: 8-32; quiz 30-31 [PMID: 22322479]
57 **Friedrich-Rust M**, Ong MF, Martens S, Sarrazin C, Bojunga J, Zeuzem S, Herrmann E. Performance of transient elastography for the staging of liver fibrosis: a meta-analysis. *Gastroenterology* 2008; **134**: 960-974 [PMID: 18395077]
58 **Castera L**, Pinzani M, Bosch J. Non invasive evaluation of portal hypertension using transient elastography. *J Hepatol* 2012; **56**: 696-703 [PMID: 21767510]
59 **Robic MA**, Procopet B, Métivier S, Péron JM, Selves J, Vinel JP, Bureau C. Liver stiffness accurately predicts portal hypertension related complications in patients with chronic liver disease: a prospective study. *J Hepatol* 2011; **55**: 1017-1024 [PMID: 21354450]
60 **Colecchia A**, Montrone L, Scaioli E, Bacchi-Reggiani ML, Colli A, Casazza G, Schiumerini R, Turco L, Di Biase AR, Mazzella G, Marzi L, Arena U, Pinzani M, Festi D. Measurement of spleen stiffness to evaluate portal hypertension and the presence of esophageal varices in patients with HCV-related cirrhosis. *Gastroenterology* 2012; **143**: 646-654 [PMID: 22643348]
61 **Kim SU**, Ahn SH, Park JY, Kim do Y, Chon CY, Choi JS, Kim KS, Han KH. Prediction of postoperative hepatic insufficiency by liver stiffness measurement (FibroScan((R))) before curative resection of hepatocellular carcinoma: a pilot study. *Hepatol Int* 2008; **2**: 471-477 [PMID: 19669322]
62 **Hoshida Y**, Toffanin S, Lachenmayer A, Villanueva A, Minguez B, Llovet JM. Molecular classification and novel targets in hepatocellular carcinoma: recent advancements. *Semin Liver Dis* 2010; **30**: 35-51 [PMID: 20175032]
63 **Tovar V**, Alsinet C, Villanueva A, Hoshida Y, Chiang DY, Solé M, Thung S, Moyano S, Toffanin S, Mínguez B, Cabellos L, Peix J, Schwartz M, Mazzaferro V, Bruix J, Llovet JM. IGF activation in a molecular subclass of hepatocellular carcinoma and pre-clinical efficacy of IGF-1R blockage. *J Hepatol* 2010; **52**: 550-559 [PMID: 20206398]
64 **Villanueva A**, Alsinet C, Yanger K, Hoshida Y, Zong Y, Toffanin S, Rodriguez-Carunchio L, Solé M, Thung S, Stanger BZ, Llovet JM. Notch signaling is activated in human hepatocellular carcinoma and induces tumor formation in mice. *Gastroenterology* 2012; **143**: 1660-1669.e7 [PMID: 22974708 DOI: 10.1053/j.gastro.2012.09.002]
65 **Villanueva A**, Hoshida Y, Battiston C, Tovar V, Sia D, Alsinet C, Cornella H, Liberzon A, Kobayashi M, Kumada H, Thung SN, Bruix J, Newell P, April C, Fan JB, Roayaie S, Mazzaferro V, Schwartz ME, Llovet JM. Combining clinical, pathology, and gene expression data to predict recurrence of hepatocellular carcinoma. *Gastroenterology* 2011; **140**: 1501-1512.e2 [PMID: 21320499]
66 **Huang GT**, Lee PH, Tsang YM, Lai MY, Yang PM, Hu RH, Chen PJ, Kao JH, Sheu JC, Lee CZ, Chen DS. Percutaneous ethanol injection versus surgical resection for the treatment of small hepatocellular carcinoma: a prospective study. *Ann Surg* 2005; **242**: 36-42 [PMID: 15973099]
67 **Huang J**, Yan L, Cheng Z, Wu H, Du L, Wang J, Xu Y, Zeng Y. A randomized trial comparing radiofrequency ablation and surgical resection for HCC conforming to the Milan criteria. *Ann Surg* 2010; **252**: 903-912 [PMID: 21107100]
68 **Feng K**, Yan J, Li X, Xia F, Ma K, Wang S, Bie P, Dong J. A randomized controlled trial of radiofrequency ablation and surgical resection in the treatment of small hepatocellular carcinoma. *J Hepatol* 2012; **57**: 794-802 [PMID: 22634125]

P- Reviewer Ruzzenente A **S- Editor** Gou SX **L- Editor** A
E- Editor Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i8.1200

World J Gastroenterol 2013 February 28; 19(8): 1200-1209
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

ORIGINAL ARTICLE

Involvement of parasympathetic pelvic efferent pathway in psychological stress-induced defecation

Kazunori Suda, Hiromi Setoyama, Masanobu Nanno, Satoshi Matsumoto, Mitsuhisa Kawai

Kazunori Suda, Hiromi Setoyama, Masanobu Nanno, Satoshi Matsumoto, Mitsuhisa Kawai, Yakult Central Institute for Microbiological Research, Tokyo 186-8650, Japan
Author contributions: Suda K and Kawai M designed experiments, carried out research and analyzed data; Setoyama H performed immunohistochemical analysis for c-Fos staining; Suda K, Matsumoto S and Kawai M prepared the manuscript; Nanno M and Matsumoto S provided helpful suggestions for all of the experiments; and all authors discussed the results and commented on the manuscript.
Correspondence to: Kazunori Suda, MSc, Yakult Central Institute for Microbiological Research, Yaho 1796, Kunitachi, Tokyo 186-8650, Japan. kazunori-suda@yakult.co.jp
Telephone: +81-42-5778960 Fax: +81-42-5773020
Received: August 3, 2012 Revised: October 30, 2012
Accepted: November 6, 2012
Published online: February 28, 2013

Abstract

AIM: To investigate the role of the pelvic nerve pathway in stress-induced acceleration of colorectal transit and defecation in rats.

METHODS: Surgical transection of rectal nerves (rectal branches of the pelvic nerve), vagotomy (Vag) or adrenalectomy (Adx) were performed bilaterally in rats. Number of fecal pellet output of these rats was measured during 1-h water avoidance stress (WAS). To evaluate the colonic transit, rats were given phenol red through the catheter indwelled in the proximal colon and subjected to WAS. After WAS session, entire colon and rectum were isolated and distribution of phenol red was measured. Distal colonic and rectal transit was evaluated using glass bead. Rats were inserted the glass bead into the distal colon and evacuation rate of the bead was measured. Neural activation was assessed by immunohistochemical staining of c-Fos and PGP9.5 in colonic whole-mount preparations of longitudinal muscle myenteric plexus (LMMP).

RESULTS: In the sham-operated rats (sham op), WAS significantly increased defecation and accelerated colorectal transit with marked elevation of plasma corticosterone level. Compared with sham-operated rats, increase in the excretion of fecal pellets during WAS was significantly reduced by rectal nerve transection (RNT) (sham op: 6.9 ± 0.8 *vs* RNT: 4.3 ± 0.6, $P < 0.05$) or Vag (sham op: 6.4 ± 0.8 *vs* Vag: 3.7 ± 1.1, $P < 0.05$), although corticosterone level remained elevated. Adx-rats significantly increased the defecation despite the lower corticosterone level. Distribution pattern of phenol red showed RNT inhibited distal colonic and rectal transit accelerated by WAS, while Vag inhibited proximal colonic transit. Suppression of distal colonic and rectal transit by RNT was further confirmed by the bead evacuation rate (sham op: 80.0% *vs* RNT: 53.8%). WAS significantly increased the number of c-Fos-immunoreactive neural cells in the LMMP of the proximal and distal colon, whereas c-Fos expression was decreased by RNT in the distal colon (sham op: 9.0 ± 2.0 *vs* RNT: 4.4 ± 1.0, $P < 0.05$) and decreased by Vag in the proximal colon.

CONCLUSION: Pelvic nerve conveys WAS stimuli from the brain to the distal colon, and directly activate the myenteric neurons, followed by the increase of its motility.

© 2013 Baishideng. All rights reserved.

Key words: Colonic transit; Fecal pellet output; Pelvic nerve; Psychological stress; Vagus nerve

Suda K, Setoyama H, Nanno M, Matsumoto S, Kawai M. Involvement of parasympathetic pelvic efferent pathway in psychological stress-induced defecation. *World J Gastroenterol* 2013; 19(8): 1200-1209 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i8/1200.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i8.1200

INTRODUCTION

Stress is a key factor in causing abnormal bowel habits, such as constipation and diarrhea, and has been known to exacerbate these symptoms[1-4]. Accumulating evidence suggests that various stressors stimulate colonic motor functions in humans and animals[5-7]. In patients with irritable bowel syndrome, psychological and physical stressors increase colonic slow-wave, contractile, and motor activities[8]. In laboratory animals, acute exposure to restraint, cold restraint, or water avoidance stress (WAS) enhances colonic motor activity (*e.g.*, colonic motility, colonic transit, and defecation)[9-11]. The stress response is a complicated process that involves the endocrine and nervous systems. Corticotropin-releasing factor (CRF) level is increased in the hypothalamic paraventricular nuclei (PVN), amygdala, and Barrington's nucleus after acute exposure to stressful stimuli[12-15]. CRF is released in the PVN, resulting in the secretion of adrenocorticotropic hormone (ACTH) from the anterior pituitary into the bloodstream. ACTH then increases the release of glucocorticoid from the cortex of the adrenal gland, which is called the hypothalamic-pituitary-adrenal axis (HPA-axis)[16]. Another endocrine system, the sympathetic-adrenal-medullary axis (SAM-axis), is activated by stress and subsequently induces the adrenal medulla to release adrenaline and noradrenaline[17]. These two major bodily pathways cause metabolic, cardiovascular, immune, and behavioral responses[18-20]. The gastrointestinal tract from the esophagus to the distal colon is innervated by parasympathetic vagal efferent fibers from the dorsal motor nucleus in the medulla oblongata[21]. Recently, it was demonstrated that vagotomy (Vag) reduced the restraint stress-induced increase in colonic transit in rats[22,23]. This evidence suggests that physical stress causes colonic dysmotility *via* parasympathetic vagal activation. On the other hand, the efferent fiber from Barrington's nucleus projects to the sacral spinal cord and terminates in the region of the preganglionic pelvic nerves[24,25]. This pelvic efferent is also classified as the parasympathetic nerve and projects to the distal colon and rectum[26]. In rats, activation of the pelvic nerve regulates distal colonic and rectal motility under physiological conditions[27], and electrical nerve stimulation elicits contractions in the rectum[28]. Million *et al*[29] demonstrated that WAS activates the sacral parasympathetic nucleus. However, it is still unclear whether activation of the pelvic nerve is involved in altered colonic and rectal motilities under stressed conditions.

In this study, we examined the role of the pelvic nerve pathway in the activation of defecation, colorectal transit, and myenteric neurons under psychological stress by surgically transecting the rectal branches of the pelvic nerve in rats. Additionally, we compared the differences between colorectal functional changes in pelvic nerve transected rats and vagotomized rats.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (Japan SLC, Shizuoka, Japan) weighing 300-400 g were maintained under a 12-h/12-h light dark cycle (8:00-20:00) with free access to food and water. All animal experiments were approved by the ethical committee for animal experiments of Yakult Institute. All animals were kept in individual cages in a controlled environment with constant temperature (23 ℃ ± 2 ℃) for at least 1 wk before experiments.

WAS

Rats were placed on a rectangular platform (11 cm × 7 cm × 8 cm) in the center of a plastic cage (41 cm × 25 cm × 20 cm) for a 1-h or 2-h period. The cage was filled with water kept at room temperature to the height of 1 cm from the top of the platform. Control rats were placed on the same platform for 1 or 2 h in a cage without water (sham stress). After loading stress, the number of fecal pellets excreted in the cage was counted. WAS and sham stress tests were carried out between 9:00 and 12:00. The animals were placed in a plastic cage for 1 h the day before the experiments, and the rats that excreted more than 5 fecal pellets were excluded from the stress experiment. Each rat was exposed to WAS only once.

Measurement of fecal pellet output and plasma corticosterone

The number of fecal pellets found in the tank was counted and blood samples were collected into heparinized tubes from the tail vein immediately after the 1-h WAS or sham stress session. Blood samples were centrifuged and plasma was stored at -80 ℃ until measurement. The plasma corticosterone concentration was analyzed by the Correlate-EIA corticosterone enzyme immunoassay kit (Enzo Life Sciences, Farmingdale, NY, United States) according to the manufacturer's instructions.

Surgical procedure

Rectal nerve transection (RNT), subdiaphragmatic Vag, or adrenalectomy (Adx) was performed in rats that were anesthetized by intraperitoneal injection (*ip*) of pentobarbital sodium (50 mg/kg; Somnopentyl, Kyoritsu Seiyaku, Tokyo, Japan). We transected the rectal nerve branches from the pelvic plexus to avoid the impairment of the micturition reflex in the present study. A midline incision was made in the abdomen and the rectal nerves were bilaterally transected at a short distance from the major pelvic ganglion. Anterior and posterior branches of the Vag were resected above the hepatic and celiac branches, and the adrenal glands were removed bilaterally. These procedures were performed using forceps under a microscope. Sham-operated animals underwent the same procedure without the above-described resection and removal. The abdominal wall and skin were sutured after completion of surgery. Rats that did not show elevated body weight during the recovery period were excluded from the experiments. Experiments were performed 7-10 d after the surgery.

Assessment of colonic transit

Rats were anesthetized by pentobarbital sodium (50 mg/

kg, *ip*). A polyethylene catheter (PE-50, Becton, Dickinson and Company, Franklin Lakes, NJ, United States) was inserted from the cecum, and the tip was positioned in the proximal colon 1 cm distal to the cecocolonic junction. The other side of the catheter was tunneled subcutaneously to the posterior neck. Rats were given phenol red (10 mg/mL in a volume of 0.4 mL, Sigma-Aldrich, St Louis, MO, United States) through the catheter that was positioned in the proximal colon and subjected to WAS or sham stress for 1-h at 7-10 d after surgery. After the stress session, rats were euthanized with an *ip* overdose of pentobarbital sodium. The entire colon and rectum were isolated and divided into 8 equal segments, where the number of the segments from 1 to 8 was consistent with each segment that was isolated from the start of the proximal colon to the rectum. Segments 1-5 were defined as proximal colon while segments 6-8 were defined as distal colon and rectum. The number of fecal pellets in segments 6-8 was counted. Each segment was transferred into tubes and washed 3 times in phosphate buffered saline (PBS), to which followed the addition of 5 mol/L NaOH by one fifth volume. The absorbance was measured at 560 nm to calculate the volume of phenol red. The geometric center was calculated using the following equation.

Geometric center = Σ (fraction of phenol red per segment × segment number)

Assessment of transit in distal colon and rectum

The rats were fasted overnight with water ad libitum before the experiment. A single 5-mm glass bead was inserted into the distal colon (3 cm proximal to the anus). The rat was subjected to WAS or sham stress for 2-h immediately after bead insertion and the evacuation rate was monitored over a 2-h period.

Immunohistochemical analysis

Rats were euthanized with an *ip* overdose of pentobarbital sodium 1 h after the WAS or sham stress session. The proximal colon (2 cm distal to the cecocolonic junction) and distal colon (4 cm proximal to the anus) were isolated and placed in 0.01 mol/L PBS (pH 7.4). The isolated colon was opened along the mesenteric border, stretched and pinned flat on a Silicon-coated petri dish (Shin-Etsu Chemical, Tokyo, Japan), and fixed overnight in 4% paraformaldehyde in 0.1 mol/L phosphate buffer at 4 ℃. The following day, tissues were washed (3 × 10 min) in PBS, the mucosa, submucosa, and circular muscle were removed, and whole mount preparations of longitudinal muscle myenteric plexus (LMMP) were prepared. The preparations were washed (3 × 10 min) in PBS and incubated in 5% normal donkey serum (Jackson Immuno Research Laboratories, West Grove, PA, United States) in PBS containing 0.3% Triton X-100 (PBS-T) for 30 min at room temperature. The preparations were incubated with rabbit anti-c-Fos (1:10000; Ab-5; Merck Calbiochem, Darmstadt, Germany) and mouse anti-protein gene product 9.5 (PGP9.5, a marker of enteric neurons, 1:50; ab8189; Abcam, Cambridge, United Kingdom) overnight at 4 ℃. The preparations were washed (3 × 10 min) in PBS-T and incubated with donkey anti-rabbit Alexa 488 (Molecular probes, Eugene, OR, United States) and donkey anti-mouse IgG Cy3-conjugated antibody (Millipore, Billerica, MA, United States). After washing (3 × 10 min) in PBS-T, the preparations were mounted on glass slides using VECTASHIELD (Vector Laboratories, Burlingame, CA, United States) and analyzed by the Zeiss LSM 510 confocal microscope with LSM Image Browser software (Carl Zeiss, Jena, Germany). The number of c-Fos immunoreactive (IR) cells was counted in 10 randomly selected ganglia in each preparation per rat and the means of c-Fos-IR cells per rat were used to calculate the mean of each group.

Statistical analysis

All results are presented as mean ± SE. Comparisons between 2 groups were evaluated using the unpaired Student's *t* test or χ^2 test. Comparisons between the mean values of multiple groups were performed with one-way analysis of variance followed by Dunnett's multiple comparison test. *P* values of < 0.05 were considered statistically significant.

RESULTS

Effect of RNT, Vag and Adx on the WAS-induced increase in defecation

When rats were subjected to acute WAS for 1-h, the number of excreted fecal pellets was significantly increased compared to rats under sham stress (4.6 ± 1.0 *vs* 0.8 ± 0.4, $P < 0.01$). Plasma corticosterone levels markedly rose in rats just after WAS compared with those under sham stress (161.6 ± 29.5 ng/mL *vs* 24.2 ± 5.3 ng/mL, $P < 0.01$) (Figure 1A).

The sham-operated rats in RNT or Vag (Figure 1B-C) evacuated more fecal pellets and showed higher level of corticosterone in plasma after WAS treatment. The WAS-induced increase in fecal pellet output was significantly suppressed by treating RNT beforehand (sham op-WAS: 6.9 ± 0.8, RNT-WAS: 4.3 ± 0.6, $P < 0.05$), although the elevation of corticosterone was almost equally observed in RNT-rats and sham-operated rats (Figure 1B). Defecation under the condition of sham stress did not differ in RNT-rats and sham-operated rats. Similarly, Vag significantly reduced the WAS-induced increase in fecal pellet output (sham op-WAS: 6.4 ± 0.8, Vag-WAS: 3.7 ± 1.1, $P < 0.05$), but did not suppress the increase in corticosterone levels induced by WAS (Figure 1C).

On the other hand, WAS significantly increased the number of fecal pellets in Adx-rats compared with sham stress (7.4 ± 0.8 *vs* 3.6 ± 0.9). The baseline levels of plasma corticosterone were lower in Adx-rats than in sham-operated rats, and an obvious change in plasma corticosterone level was not observed after WAS in Adx-rats (Figure 1D).

Effect of RNT and Vag on WAS-induced acceleration of colonic transit

We evaluated whether the pelvic nerve pathway is in-

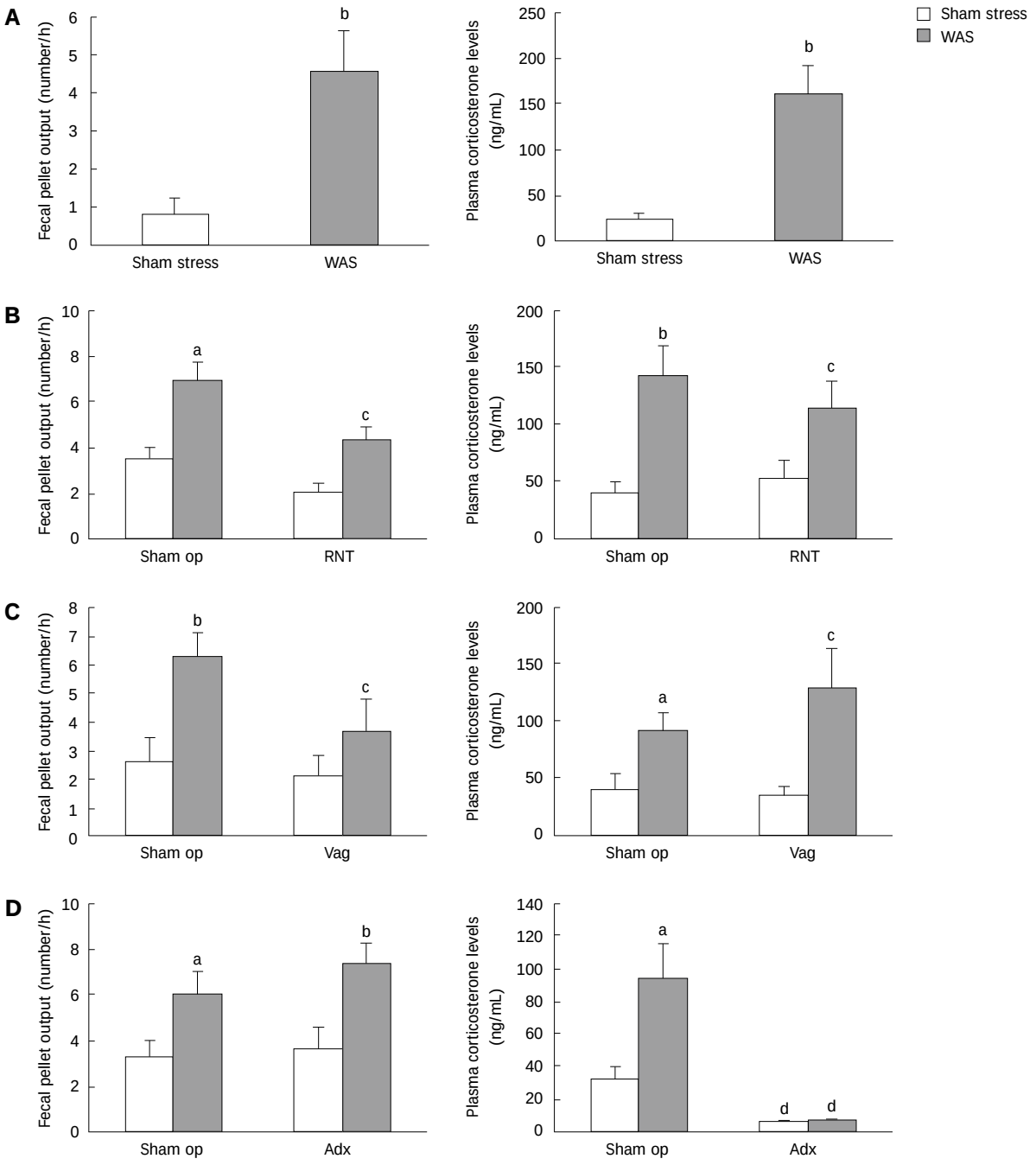


Figure 1 Involvement of parasympathetic nerves and corticosterone in the accelerated defecation induced by acute water avoidance stress. A: The naive rats showed increases in defecation and corticosterone levels after 1-h of water avoidance stress (WAS) compared with sham stress; B-D: Effects of rectal nerve transection (RNT), vagotomy (Vag) and adrenalectomy (Adx) on WAS-induced increases in defecation and plasma corticosterone in rats. Each value represents the mean ± SE (n = 6-12 per group). [a]$P < 0.05$, [b]$P < 0.01$ *vs* the response of sham stress rats; [c]$P < 0.05$, [d]$P < 0.01$ *vs* sham-operated (sham op) rats.

volved in the colonic transit during the stress session. Phenol red, a non-absorbable marker, was injected into the proximal colon *via* a catheter before stress, and geometric center was measured in the rats under the different stress conditions. In sham-operated rats, WAS significantly increased the geometric center (4.6 ± 0.4 *vs* 3.5 ± 0.1, $P < 0.05$, Figure 2A), and also RNT-rats were found to exhibit the higher geometric center after WAS treatment although the difference was not statistically significant (Figure 2A). In contrast, WAS-induced increase of geometric center was completely suppressed in Vag-rats (sham op-WAS: 5.2 ± 0.4, Vag-WAS: 3.8 ± 0.2, $P < 0.001$, Figure 2B).

In sham-stressed RNT- and Vag-rats, the phenol red was distributed over a wide range from segments 1-8 with peaks at segments 3-4. The phenol red contents decreased in segments 1-4 and increased in segments 6-8 in sham-operated rats after WAS loading compared with sham stress (Figure 2C and E). In RNT-rats, the peak of phenol red at segment 3 during sham stress shifted to segments 4-6 after WAS loading (Figure 2D). However, the distribution pattern of phenol red did not change in

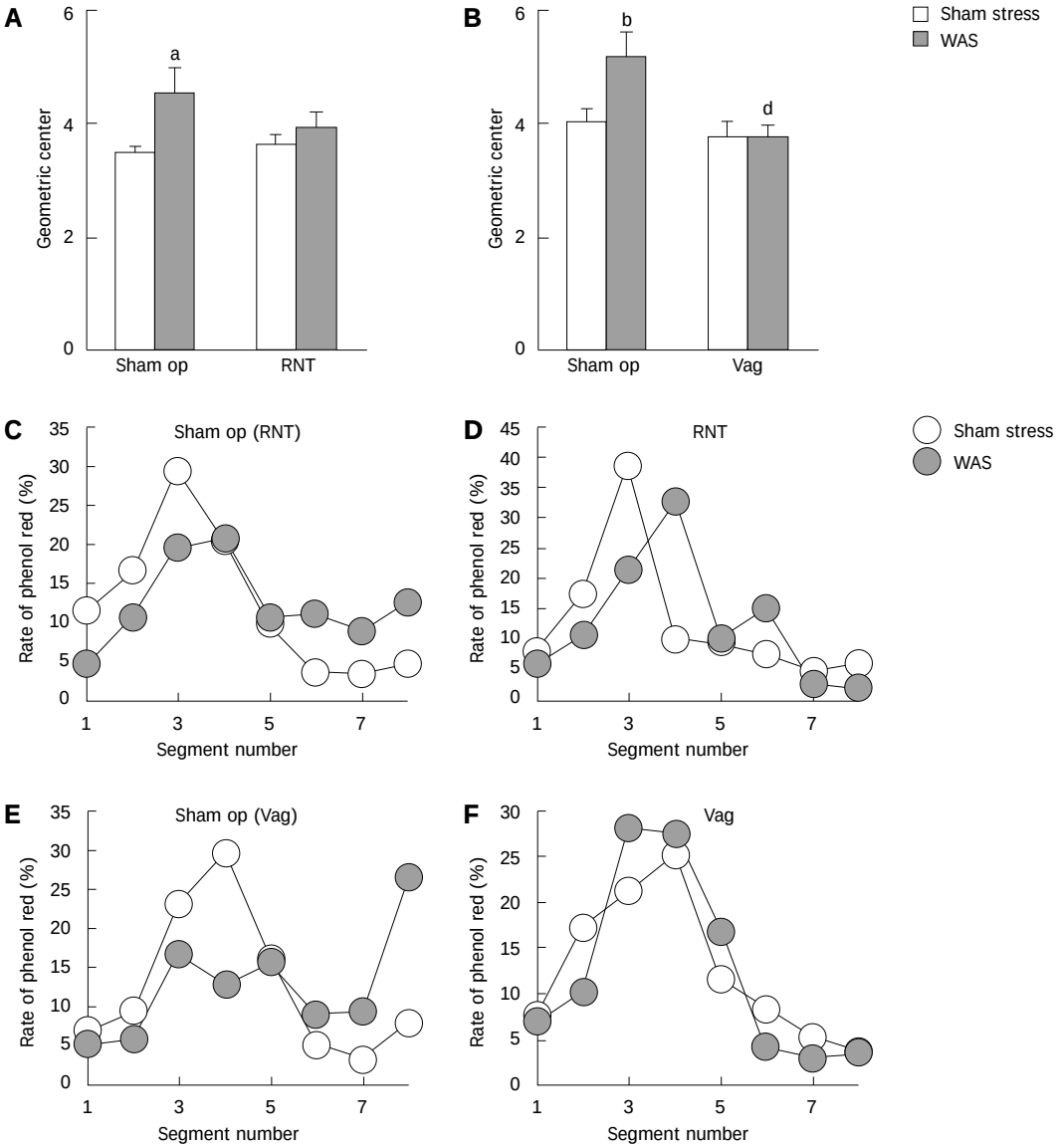


Figure 2 Involvement of parasympathetic nerves in the bowel movement. A, B: Effects of rectal nerve transection (RNT) (A) and vagotomy (Vag) (B) on the colonic transit presented by geometric center of moved phenol red; C-F: Relative distribution of phenol red through the entire colon and rectum after water avoidance stress (WAS) in sham-operated rats (C, E), RNT-rats (D) and Vag-rats (F). Each value represents the mean of 6-8 rats. [a]$P < 0.05$, [b]$P < 0.01$ *vs* the response of sham stress rats; [d]$P < 0.01$ *vs* sham-operated (sham op) rats.

Vag-rats even after WAS was treated (Figure 2F).

The number of luminal feces in the distal colon and rectum was higher in RNT-rats and Vag-rats than in sham-operated rats during sham stress (baseline values) [sham op (RNT): 2.3 ± 0.5, RNT-rats: 4.1 ± 0.4, sham op (Vag): 2.1 ± 0.4, Vag-rats: 3.0 ± 0.5, Table 1]. WAS tended to reduce luminal feces in sham-operated and Vag-rats, with differences of -0.6 and -1.8 between luminal feces in sham stress and in WAS, respectively (Table 1). The number of fecal pellets was increased by WAS in RNT-rats, but was decreased in sham op- and Vag-rats. (B)-(A) indicates the differences of the influx and efflux of fecal pellets from the distal colon and rectum. However, the difference between sham stress and WAS was 0.1 feces in RNT-rats, indicating that the luminal feces in segments 6-8 did not decrease under WAS in these rats.

Effect of RNT and Vag on WAS-induced acceleration of distal colonic and rectal transit

Preganglionic parasympathetic pelvic nerves project from the sacral spinal cord to the distal colon and rectum[26]. Thus, to investigate the effect of RNT on WAS-induced distal colonic and rectal transit, a glass bead was inserted until 3 cm depth from the anus into the distal colon, and the evacuation rate of the bead was monitored over a 2-h period.

In sham-operated rats, WAS significantly increased the rate of bead evacuation to 80% compared to 38% in sham stress, but this increase was not observed in RNT-rats subjected to WAS (Figure 3A). On the other hand, Vag did not affect the WAS-induced increase in the bead evacuation rate (Figure 3B).

Table 1 Number of luminal fecal pellets remaining in the distal colon and rectum after sham stress or water-avoidance stress

	Number of fecal pellets in the distal colon and rectum		
	Sham stress (A)	WAS (B)	(B) - (A)
Sham op (Vag)	2.1 ± 0.4	1.5 ± 0.2	-0.6
Vag	3.0 ± 0.4	1.2 ± 0.5[a]	-1.8
Sham op (RNT)	2.3 ± 0.5	1.1 ± 0.3	-1.2
RNT	4.1 ± 0.4[c]	4.3 ± 0.4[d]	0.1

Each value represents the mean ± SE (n = 6-8 per group). [a]P < 0.05 *vs* sham stress; [c]P < 0.05 , [d]P < 0.01 *vs* sham operated (sham op) rats. WAS: Water-avoidance stress; Vag: Vagotomy; RNT: Rectal nerve transection.

c-Fos expression in LMMP of RNT- and Vag-rats after WAS treatment

After rats were exposed to WAS for 1 h, the number of c-Fos-IR cells was examined in whole mount preparations of LMMP from the distal colon at 0, 1, 2 and 4 h later. The maximum increase after WAS was found at 0 h, and this number returned to a normal level within 4 h (data not shown). Therefore, we measured c-Fos expression immediately after the WAS session (1-h) in all of the following experiments using denervated rats.

The number of c-Fos-IR cells per ganglia in the LMMP of both the proximal and distal colon (Figures 4 and 5) was increased by WAS compared with sham stress. RNT significantly reduced the WAS-induced increase of c-Fos-IR cells in the LMMP by 52% in the distal colon compared with sham-operated rats (4.4 ± 1.0 *vs* 9.0 ± 2.0, P < 0.05, Figure 5B). In contrast, Vag inhibited the increase in c-Fos-IR cells after WAS in the LMMP of proximal colon but not of distal colon (1.7 ± 1.0 *vs* 5.2 ± 1.3, Figure 5C and D).

DISCUSSION

In the present study, we demonstrated the functional importance of the pelvic nerve pathway in the response to psychological stress by showing that transection of the rectal branches of the pelvic nerve (RNT) inhibited the WAS-induced increase in fecal pellet output in rats. This inhibitory effect is supported further by the observation that the RNT inhibited the increased propulsive activity of the distal colon and rectum as shown in the analysis of the colorectal excretion rate of glass beads. Therefore, it is likely that WAS accelerates distal colonic and rectal motilities *via* activation of the pelvic nerve, resulting in increased defecation in the rat. Lenz[22] and Nakade *et al*[23] showed that Vag reduced the restraint stress-induced increase in colonic motility in rats, suggesting that physical stress causes colonic dysmotility *via* parasympathetic vagal activation. Vag also suppressed the WAS-induced increase in fecal pellet output in our study, which is consistent with their results. Our study also confirmed that proximal part of the colonic transit was accelerated in RNT-rats, whereas it was inhibited in Vag-rats under stressed conditions. In fact, many hard fecal pellets remained in the distal colon and rectum of RNT-rats in spite of WAS loading. In contrast, luminal fecal pellets in the distal part of the colon and rectum were decreased in sham-operated and Vag-rats after WAS. These results suggest that pelvic nerve activation by WAS accelerates motor activities of distal colon and rectum, but not in those of proximal colon. On the other hand, Vagus nerve activation accelerates the proximal colonic transit, but not the distal colonic and rectal transit. Therefore, the difference in projection sites of the gut between the pelvic and vagal efferent nerves may be responsible for the differential actions in the gut[21,26]. The pelvic nerve innervates the distal colon and rectum, and regulates rectal motility in the physiological conditions[27]. In this study, a larger number of fecal pellets remained in the distal colon and rectum in RNT-rats without stress, compared with sham-operated rats. These observations suggest that the pelvic nerve pathway may be involved in colorectal motility in the non-stressed conditions. In addition, our findings demonstrate that acute psychological stress stimulates colorectal transit *via* the pelvic nerve pathway.

Recently, c-Fos protein expression has been used as a marker of neural cell activation[30]. c-Fos expression was significantly increased in PGP9.5-positive myenteric neurons in the proximal and distal colon after WAS. This finding is similar to that of a study by Miampamba *et al*[31], who reported increased c-Fos expression in the

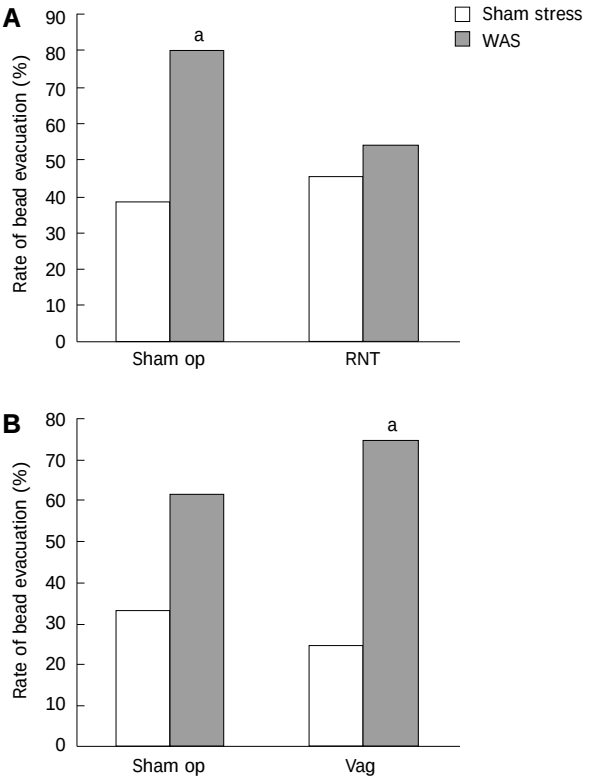


Figure 3 Involvement of parasympathetic nerves in the fecal expulsion through the distal colon. A, B: Effects of rectal nerve transection (RNT) (A) and vagotomy (Vag) (B) on the rate of bead evacuation from the distal colon during 2-h water avoidance stress (WAS). The increased rate of bead evacuation in sham-operated rats was inhibited in RNT-rats. Each value represents the mean ± SE (n = 11-15 per group). [a]P < 0.05 *vs* sham stress rats.

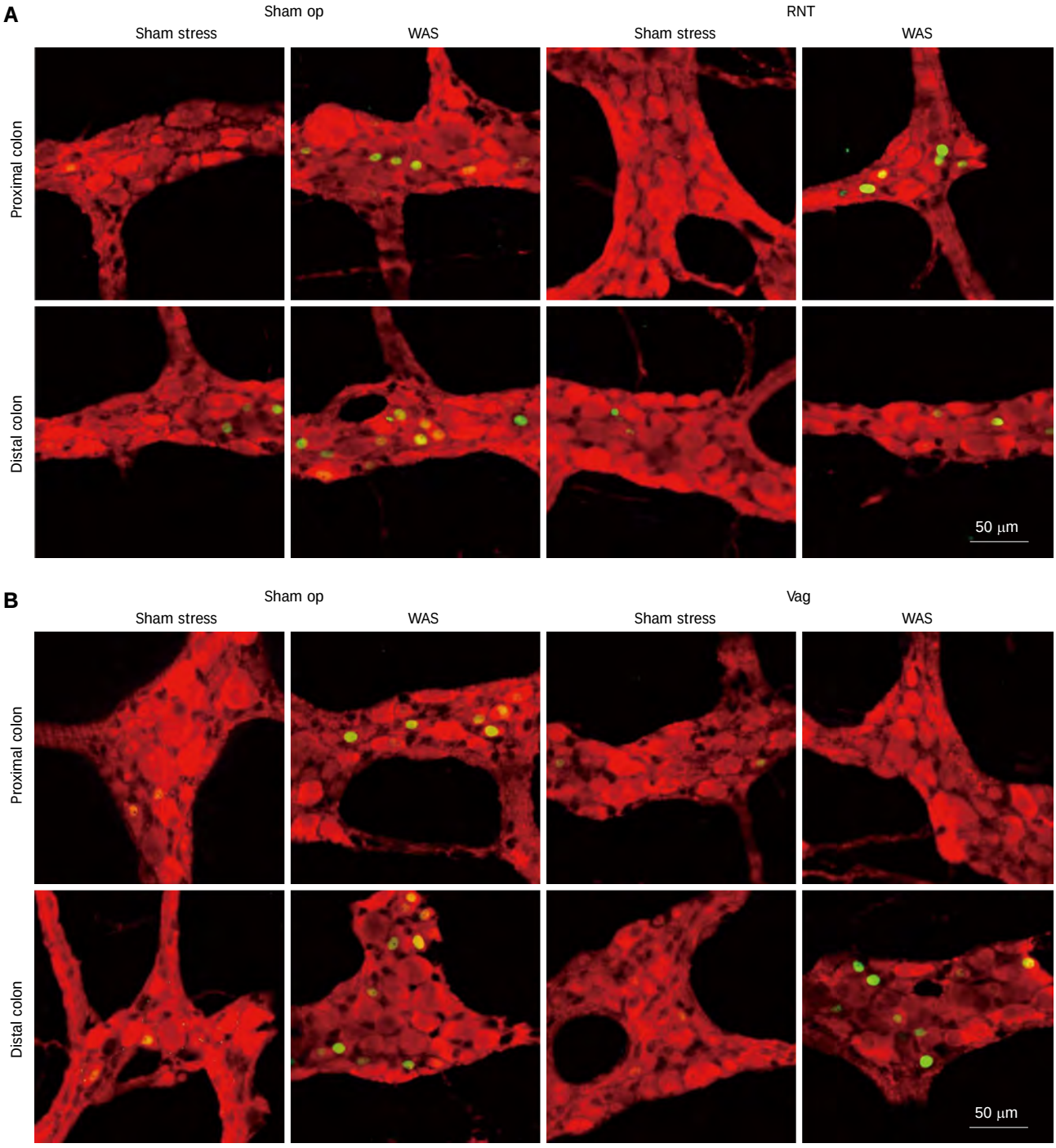


Figure 4 Induction of c-Fos expression after exposure to water avoidance stress. Confocal microscope images showing the staining of c-Fos (green) and protein gene product 9.5 (PGP9.5, red) in whole mount preparations of longitudinal muscle myenteric plexus in rectal nerve transection (RNT) (A) and vagotomy (Vag) rats (B). WAS: Water avoidance stress; Sham op: Sham-operated.

myenteric ganglia of the proximal and distal colon after WAS. Enhanced c-Fos expression was correlated with the number of fecal pellets in the rats and was significantly suppressed in the distal, but not proximal, colon by the denervation of pelvic nerve branches after WAS. On the other hand, Vag suppressed c-Fos expression in the proximal colon but had no effect in the distal colon. These results concerning c-Fos expression are consistent with the differences in the functional site of action between the vagus and pelvic nerves, as discussed above. It is yet unclear whether c-Fos expression, which was induced by WAS-loading, is a direct or indirect response to pelvic efferent nerve activation. As a direct effect, c-Fos expression could be due to inputs from the preganglionic efferent fibers to the myenteric neurons[21,26]. As indirect effect, this may be due to mechanisms such as colonic motility and secretory responses, sensory neurons and interneurons in enteric nervous systems, or the release of gastrointestinal hormones and other factors that activate myenteric neurons. Although we did not clarify the

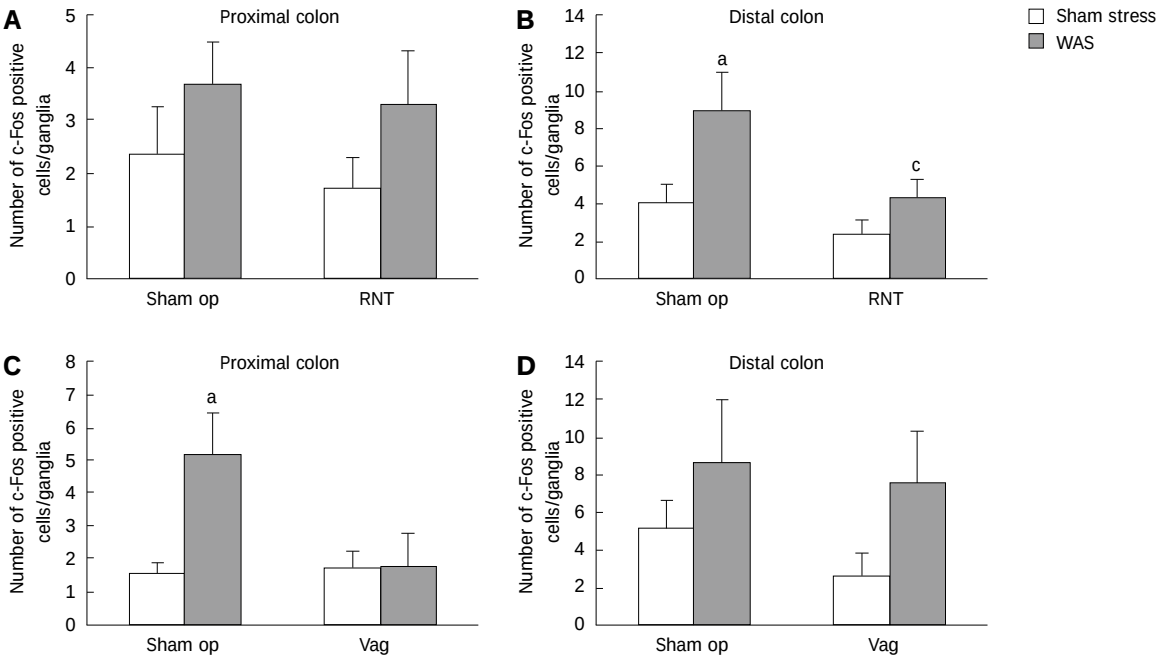


Figure 5 Effects of vagotomy and rectal nerve transection on the induction of c-Fos-expressing cells in the colon after water avoidance stress. Using whole mount preparations of longitudinal muscle myenteric plexus in the proximal (A, C) and distal colons (B, D), numbers of c-Fos immunoreactive cells per ganglia in the proximal and distal colons of rectal nerve transection (RNT) rats (A, B) and vagotomy (Vag) rats (C, D) after water avoidance stress (WAS) treatment were counted and compared to those of sham stress rats. Each value represents the mean ± SE (n = 6-12 per group). [a]$P < 0.05$ *vs* sham stress rats; [c]$P < 0.05$ *vs* sham-operated (sham op) rats.

types of neurons that expressed c-Fos protein in these experiments, myenteric neurons are generally classified as motor neurons, sensory neurons, and interneurons based on histological and morphological properties[31,32]. A double-labeling study revealed that 24%-25% of the total peripheral-choline acetyltransferase-positive neurons, which are mostly motor neurons in the myenteric plexus[32], were c-Fos positive in the proximal and distal colon after WAS[31]. Furthermore, the muscarinic receptor antagonist atropine inhibits the WAS-induced increase in fecal pellets without reducing the elevation of c-Fos-IR nuclei in the proximal and distal colonic LMMP[31,33]. Taken together, these results suggest that myenteric neurons in the distal and proximal colon are activated directly *via* the pelvic and vagal efferent, respectively, followed by acceleration of colonic transit and defecation.

The HPA-axis is also known as a key stress response pathway. HPA-axis activation ultimately induces corticosterone release from the adrenal gland[16]. In this study, the failure to release corticosterone by bilateral Adx did not affect the WAS-induced increase in fecal pellets. Furthermore, Lenz[22] showed that Adx or hypophysectomy did not alter the restraint stress-induced acceleration of colonic transit. These results indicate that the HPA-axis is not mainly involved in acute stress-induced acceleration of colorectal transit and defecation. Our results from adrenalectomized rats in this study also imply that the SAM-axis is of little importance in acute stress-induced colonic dysmotility because Adx inhibits the SAM-axis in addition to the HPA-axis[34]. It was shown recently that a corticoid-receptor antagonist inhibits chronic stress-induced defecation, indicating that the HPA-axis could be involved in the acceleration of colonic motor function by stress[35]. It is not clear why this corticoid-receptor antagonist decreased stress-induced colonic motor function, but this might be due to the different mechanisms that are involved in acute and chronic stress. In fact, it has been reported that chronic exposure of the amygdala to corticosterone delayed colorectal transit after WAS[36]. The current study has only examined the involvement of the parasympathetic nerves in stress-induced acceleration of colonic transit and defecation. The colon and rectum also receives sympathetic inputs from the lumbar colonic nerves and hypogastric nerves. It has been shown that stimulation of sympathetic nerves inhibits rectal motility in guinea pigs and cats[37-39]. Furthermore, it has been reported that sympathetic nerve denervation increased the c-Fos immunoreactivity in the LMMP[40] and the rectal motility[27]. These results suggested that sympathetic nerves may negatively regulate the colonic motor function. In the stressed-condition, however, it remains to be determined whether sympathetic nerve activity participates in the acceleration of colorectal transit and defecation induced by stress. Further experimental investigations are needed to understand the involvement of sympathetic nerves in stress-induced colonic dysmotility.

In conclusion, we have shown that WAS-induced acceleration of distal colonic transit and defecation is mediated by the parasympathetic pelvic efferent. The pelvic nerve may convey stress stimuli from the brain to the distal colon and rectum, directly activating myenteric neurons and subsequently accelerating distal colonic motility.

In contrast, WAS-induced acceleration of colonic transit and activation of myenteric neurons in proximal colon is mediated by parasympathetic vagal efferent.

ACKNOWLEDGMENTS

We thank Dr. Mamoru Tanida from the Department of Biomedical Sciences, College of Life Sciences, Ritsumeikan University, for his technical advice on the subdiaphragmatic vagotomy, and the entire staff at the animal care facility of Yakult Central Institute for Microbiological Research.

COMMENTS

Background

Stress is a key factor in causing abnormal bowel habits, such as constipation and diarrhea, and has been known to exacerbate these symptoms. It has been described that various stressors stimulate colonic motor functions. Although it is reported that the activation of colonic motor function is mediated *via* the parasympathetic vagus nerve, there are few studies that have reported the involvement of the parasympathetic pelvic nerve in stress-induced gut dysmotility.

Research frontiers

The pelvic efferent projects from the sacral parasympathetic nucleus to the distal colon and rectum. It is reported that water avoidance stress (WAS) activate the sacral parasympathetic nucleus, however, it is still unclear whether activation of the pelvic nerve is involved in altered colonic and rectal motilities under stressed conditions.

Innovations and breakthroughs

This study demonstrated the importance of the pelvic nerve in the response to WAS-induced acceleration of distal colonic transit and increase of defecation. Furthermore, the results confirmed the involvement of vagus nerve to WAS-induced acceleration of proximal colonic transit. This is the first study to report the differences in the functional site of action between two nerves under stressed condition.

Applications

By understanding how stress stimuli cause the colonic dysmotility, these results would be useful for the therapeutic applications of stress-related colonic motor dysfunction, such as irritable bowel syndrome.

Terminology

Longitudinal muscle myenteric plexus (LMMP): LMMP is generally used as immunohistochemical study of the myenteric plexus; c-Fos: c-Fos protein is the product of immediate-early gene, c-fos, which is used as a marker of neural activation.

Peer review

The authors clearly demonstrated involvement of pelvic nerve in the psychological stress-induced acceleration of distal colonic transit and defecation. Additionally this study suggested that pelvic nerve directly activate the myenteric neurons and subsequently accelerating distal colonic motility. This is an interesting study, that clarify the role of the pelvic and vagus nerve in the stress-induced colonic dysmotility.

REFERENCES

1 **Hislop IG**. Psychological significance of the irritable colon syndrome. *Gut* 1971; **12**: 452-457 [PMID: 5090869 DOI: 10.1136/gut.12.6.452]
2 **Guthrie E**, Creed F, Dawson D, Tomenson B. A controlled trial of psychological treatment for the irritable bowel syndrome. *Gastroenterology* 1991; **100**: 450-457 [PMID: 1985041]
3 **Chan AO**, Cheng C, Hui WM, Hu WH, Wong NY, Lam KF, Wong WM, Lai KC, Lam SK, Wong BC. Differing coping mechanisms, stress level and anorectal physiology in patients with functional constipation. *World J Gastroenterol* 2005; **11**: 5362-5366 [PMID: 16149147]
4 **Murray CD**, Flynn J, Ratcliffe L, Jacyna MR, Kamm MA, Emmanuel AV. Effect of acute physical and psychological stress on gut autonomic innervation in irritable bowel syndrome. *Gastroenterology* 2004; **127**: 1695-1703 [PMID: 15578507 DOI: 10.1053/j.gastro.2004.08.057]
5 **Rao SS**, Hatfield RA, Suls JM, Chamberlain MJ. Psychological and physical stress induce differential effects on human colonic motility. *Am J Gastroenterol* 1998; **93**: 985-990 [PMID: 9647034 DOI: 10.1016/S0002-9270(98)00174-9]
6 **Wittmann T**, Crenner F, Angel F, Hanusz L, Ringwald C, Grenier JF. Long-duration stress. Immediate and late effects on small and large bowel motility in rat. *Dig Dis Sci* 1990; **35**: 495-500 [PMID: 2318096 DOI: 10.1007/BF01536925]
7 **Stam R**, Croiset G, Akkermans LM, Wiegant VM. Effects of novelty and conditioned fear on small intestinal and colonic motility and behaviour in the rat. *Physiol Behav* 1995; **58**: 803-809 [PMID: 8559795 DOI: 10.1016/0031-9384(95)00137-8]
8 **Welgan P**, Meshkinpour H, Hoehler F. The effect of stress on colon motor and electrical activity in irritable bowel syndrome. *Psychosom Med* 1985; **47**: 139-149 [PMID: 4048360]
9 **Miyata K**, Kamato T, Nishida A, Ito H, Yuki H, Yamano M, Tsutsumi R, Katsuyama Y, Honda K. Role of the serotonin3 receptor in stress-induced defecation. *J Pharmacol Exp Ther* 1992; **261**: 297-303 [PMID: 1560375]
10 **Barone FC**, Deegan JF, Price WJ, Fowler PJ, Fondacaro JD, Ormsbee HS. Cold-restraint stress increases rat fecal pellet output and colonic transit. *Am J Physiol* 1990; **258**: G329-G337 [PMID: 2316647]
11 **Bonaz B**, Taché Y. Water-avoidance stress-induced c-fos expression in the rat brain and stimulation of fecal output: role of corticotropin-releasing factor. *Brain Res* 1994; **641**: 21-28 [PMID: 8019847 DOI: 10.1016/0006-8993(94)91810-4]
12 **Kalin NH**, Takahashi LK, Chen FL. Restraint stress increases corticotropin-releasing hormone mRNA content in the amygdala and paraventricular nucleus. *Brain Res* 1994; **656**: 182-186 [PMID: 7804835 DOI: 10.1016/0006-8993(94)91382-X]
13 **Merlo Pich E**, Lorang M, Yeganeh M, Rodriguez de Fonseca F, Raber J, Koob GF, Weiss F. Increase of extracellular corticotropin-releasing factor-like immunoreactivity levels in the amygdala of awake rats during restraint stress and ethanol withdrawal as measured by microdialysis. *J Neurosci* 1995; **15**: 5439-5447 [PMID: 7643193]
14 **Yamano Y**, Yoshioka M, Toda Y, Oshida Y, Chaki S, Hamamoto K, Morishima I. Regulation of CRF, POMC and MC4R gene expression after electrical foot shock stress in the rat amygdala and hypothalamus. *J Vet Med Sci* 2004; **66**: 1323-1327 [PMID: 15585943 DOI: 10.1292/jvms.66.1323]
15 **Imaki T**, Nahan JL, Rivier C, Sawchenko PE, Vale W. Differential regulation of corticotropin-releasing factor mRNA in rat brain regions by glucocorticoids and stress. *J Neurosci* 1991; **11**: 585-599 [PMID: 2002354]
16 **Lightman SL**. The neuroendocrinology of stress: a never ending story. *J Neuroendocrinol* 2008; **20**: 880-884 [PMID: 18601712 DOI: 10.1111/j.1365-2826.2008.01711.x]
17 **Buske-Kirschbaum A**, Geiben A, Höllig H, Morschhäuser E, Hellhammer D. Altered responsiveness of the hypothalamus-pituitary-adrenal axis and the sympathetic adrenomedullary system to stress in patients with atopic dermatitis. *J Clin Endocrinol Metab* 2002; **87**: 4245-4251 [PMID: 12213879 DOI: 10.1210/jc.2001-010872]
18 **Charmandari E**, Tsigos C, Chrousos G. Endocrinology of the stress response. *Annu Rev Physiol* 2005; **67**: 259-284 [PMID: 15709959 DOI: 10.1146/annurev.physiol.67.040403.120816]
19 **Yang EV**, Glaser R. Stress-induced immunomodulation: impact on immune defenses against infectious disease. *Biomed Pharmacother* 2000; **54**: 245-250 [PMID: 10917461 DOI: 10.1016/S0753-3322(00)80066-9]
20 **De Vente W**, Olff M, Van Amsterdam JG, Kamphuis JH, Emmelkamp PM. Physiological differences between burnout patients and healthy controls: blood pressure, heart rate,

and cortisol responses. *Occup Environ Med* 2003; **60** Suppl 1: i54-i61 [PMID: 12782748 DOI: 10.1136/oem.60.suppl_1.i54]
21 **Berthoud HR**, Carlson NR, Powley TL. Topography of efferent vagal innervation of the rat gastrointestinal tract. *Am J Physiol* 1991; **260**: R200-R207 [PMID: 1992820]
22 **Lenz HJ**. Neurohumoral pathways mediating stress-induced changes in rat gastrointestinal transit. *Gastroenterology* 1989; **97**: 216-218 [PMID: 2566550]
23 **Nakade Y**, Fukuda H, Iwa M, Tsukamoto K, Yanagi H, Yamamura T, Mantyh C, Pappas TN, Takahashi T. Restraint stress stimulates colonic motility via central corticotropin-releasing factor and peripheral 5-HT3 receptors in conscious rats. *Am J Physiol Gastrointest Liver Physiol* 2007; **292**: G1037-G1044 [PMID: 17158256 DOI: 10.1152/ajpgi.00419.2006]
24 **Loewy AD**, Saper CB, Baker RP. Descending projections from the pontine micturition center. *Brain Res* 1979; **172**: 533-538 [PMID: 476495 DOI: 10.1016/0006-8993(79)90584-5]
25 **Blok BF**, Holstege G. Ultrastructural evidence for a direct pathway from the pontine micturition center to the parasympathetic preganglionic motoneurons of the bladder of the cat. *Neurosci Lett* 1997; **222**: 195-198 [PMID: 9148248 DOI: 10.1016/S0304-3940(97)13384-5]
26 **Luckensmeyer GB**, Keast JR. Projections of pelvic autonomic neurons within the lower bowel of the male rat: an anterograde labelling study. *Neuroscience* 1998; **84**: 263-280 [PMID: 9522380 DOI: 10.1016/S0306-4522(97)89502-4]
27 **Ridolfi TJ**, Tong WD, Takahashi T, Kosinski L, Ludwig KA. Sympathetic and parasympathetic regulation of rectal motility in rats. *J Gastrointest Surg* 2009; **13**: 2027-2033; discussion 2033 [PMID: 19760300 DOI: 10.1007/s11605-009-0999-z]
28 **Tong WD**, Ridolfi TJ, Kosinski L, Ludwig K, Takahashi T. Effects of autonomic nerve stimulation on colorectal motility in rats. *Neurogastroenterol Motil* 2010; **22**: 688-693 [PMID: 20067587 DOI: 10.1111/j.1365-2982.2009.01461.x]
29 **Million M**, Wang L, Martinez V, Taché Y. Differential Fos expression in the paraventricular nucleus of the hypothalamus, sacral parasympathetic nucleus and colonic motor response to water avoidance stress in Fischer and Lewis rats. *Brain Res* 2000; **877**: 345-353 [PMID: 10986349 DOI: 10.1016/S0006-8993(00)02719-0]
30 **Sagar SM**, Sharp FR, Curran T. Expression of c-fos protein in brain: metabolic mapping at the cellular level. *Science* 1988; **240**: 1328-1331 [PMID: 3131879 DOI: 10.1126/science.3131879]
31 **Miampamba M**, Million M, Yuan PQ, Larauche M, Taché Y. Water avoidance stress activates colonic myenteric neurons in female rats. *Neuroreport* 2007; **18**: 679-682 [PMID: 17426598 DOI: 10.1097/WNR.0b013e3280bef7f8]
32 **Gwynne RM**, Bornstein JC. Synaptic transmission at functionally identified synapses in the enteric nervous system: roles for both ionotropic and metabotropic receptors. *Curr Neuropharmacol* 2007; **5**: 1-17 [PMID: 18615154 DOI: 10.2174/157015907780077141]
33 **Mönnikes H**, Schmidt BG, Taché Y. Psychological stress-induced accelerated colonic transit in rats involves hypothalamic corticotropin-releasing factor. *Gastroenterology* 1993; **104**: 716-723 [PMID: 8440432]
34 **Ismahan G**, Parvez H, Parvez S, Youdim MB. Comparative effects of hypophysectomy and adrenalectomy upon plasma and adrenal monoamines in pregnant and non-pregnant rats. *Br J Pharmacol* 1977; **60**: 385-391 [PMID: 196710 DOI: 10.1111/j.1476-5381.1977.tb07513.x]
35 **Hong S**, Zheng G, Wu X, Snider NT, Owyang C, Wiley JW. Corticosterone mediates reciprocal changes in CB 1 and TRPV1 receptors in primary sensory neurons in the chronically stressed rat. *Gastroenterology* 2011; **140**: 627-637.e4 [PMID: 21070780 DOI: 10.1053/j.gastro.2010.11.003]
36 **Venkova K**, Johnson AC, Myers B, Greenwood-Van Meerveld B. Exposure of the amygdala to elevated levels of corticosterone alters colonic motility in response to acute psychological stress. *Neuropharmacology* 2010; **58**: 1161-1167 [PMID: 20170666 DOI: 10.1016/j.neuropharm.2010.02.012]
37 **Takaki M**, Neya T, Nakayama S. Sympathetic activity in the recto-rectal reflex of the guinea pig. *Pflugers Arch* 1980; **388**: 45-52 [PMID: 7192388 DOI: 10.1007/BF00582627]
38 **Neya T**, Takaki M, Nakayama S. Mechanism of rectal contraction mediated by sympathetic efferents from rectoanal pelvic afferents in guinea pigs. *Acta Med Okayama* 1984; **38**: 21-27 [PMID: 6702482]
39 **Carlstedt A**, Fasth S, Hultén L, Nordgren S. The sympathetic innervation of the internal anal sphincter and rectum in the cat. *Acta Physiol Scand* 1988; **133**: 423-431 [PMID: 2906510 DOI: 10.1111/j.1748-1716.1988.tb08425.x]
40 **Yuyama N**, Mizuno J, Tsuzuki H, Wada-Takahashi S, Takahashi O, Tamura K. Effects of extrinsic autonomic inputs on expression of c-Fos immunoreactivity in myenteric neurons of the guinea pig distal colon. *Brain Res* 2002; **948**: 8-16 [PMID: 12383950 DOI: 10.1016/S0006-8993(02)02943-8]

P- Reviewer Lohsiriwat V **S- Editor** Wen LL **L- Editor** A **E- Editor** Li JY



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i8.1210

World J Gastroenterol 2013 February 28; 19(8): 1210-1218
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

ORIGINAL ARTICLE

Inhibition of pacemaker activity in interstitial cells of Cajal by LPS *via* NF-κB and MAP kinase

Dong Chuan Zuo, Seok Choi, Pawan Kumar Shahi, Man Yoo Kim, Chan Guk Park, Young Dae Kim, Jun Lee, In Yeoup Chang, Insuk So, Jae Yeoul Jun

Dong Chuan Zuo, Seok Choi, Pawan Kumar Shahi, Jae Yeoul Jun, Department of Physiology, College of Medicine, Chosun University, Gwangju 501-759, South Korea
Man Yoo Kim, Chan Guk Park, Young Dae Kim, Jun Lee, Department of Internal Medicine, College of Medicine, Chosun University, Gwangju 501-759, South Korea
In Yeoup Chang, Department of Anatomy, College of Medicine, Chosun University, Gwangju 501-759, South Korea
Insuk So, Department of Physiology and Biophysics, College of Medicine, Seoul National University, Seoul 56-1, South Korea
Author contributions: Zuo DC and Choi S contributed equally to this work; Jun JY designed research; Zuo DC, Choi S, Shahi PK, Kim MY, Park CG, Kim YD and Lee J performed research; Jun JY wrote the paper; So I and Chang IY contributed to the experiment analysis.
Supported by Basic Science Research Program through the National Research Foundation of Korea funded by the Ministry of Education, Science and Technology, No. 2012-0001335
Correspondence to: Jae Yeoul Jun, MD, PhD, Professor, Department of Physiology, College of Medicine, Chosun University, 375 Seosuk-dong, Gwangju 501-759, South Korea. jyjun@chosun.ac.kr
Telephone: +82-62-2306412 Fax: +82-62-2324943
Received: November 28, 2012 Revised: January 7, 2013
Accepted: January 18, 2013
Published online: February 28, 2013

Abstract

AIM: To investigate lipopolysaccharide (LPS) related signal transduction in interstitial cells of Cajal (ICCs) from mouse small intestine.

METHODS: For this study, primary culture of ICCs was prepared from the small intestine of the mouse. LPS was treated to the cells prior to measurement of the membrane currents by using whole-cell patch clamp technique. Immunocytochemistry was used to examine the expression of the proteins in ICCs.

RESULTS: LPS suppressed the pacemaker currents of ICCs and this could be blocked by AH6809, a prostaglandin E2-EP2 receptor antagonist or NG-Nitro-L-arginine Methyl Ester, an inhibitor of nitric oxide (NO) synthase. Toll-like receptor 4, inducible NO synthase or cyclooxygenase-2 immunoreactivity by specific antibodies was detected on ICCs. Catalase (antioxidant agent) had no action on LPS-induced action in ICCs. LPS actions were blocked by nuclear factor κB (NF-κB) inhibitor, actinomycin D (a gene transcription inhibitor), PD 98059 (a p42/44 mitogen-activated protein kinases inhibitor) or SB 203580 [a p38 mitogen-activated protein kinases (MAPK) inhibitor]. SB 203580 also blocked the prostaglandin E2-induced action on pacemaker currents in ICCs but not NO.

CONCLUSION: LPS inhibit the pacemaker currents in ICCs *via* prostaglandin E2- and NO-dependent mechanism through toll-like receptor 4 and suggest that MAPK and NF-κB are implicated in these actions.

© 2013 Baishideng. All rights reserved.

Key words: Interstitial cells of Cajal; Lipopolysaccharide; Mitogen-activated protein kinases; Nuclear factor κB; Small intestine

Zuo DC, Choi S, Shahi PK, Kim MY, Park CG, Kim YD, Lee J, Chang IY, So I, Jun JY. Inhibition of pacemaker activity in interstitial cells of Cajal by LPS *via* NF-κB and MAP kinase. *World J Gastroenterol* 2013; 19(8): 1210-1218 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i8/1210.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i8.1210

INTRODUCTION

Many morphological or functional studies have disclosed the roles of interstitial cells of Cajal (ICCs) in many organs including gastrointestinal (GI) tract. An accumulation of

evidence from these studies has revealed ICCs has the distinct role in pacemaker activity[1-3] and as mediators of enteric motor neurotransmission in GI tract[4-6]. For development and maintenance, the ICCs express the receptor tyrosine kinase Kit[1,3,7]. And, it is well known that c-Kit or ANO1 can be a selective molecular marker for researching the distribution and function of ICCs[8].

GI inflammation can be cause by inflammatory cytokines such as interleukins, chemical inflammatory mediators *etc* and lipopolysaccharide (LPS) from gram-negative bacteria is a major causative factor of GI inflammation[9]. Various *vivo* and *vitro* experiments showed LPS could play a major role in GI motility disorders[10-13]. Also, there are several reports that the morphological and functional changes of ICCs are involved in inflammation-induced GI motility[14-16]. These indicate ICCs may be important target for inflammation-induced motility disorders. Recently we reported that LPS inhibited the pacemaker currents in cultured ICCs from mouse small intestine. LPS-action was blocked by cyclooxygenase (COX)-2 inhibitor or nitric oxide (NO) synthase inhibitor, suggesting prostaglandins (PGs) and NO are involve in these actions[17], can verify this.

Generally, the stimulation of LPS rouses regulatory pathways such as the nuclear factor κB (NF-κB) pathway, as well as reactive oxygen species (ROS) signaling cascades *via* a toll like receptor-4-mediated signaling pathway[18]. Furthermore, the stimulation of LPS results in the activation of kinases including extracellular signal-regulated kinases, c-Jun N-terminal kinase (JNK) and p38 mitogen-activated protein kinases (MAPKs) critical for growth and cytokine production that induces inflammatory-related substances, such as interleukin, PGs, inducible NO synthase (iNOS) *etc*[19-21]. Our previous report suggested production of inflammatory-related substances, such as prostaglandin E2 (PGE_2) or NO are involved in LPS-induced action on ICCs[17]. However, the details of the PGs or iNOS induction in ICCs by LPS stimulation remain unclear.

In the present study, we aimed to clarify the involvement of NF-κB, ROS and MAPKs that contribute to PGs or iNOS induction in ICCs and changing of pacemaker activity in LPS-treated ICCs.

MATERIALS AND METHODS

Solutions and drugs

The cells were bathed in a solution containing 5 mmol/L KCl, 135 mmol/L NaCl, 2 mmol/L $CaCl_2$, 10 mmol/L glucose, 1.2 mmol/L $MgCl_2$, and 10 mmol/L HEPES, adjusted to pH 7.2 with Tris. NaCl was replaced with equimolar *N*-methyl-D-glucamine for making Na^+-free solution, and $CaCl_2$ was omitted in the bath solution for Ca^{2+}-free solution. The pipette solution contained 20 mmol/L K-aspartate, 120 mmol/L KCl, 5 mmol/L $MgCl_2$, 2.7 mmol/L K_2ATP, 0.1 mmol/L Na_2GTP, 2.5 mmol/L creatine phosphate disodium, 5 mmol/L 4-(2-Hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES), and 0.1 mmol/L EGTA, adjusted to pH 7.2 with Tris.

The drugs used were LPS (salmonella enteric serotype typhimurium), JNK inhibitor Ⅱ, SN-50 (NF-κB inhibitor) and catalase from Calbiochem, (San Diego, CA, United States) and the other compounds were purchased from Sigma.

Preparation of cells and tissues

Balb/C mice (3-8 d old) of either sex were anesthetized with ether and were sacrificed by cervical dislocation. The small intestines from 1 cm below the pyloric ring to the cecum were removed and opened along the mesenteric border. The luminal contents were washed away with Krebs-Ringer bicarbonate solution. The tissues were pinned to the base of a Sylgard dish and the mucosa was removed by sharp dissection. Small strips of intestinal muscle were equilibrated in Ca^{2+}-free Hank's solution containing 5.36 mmol/L KCl, 125 mmol/L NaCl, 0.34 mmol/L NaOH, 0.44 mmol/L Na_2HCO_3, 10 mmol/L glucose, 2.9 mmol/L sucrose, and 11 mmol/L HEPES for 30 min, and the cells were dispersed with an enzyme solution containing 1.3 mg/mL collagenase (Worthington Biochemical Co, Lakewood, NJ, United States), 2 mg/mL bovine serum albumin (Sigma Chemical Co., St. Louis, MO, United States), 2 mg/mL trypsin inhibitor (Sigma) and 0.27 mg/mL ATP. Cells were plated onto sterile glass coverslips coated with poly L-lysine (2.5 μg/mL, Sigma) in 35 mm culture dishes. The cells were then cultured at 37 ℃ in a 5% CO_2 incubator in SMGM (smooth muscle growth medium) (Clonetics Co., Walkersville, MD, United States) supplemented with 2% antibiotics/antimycotics (Gibco, Grand Island, NY, United States) and murine stem cell factor (SCF, 5 ng/mL, Sigma). ICCs were identified immunologically with the use of a monoclonal antibody for kit protein (ACK2) labeled with Alexa Fluor 488 (Molecular Probes, Eugene, OR, United States).

Patch clamp experiments

The whole-cell configuration of the patch clamp technique was used to record membrane currents (voltage clamp) and membrane potentials (current clamp) from cultured ICC after 2-3 d in culture. We recorded from small clusters of ICC, as spontaneous inward currents from small groups of cells are more robust and regular than from single cells. Currents or potentials were amplified by Axopatch 200B (Axon Instruments, Foster, CA, United States). A command pulse was applied using an IBM compatible personal computer and pClamp software (version 9.2; Axon Instruments). The data were filtered at 5 kHz, and the recorded data were displayed on a computer monitor saved for data analysis and future references. Results were analyzed using the Clampfit program (Axon Instruments) and Graph Pad Prism (version 2.01) software. All experiments were performed at 30 ℃.

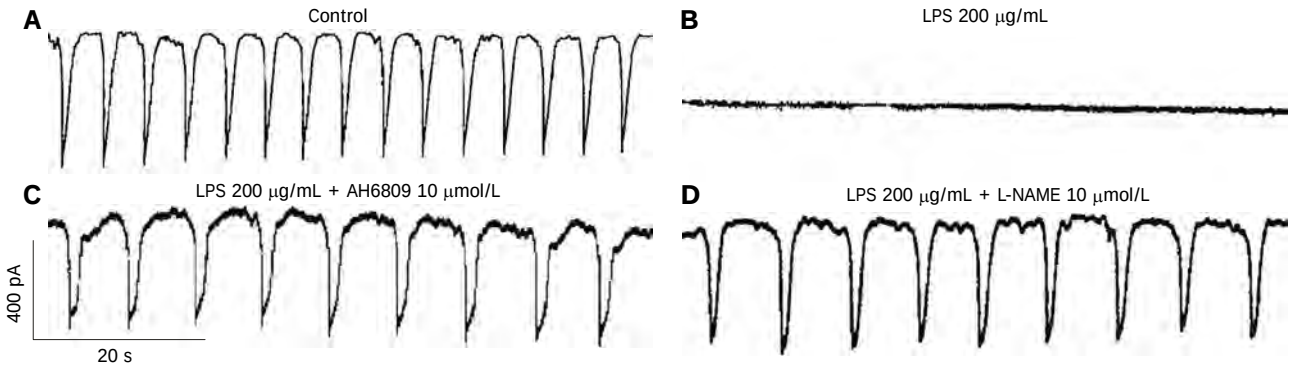


Figure 1 Effects of AH6809 or NG-Nitro-L-arginine Methyl Ester on lipopolysaccharide-induced action in interstitial cells of Cajal. A: Pacemaker currents of interstitial cells of Cajal (ICCs) at a holding potential of -70 mV in control condition; B: Pacemaker currents of ICCs incubated at 37 ℃ with 200 μg/mL of lipopolysaccharide (LPS) for 12 h; C: Pacemaker currents in ICCs pretreated with AH6809 (10 μmol/L) for 2 h prior to LPS incubation; D: Pacemaker currents in ICCs pretreated with NG-Nitro-L-arginine Methyl Ester (10 μmol/L) for 2 h prior to LPS incubation. LPS: Lipopolysaccharide; L-NAME: NG-Nitro-L-arginine Methyl Ester.

Immunocytochemistry

Cultured cells were fixed in acetone (20 ℃/5 min). Following fixation, preparations were washed for 60 min in phosphate buffered saline (PBS; 0.01 mol, pH 7.4). Cultured cells were then incubated in 10% goat serum containing 1% bovine serum albumin for 1 h at RT to reduce nonspecific antibody binding. For double immunostaining, cells were incubated overnight at 4 ℃ in a mixture of two primary antibodies raised in different species (a rat monoclonal c-Kit antibody, 1:200, Gibco-BRL, Gaithersburg, MD, United States; a goat polyclonal TLR4 antibody, 1:100, Santa Cruz Biotechnology, CA, United States; a goat polyclonal iNOS antibody, 1:100, Santa Cruz Biotechnology; a goat polyclonal COX-2 antibody, 1:100, Santa Cruz Biotechnology). The secondary antibodies were fluorescein isothiocyanate (FITC) for c-Kit and Texas Red for anti-TLR4, iNOS or COX-2, diluted 1:100 (all reagents from Vector Laboratories, Burlingame, CA). Immunoreactivity was detected using FITC-conjugated secondary antibody (FITC-anti-rat; Vector Laboratories, 1:100 in PBS, 1 h, room temperature). Control cultured ICCs were prepared in a similar manner, but, omitting ACK2 from the incubation medium. After washing with PBS, the mixture of labeled secondary antibodies was treated with FITC-conjugated donkey anti-rabbit IgG (1:100, Chemicon, Temecula, CA, United States) and Texas red-conjugated donkey anti-goat IgG (1:100, Jackson Immunoresearch Laboratories, Baltimore, PA, United States). The incubation of labeled secondary antibodies was performed for 1 h at room temperature. Cells were examined with a FV300 confocal microscope (Olympus, Tokyo, Japan) with an excitation wavelength appropriate for FITC (488 nm) and Texas Red (568 nm). Final images were constructed with Flow View Software Program (Olympus, Tokyo, Japan).

Ethics

All experiments were performed according to the Guiding Principles for the Care and Use of Animals approved by the Ethics Committee of Chosun University and the National Institute of Health Guide for the Care and Use of Laboratory Animals. Every effort was made to minimize both the number of animals used and the suffering of the animals.

Statistical analysis

Data are expressed as the mean ± SE. Differences in the data were evaluated by use of the Student's *t* test. *P* values less than 0.05 are considered a statistically significant difference. The *n* values reported in the text refer to the number of cells used in the patch clamp experiments.

RESULTS

Confirmation of prostaglandin or NO production involvement on LPS-induced action in ICCs

First, we verified inhibitory action of LPS on ICCs and the involvement of PG or NO production on LPS-induced action. Recordings were made from cells within networks that had morphologies similar to the cells that were immunopositive for c-Kit. Under voltage clamp mode at a holding potential of -70 mV, ICCs showed spontaneous inward pacemaker currents (Figure 1A) and this inhibited by 200 μg/mL LPS incubation for 12 h (Figure 1B). And inhibitory action of LPS on pacemaker activity in ICCs was blocked by 10 μmol/L AH6809 (a PGE_2-EP2 receptor antagonist) or 10 μmol/L NG-Nitro-L-arginine Methyl Ester (L-NAME) (an inhibitor of NO synthase) (n = 5, Figure 1C and D, bar graph not shown). These could make sure the involvement of PG or NO production on LPS-induced action in ICCs.

Localization of toll like receptor-4, inducible NO synthase and COX-2 in ICCs

For searching the ability that can interact with TLR4 and produce NO or PGs in ICCs, we tried immunocytochemistry with specific antibody for TLR4, iNOS and COX-2 proteins. Double staining with anti-c-Kit and anti-TLR4, anti-iNOS or anti-COX-2 antibodies revealed TLR4, iNOS and COX-2 immunoreactivity in c-Kit immune-positive ICCs (Figure 2A-C).

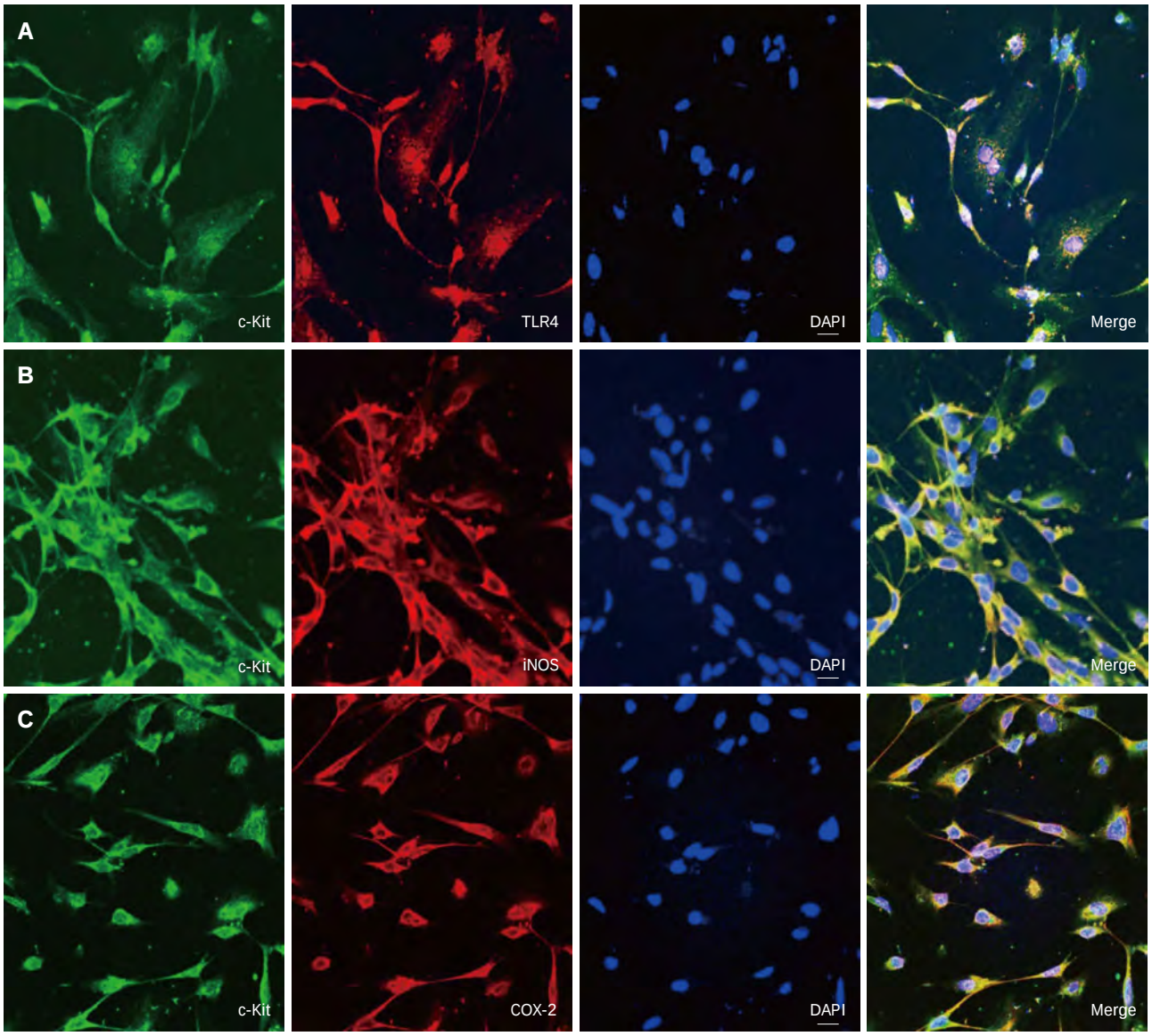


Figure 2 Expression of TLR4, iNOS and COX-2 in c-Kit positive cells. A: Double labeling of TLR4- and c-Kit-like immunoreactivity in cultured interstitial cells of Cajal (ICCs). Green (c-Kit) and red (TLR4) result in the mixed color yellow, indicating the colocalization of both peptides; B: Double labeling of iNOS and c-Kit-like immunoreactivity in ICCs. Green (c-Kit) and red (iNOS) result in the mixed color yellow, indicating the colocalization of both peptides; C: Double labeling of COX-2 and c-Kit-like immunoreactivity in ICCs. Green (c-Kit) and red (COX-2) result in the mixed color yellow, indicating the colocalization of both peptides (bar = 20 μm).

Effects of catalase on LPS-induced action in ICCs

For finding the involvement of ROS production on LPS-induced action, we used the antioxidant reagent, catalase. To investigate the effect of LPS, ICCs were incubated with 200 μg/mL LPS for 12 h and we could reconfirm the disappearance of pacemaker currents (Figure 3A). Pretreatment with catalase (3000 unit/mL) for 2 h prior to 200 μg/mL LPS incubation could not show any difference compared with LPS alone (Figure 3B). The frequency and amplitude values of pacemaker currents in catalase treatment with LPS were not significantly different compared with LPS alone (n = 4, Figure 3C and D).

Effects of SN-50 or actinomycin D on lipopolysacchardie-induced action in ICCs

For detecting the role for NF-κB or involvement of gene transcriptions on LPS-induced action, we used SN-50, the NF-κB inhibitor and actinomycin D, the gene transcription inhibitor. The incubation with LPS 200 μg/mL alone, the pacemaker currents were completely inhibited (Figure 4A). But when SN-50 (10 μmol/L) was treated for 2 h prior to LPS incubation, LPS-induced action on pacemaker currents in ICCs was blocked (Figure 4B). Also, we could find that actinomycin D (10 μmol/L) blocked the LPS-induced pacemaker currents suppression (Figure 4C). The values of frequency and amplitude induced by LPS in the presence of SN-50 or actinomycin D were significantly different from those obtained in the absence of NF-κB inhibitor (n = 5, Figure 4D and E).

Effects of MAPK inhibitors on LPS-induced action in ICCs

For understanding the involvement of MAPKs on LPS-induced action, specific MAPKs inhibitors were used. When the pretreatments of ICCs with PD 98059 (a

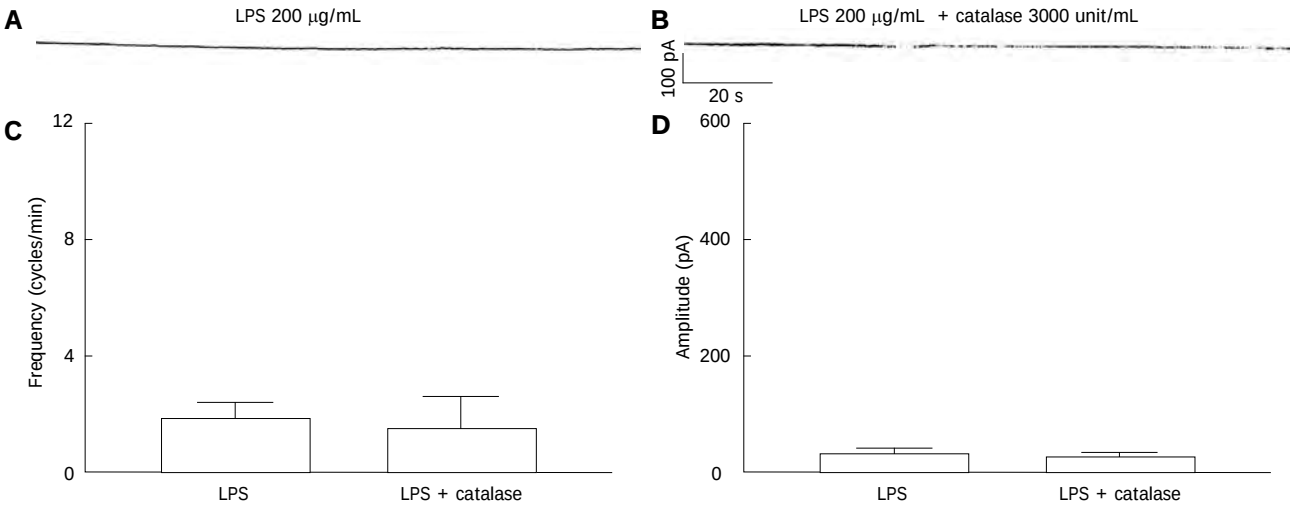


Figure 3 Effects of catalase on lipopolysaccharide-induced action in interstitial cells of Cajal. A: Pacemaker currents of interstitial cells of Cajal (ICCs) incubated at 37 ℃ with 200 μg/mL of lipopolysaccharide (LPS) for 12 h; B: Pacemaker currents in ICCs pretreated with catalase (3000 unit/mL) for 2 h prior to LPS incubation. The effects of catalase on the LPS-induced action in ICCs are summarized in C and D. Bars represent mean ± SE values (n = 4 per group).

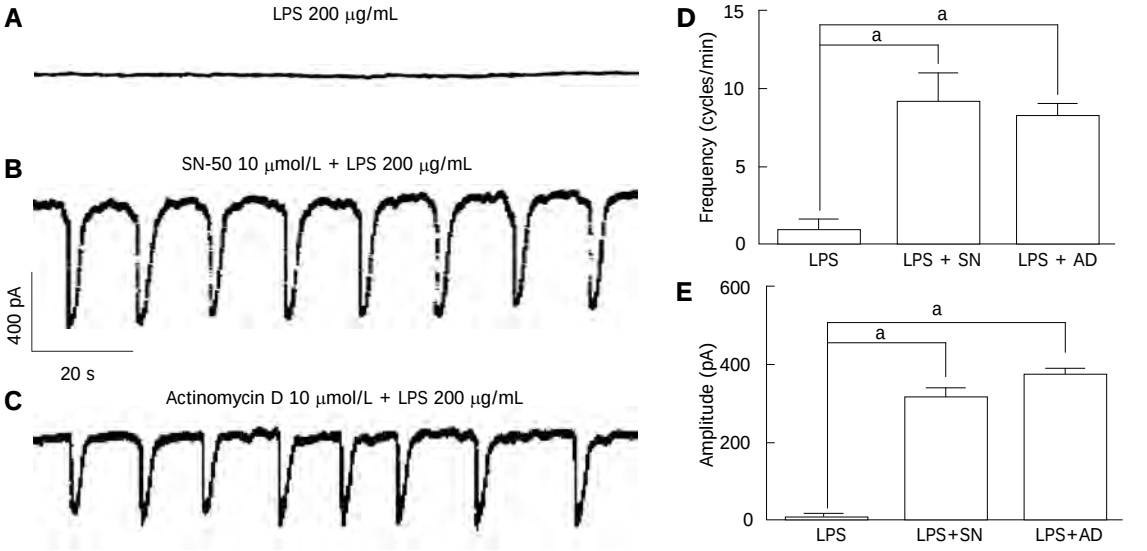


Figure 4 Effects of SN-50 or actinomycin D on lipopolysaccharide-induced action in interstitial cells of Cajal. A: Pacemaker currents of interstitial cells of Cajal (ICCs) incubated at 37 ℃ with 200 μg/mL of lipopolysaccharide (LPS) for 12 h at a holding potential of -70 mV; B: Pacemaker currents in ICCs pretreated with SN-50 (10 μmol/L) for 2 h prior to LPS incubation; C: Pacemaker currents in ICCs pretreated with actinomycin D (10 μmol/L) for 2 h prior to LPS incubation. The effects of SN-50 or actinomycin D on the LPS-induced action in ICCs are summarized in D and E. Bars represent mean ± SE values (n = 5 per group). $^aP < 0.05$ *vs* LPS alone treatment. SN: SN-50, AD: Actinomycin D.

selective p42/44 inhibitor), SB 203580 (a selective p38 inhibitor) or JNK inhibitor Ⅱ for 2 h prior to 200 μg/mL LPS incubation, the LPS-induced inhibitory action on pacemaker currents were blocked by PD 98059 (10 μmol/L), SB 203580 (10 μmol/L) but not by JNK inhibitor Ⅱ (10 μmol/L) (Figure 5B-D). The values of frequency, amplitude and resting currents induced by LPS in the presence of PD 98059 or SB 203580 were significantly different from those obtained in the absence of these (n = 7, Figure 5E and F).

Effects of MAPK inhibitors on Prostaglandin E2-induced action in ICCs

For examining the involvement of MAPKs on PGE_2 production by LPS stimulation in ICCs, we used p42/44 inhibitor and p38 inhibitor with PGE_2. Because JNK inhibitor Ⅱ did not show any influence on LPS-induced action in ICCs, we did not test with JNK inhibitor Ⅱ in here. At first, we could see PGE_2 (5 μmol/L) itself has the inhibitory effect on pacemaker currents in ICCs (Figure 6A). Then, we examined the effects of PGE_2 on pacemaker currents in presence of PD 98059 or SB203580. The pretreatments of ICCs with PD 98059 (10 μmol/L) blocked the 5 μmol/L PGE_2-induced inhibitory action on pacemaker currents but not SB 203580 (10 μmol/L) (Figure 6B and C). The values of frequency and amplitude currents induced by PGE_2 in the presence of PD 98059 were significantly different from those obtained in the absence

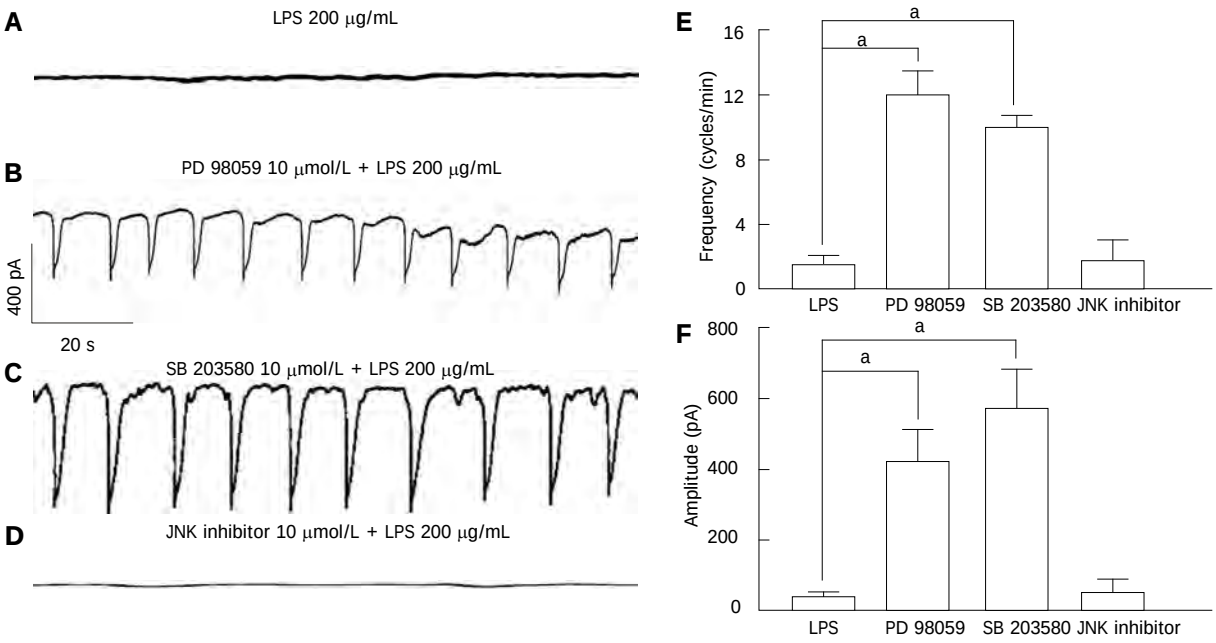


Figure 5 Effects of Mitogen-activated protein kinases inhibitors on lipopolysaccharide-induced action in interstitial cells of Cajal. A: Pacemaker currents of interstitial cells of Cajal (ICCs) incubated at 37 ℃ with 200 μg/mL of lipopolysaccharide (LPS) for 12 h at a holding potential of -70 mV; B: Pacemaker currents after pretreatment with PD 98059 (10 μmol/L) for 2 h prior to LPS incubation; C: Pacemaker currents after pretreatment with SB 203580 (10 μmol/L) for 2 h prior to LPS incubation; D: Pacemaker currents after pretreatment with c-Jun N-terminal kinase (JNK) inhibitor II (10 μmol/L) for 2 h prior to LPS incubation. The effects of mitogen-activated protein kinases inhibitors on the LPS-induced action are summarized in E and F. Bars represent mean ± SE values (n = 7 per group). $^aP < 0.05$ *vs* LPS.

of PD 98059 (n = 4, Figure 6D and E).

Effects of MAPK inhibitors on NO-induced action in ICCs

For examing the role of MAPKs on NO production by LPS stimulation in ICCs, we also used p42/44 inhibitor and p38 inhibitor with (±)-S-nitroso-N-acetylpenicillamine (SNAP), a NO donor. In our previous report[22], we already showed SNAP has inhibitory action on pacemaker currents in ICCs like as PGE_2. In here, we also could see the inhibitory action of 100 μmol/L SNAP on pacemaker currents (Figure 7A). We examined the effects of SNAP on pacemaker currents in presence of PD 98059 or SB 203580. The pretreatments of ICCs with PD 98059 (10 μmol/L) or SB 203580 (10 μmol/L) did not show any influence on 5 μmol/L SNAP-induced inhibitory action on pacemaker currents (Figure 7B and C). The values of frequency and amplitude induced by SNAP in the presence of PD 98059 or SB 203580 were not significantly different from those obtained in the absence of PD 98059 or SB 203580 (n = 5, Figure 7E and F). Furthermore, we checked the reciprocal relation between PG and NO with PGE_2 and L-NAME. In presence of 10 μmol/L L-NAME, 5 μmol/L PGE_2 still inhibited the pacemaker current (n = 4, Figure 7D, bar graph not shown).

DISCUSSION

PGE_2 and NO are inhibitory mediators on intestinal motility and produced by COX (COX-1 or 2) and NOS (nNOS/eNOS/iNOS), respectively. We have shown that LPS inhibited pacemaker currents of intestinal ICCs by activating ATP-sensitive K^+ channels through the release of PGE_2 and NO[17]. In this experiment, we further found that MAPKs are involving in LPS-induced inhibition of pacemaker currents through NF-κB-dependent mechanisms in ICCs.

The binding of TLR4 with LPS activates NF-κB through myeloid differentiation primary response gene-88 (Myd88)-dependent pathway. LPS has been shown to induce NF-κB nuclear translocation in intestinal smooth muscle and myenteric plexus cells, but not in TLR4 knockout mice[23]. The activated NF-κB then induces various transcriptional genes including COX-2 and iNOS in GI tracts. In this study, LPS-induced inhibition of pacemaker currents was blocked by COX-2 inhibitor or iNOS inhibitor. And a specific NF-κB blocker or actinomycin D, a gene transcriptional blocker, suppressed LPS-induced inhibition of pacemaker currents. Furthermore, double staining with anti-c-Kit and anti-COX-2 antibodies or anti-iNOS antibodies revealed COX-2 or iNOS immunoreactivity in c-Kit immunopositive ICCs in cultured ICCs. These results suggest that NF-κB activation is essential to induce expression of COX-2 and iNOS exposed to LPS in ICCs.

MAPKs play an important role in the mediation of cellular responses, including transcriptional responses. Three types of MAPKs (p42/44 MAPK, p38 MAPK, and JNK) are involved in LPS-induced NF-κB or JNK activator protein-1 activation in various cells[24-27]. Recently, it was reported that three types of MAPKs were

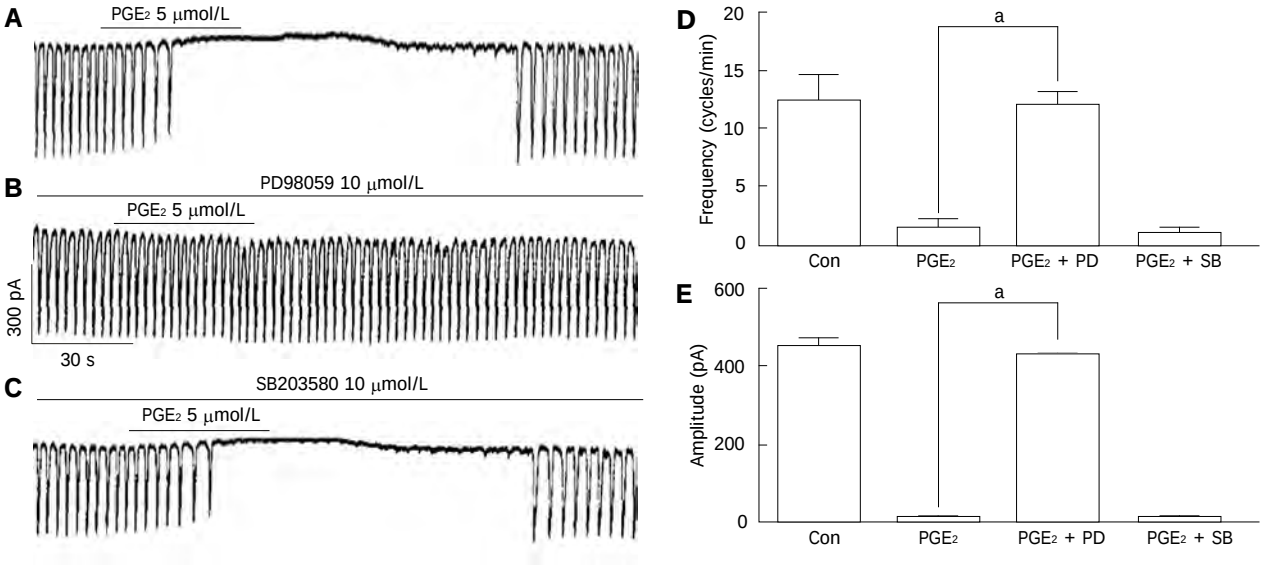


Figure 6 Effects of mitogen-activated protein kinases inhibitors on prostaglandin E2-induced action in interstitial cells of Cajal. A: Pacemaker currents of interstitial cells of Cajal (ICCs) treated with 5 μmol/L prostaglandin E2 (PGE_2) at a holding potential of -70 mV; B: Pacemaker currents after pretreatment with PD 98059 (10 μmol/L) prior to PGE_2 treatment; C: Pacemaker currents after pretreatment with SB 203580 (10 μmol/L) prior to PGE_2 treatment. The effects of mitogen-activated protein kinases inhibitors on the PGE_2-induced action are summarized in D and E. Bars represent mean ± SE values (n = 4 per group). $^aP < 0.05$ *vs* PGE_2. Con: Control, PD: PD 98059, SB: SB203580.

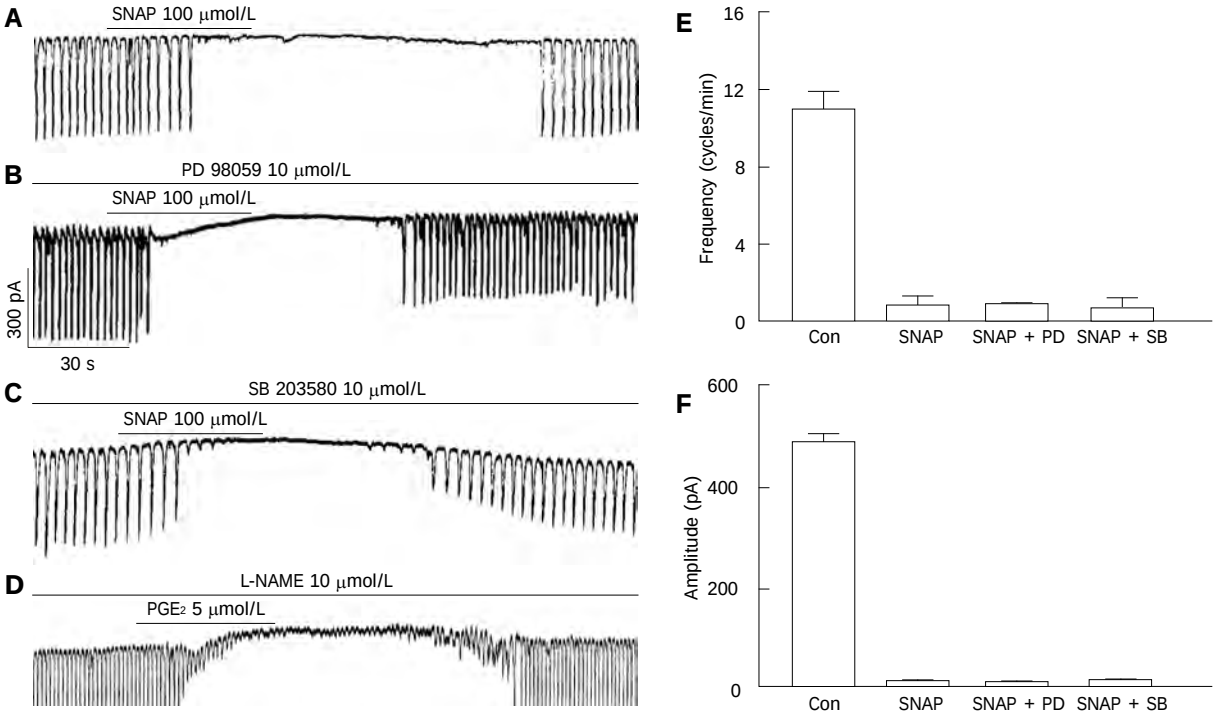


Figure 7 Effects of mitogen-activated protein kinases inhibitors on (±)-S-nitroso-N-acetylpenicillamine-induced action in interstitial cells of Cajal. A: Pacemaker currents of interstitial cells of Cajal (ICCs) treated with 100 μmol/L SNAP at a holding potential of -70 mV; B: Pacemaker currents after pretreatment with PD 98059 (10 μmol/L) prior to SNAP treatment; C: Pacemaker currents after pretreatment with SB 203580 (10 μmol/L) prior to SNAP treatment; D: Pacemaker currents after pretreatment with L-NAME (10 μmol/L) prior to prostaglandin E2 (PGE_2) (5 μmol/L) treatment. The effects of mitogen-activated protein kinases inhibitors on the (±)-S-nitroso-N-acetylpenicillamine-induced action are summarized in E and F. Bars represent mean ± SE values (n = 5 per group). Con: Control, PD: PD 98059, SB: SB203580; SNAP: S-nitroso-N-acetylpenicillamine; L-NAME: NG-Nitro-L-arginine Methyl Ester.

involved in the mechanism of LPS actions in rabbit ileum and the blockade of p38, JNK or p42/44 pathways suppressed the effects of LPS on the acetylcholine-induced contractions[28]. In our study, the LPS-induced effects in ICCs were blocked by PD98059 or SB20356, but not by JNK inhibitor Ⅱ, indicating that activation of p42/44 and p38 MAPKs are involved in LPS-induced activation of NF-κB in ICCs. These results are consistent with those of others showing that p42/44 and p38 MAPKs are involved in COX-2 and iNOS induction by

LPS in tracheal smooth muscle cells[26]. However, this study showed PGE_2-induced inhibition of pacemaker currents is blocked by p42/44 MAPK inhibitor, but not by p38 MAPK inhibitor in intestinal ICCs and NO-induced inhibition of pacemaker currents was not blocked by both p42/44 MAPK inhibitor and p38 MAPK inhibitor. These results suggest that p42/44 MAPK and p38 MAPK play differential role in regulation of LPS-induced actions in ICCs. Thus, it seems that p38 MAPK may be involved in LPS-induced NF-κB activation to express COX-2 and iNOS, whereas p42/44 MAPK as a downstream pathway of NF-κB may be involved in PGE_2-induced effects in ICCs not NO signaling. Our previous study could explains the possibility that NO-induced action on pacemaker currents is *via* activating ATP-sensitive K^+ channels through cGMP-dependent mechanism in ICCs but not involvement of MAPKs[22].

LPS action was also mediated by ROS production through a nicotinamide adenine dinucleotide phosphate oxidase pathway. In rat aortic smooth muscles, LPS could induce the hydrogen peroxide signaling[29]. Also, we found that H_2O_2 inhibited pacemaker currents by activating ATP-sensitive K^+ channels in ICCs, which was mediated through COX-2-dependent PGE_2 production[30]. Therefore, in order to investigate whether H_2O_2 is involved in LPS-induced PGE_2 production, we treated catalase, a hydrogen peroxide scavenger. However, catalase did not block the LPS-induced inhibition of pacemaker currents. This result suggests that hydrogen peroxide do not involve in LPS-induced actions in intestinal ICCs.

Many reports are arguing the relation between the production of COX and NOS. NO production by NOS enzyme could stimulate or inhibit the COX enzyme induction[31,32]. And some reports suggested products by COX enzyme stimulate the NOS pathway[33,34]. In our opinion, the cross talk between NOS and COX may depend on the cell type or enzyme type. In here, we need to check whether inhibitory effect of PGE_2 on pacemaker activity is through induction of NO synthesis in ICCs. However, the proofs that PGE_2 alone could inhibit the pacemaker activity in ICCs in our previous study and L-NAME could not show any influence on PGE_2-induced action in here could establish no-interaction between the products of COX and NOS by LPS stimulation in ICCs.

In summary, the current study provides evidence that LPS inhibited pacemaker currents in cultured ICCs *via* PGE_2 and NO-dependent pathways. NF-κB and MAP kinases are involved in this inhibitory action.

COMMENTS

Background

Interstitial cells of Cajal (ICCs) are the pacemaker cells present in the gastrointestinal tract (GI) that generate the pacemaker currents that regulate the GI motility. Lipopolysaccharides (LPS) are endotoxins and pro-inflammatory agents present in the cell wall of the Gram-negative bacteria responsible for various alterations of gastrointestinal functions including intestinal dysmotility.

Innovations and breakthroughs

LPS altered GI motility through release of prostaglandins or nitric oxide (NO) by nuclear factor κB (NF-κB) dependent gene transcription. This study showed LPS inhibited pacemaker currents of ICC. Prostaglandin E2 and NO contributed to the inhibitory effects of LPS together with mitogen-activated protein kinases and NF-κB.

Applications

LPS-induced inhibitory actions on pacemaker activities of ICC may be one possible mechanism to explain LPS-induced GI dysmotility.

Terminology

ICCs refer to the several types of cells present on the musculature of the GI tract. ICCs act as pacemaker cells in gastrointestinal tracts, regulating rhythmicity by activating calcium activated chloride channel or nonselective cation channels.

Peer review

The functional regulation of ICCs is still not fully understood and this study provides novel information on the TLR 4-mediated signaling pathways on LPS–suppressed pacemaker currents of ICCs.

REFERENCES

1 **Huizinga JD**, Thuneberg L, Klüppel M, Malysz J, Mikkelsen HB, Bernstein A. W/kit gene required for interstitial cells of Cajal and for intestinal pacemaker activity. *Nature* 1995; **373**: 347-349 [PMID: 7530333 DOI: 10.1038/373347a0]

2 **Ordög T**, Ward SM, Sanders KM. Interstitial cells of cajal generate electrical slow waves in the murine stomach. *J Physiol* 1999; **518** (Pt 1): 257-269 [PMID: 10373707 DOI: 10.1111/j.1469-7793.1999.0257r.x]

3 **Ward SM**, Burns AJ, Torihashi S, Sanders KM. Mutation of the proto-oncogene c-kit blocks development of interstitial cells and electrical rhythmicity in murine intestine. *J Physiol* 1994; **480** (Pt 1): 91-97 [PMID: 7853230]

4 **Beckett EA**, Horiguchi K, Khoyi M, Sanders KM, Ward SM. Loss of enteric motor neurotransmission in the gastric fundus of Sl/Sl(d) mice. *J Physiol* 2002; **543**: 871-887 [PMID: 12231645 DOI: 10.1113/jphysiol.2002.021915]

5 **Burns AJ**, Lomax AE, Torihashi S, Sanders KM, Ward SM. Interstitial cells of Cajal mediate inhibitory neurotransmission in the stomach. *Proc Natl Acad Sci USA* 1996; **93**: 12008-12013 [PMID: 8876253 DOI: 10.1073/pnas.93.21.12008]

6 **Ward SM**, Beckett EA, Wang X, Baker F, Khoyi M, Sanders KM. Interstitial cells of Cajal mediate cholinergic neurotransmission from enteric motor neurons. *J Neurosci* 2000; **20**: 1393-1403 [PMID: 10662830]

7 **Maeda H**, Yamagata A, Nishikawa S, Yoshinaga K, Kobayashi S, Nishi K, Nishikawa S. Requirement of c-kit for development of intestinal pacemaker system. *Development* 1992; **116**: 369-375 [PMID: 1283735]

8 **Gomez-Pinilla PJ**, Gibbons SJ, Bardsley MR, Lorincz A, Pozo MJ, Pasricha PJ, Van de Rijn M, West RB, Sarr MG, Kendrick ML, Cima RR, Dozois EJ, Larson DW, Ordog T, Farrugia G. Ano1 is a selective marker of interstitial cells of Cajal in the human and mouse gastrointestinal tract. *Am J Physiol Gastrointest Liver Physiol* 2009; **296**: G1370-G1381 [PMID: 19372102 DOI: 10.1152/ajpgi.00074.2009]

9 **Deitch EA**, Xu D, Franko L, Ayala A, Chaudry IH. Evidence favoring the role of the gut as a cytokine-generating organ in rats subjected to hemorrhagic shock. *Shock* 1994; **1**: 141-145 [PMID: 7749933 DOI: 10.1097/00024382-199402000-00010]

10 **Park H**, Calrk E, Cullen JJ, Conklin JL. Effect of endotoxin on opossum oesophageal motor function. *Neurogastroenterol Motil* 2000; **12**: 215-221 [PMID: 10867618 DOI: 10.1046/j.1365-2982.2000.00202.x]

11 **van Miert AS**, van Duin CT. Endotoxin-induced inhibition of gastric emptying rate in the rat. The effect of repeated administration and the influence of some antipyretic agents. *Arch Int Pharmacodyn Ther* 1980; **246**: 19-27 [PMID: 7004366]

12 **Wechsung E**. [The involvement of prostaglandins in the inhibiting effect of endotoxin on the myoelectric activity of the gastrointestinal system in pigs]. *Verh K Acad Geneeskd Belg* 1996; **58**: 711-738 [PMID: 9157744]

13 **Weisbrodt NW**, Pressley TA, Li YF, Zembowicz MJ, Hi-gham SC, Zembowicz A, Lodato RF, Moody FG. Decreased ileal muscle contractility and increased NOS II expression induced by lipopolysaccharide. *Am J Physiol* 1996; **271**: G454-G460 [PMID: 8843770]

14 **Der T**, Bercik P, Donnelly G, Jackson T, Berezin I, Collins SM, Huizinga JD. Interstitial cells of cajal and inflammation-induced motor dysfunction in the mouse small intestine. *Gastroenterology* 2000; **119**: 1590-1599 [PMID: 11113080 DOI: 10.1053/gast.2000.20221]

15 **Lu G**, Qian X, Berezin I, Telford GL, Huizinga JD, Sarna SK. Inflammation modulates in vitro colonic myoelectric and contractile activity and interstitial cells of Cajal. *Am J Physiol* 1997; **273**: G1233-G1245 [PMID: 9435548]

16 **Wang XY**, Berezin I, Mikkelsen HB, Der T, Bercik P, Collins SM, Huizinga JD. Pathology of interstitial cells of Cajal in relation to inflammation revealed by ultrastructure but not immunohistochemistry. *Am J Pathol* 2002; **160**: 1529-1540 [PMID: 11943737 DOI: 10.1016/S0002-9440(10)62579-5]

17 **Zuo DC**, Choi S, Shahi PK, Kim MY, Park CG, Kim YD, Lee J, Chang IY, Lee HS, Yeom SC, Moon HJ, Seong SY, So I, Jun JY. Action of lipopolysaccharide on interstitial cells of cajal from mouse small intestine. *Pharmacology* 2012; **90**: 151-159 [PMID: 22890360 DOI: 10.1159/000340018]

18 **Abreu MT**, Fukata M, Arditi M. TLR signaling in the gut in health and disease. *J Immunol* 2005; **174**: 4453-4460 [PMID: 15814663]

19 **Kaisho T**, Akira S. Toll-like receptor function and signaling. *J Allergy Clin Immunol* 2006; **117**: 979-987; quiz 988 [PMID: 16675322 DOI: 10.1016/j.jaci.2006.02.023]

20 **Kawai T**, Akira S. Signaling to NF-kappaB by Toll-like receptors. *Trends Mol Med* 2007; **13**: 460-469 [PMID: 18029230 DOI: 10.1016/j.molmed.2007.09.002]

21 **Wang C**, Deng L, Hong M, Akkaraju GR, Inoue J, Chen ZJ. TAK1 is a ubiquitin-dependent kinase of MKK and IKK. *Nature* 2001; **412**: 346-351 [PMID: 11460167 DOI: 10.1038/35085597]

22 **Park CG**, Kim YD, Kim MY, Kim JS, Choi S, Yeum CH, Parajuli SP, Park JS, Jeong HS, So I, Kim KW, Jun JY. Inhibition of pacemaker currents by nitric oxide via activation of ATP-sensitive K+ channels in cultured interstitial cells of Cajal from the mouse small intestine. *Naunyn Schmiedebergs Arch Pharmacol* 2007; **376**: 175-184 [PMID: 17932655 DOI: 10.1007/s00210-007-0187-1]

23 **Rumio C**, Besusso D, Arnaboldi F, Palazzo M, Selleri S, Gariboldi S, Akira S, Uematsu S, Bignami P, Ceriani V, Ménard S, Balsari A. Activation of smooth muscle and myenteric plexus cells of jejunum via Toll-like receptor 4. *J Cell Physiol* 2006; **208**: 47-54 [PMID: 16523497 DOI: 10.1002/jcp.20632]

24 **Chen CC**, Wang JK. p38 but not p44/42 mitogen-activated protein kinase is required for nitric oxide synthase induction mediated by lipopolysaccharide in RAW 264.7 macrophages. *Mol Pharmacol* 1999; **55**: 481-488 [PMID: 10051531]

25 **Huang KT**, Kuo L, Liao JC. Lipopolysaccharide activates endothelial nitric oxide synthase through protein tyrosine kinase. *Biochem Biophys Res Commun* 1998; **245**: 33-37 [PMID: 9535778 DOI: 10.1006/bbrc.1998.8384]

26 **Luo SF**, Wang CC, Chien CS, Hsiao LD, Yang CM. Induction of cyclooxygenase-2 by lipopolysaccharide in canine tracheal smooth muscle cells: involvement of p42/p44 and p38 mitogen-activated protein kinases and nuclear factor-kappaB pathways. *Cell Signal* 2003; **15**: 497-509 [PMID: 12639713 DOI: 10.1016/S0898-6568(02)00135-3]

27 **Luo SF**, Lin WN, Yang CM, Lee CW, Liao CH, Leu YL, Hsiao LD. Induction of cytosolic phospholipase A2 by lipopolysaccharide in canine tracheal smooth muscle cells: involvement of MAPKs and NF-kappaB pathways. *Cell Signal* 2006; **18**: 1201-1211 [PMID: 16278065 DOI: 10.1016/j.cellsig.2005.09.011]

28 **Gonzalo S**, Grasa L, Hernández LV, Arruebo MP, Plaza MÁ, Murillo MD. Mitogen activated protein kinases blockade improves lipopolysaccharide-induced ileal motor disturbances. *Rev Esp Enferm Dig* 2012; **104**: 305-309 [PMID: 22738700 DOI: 10.4321/S1130-01082012000600004]

29 **Torrie LJ**, MacKenzie CJ, Paul A, Plevin R. Hydrogen peroxide-mediated inhibition of lipopolysaccharide-stimulated inhibitory kappa B kinase activity in rat aortic smooth muscle cells. *Br J Pharmacol* 2001; **134**: 393-401 [PMID: 11564658 DOI: 10.1038/sj.bjp.0704259]

30 **Choi S**, Yeum CH, Kim YD, Park CG, Kim MY, Park JS, Jeong HS, Kim BJ, So I, Kim KW. Receptor tyrosine and MAP kinase are involved in effects of H(2)O(2) on interstitial cells of Cajal in murine intestine. *J Cell Mol Med* 2010; **14**: 257-266 [PMID: 20414970 DOI: 10.1111/j.1582-4934.2008.00403.x]

31 **Coffey MJ**, Coles B, O'Donnell VB. Interactions of nitric oxide-derived reactive nitrogen species with peroxidases and lipoxygenases. *Free Radic Res* 2001; **35**: 447-464 [PMID: 11767404 DOI: 10.1080/10715760100301471]

32 **Goodwin DC**, Landino LM, Marnett LJ. Effects of nitric oxide and nitric oxide-derived species on prostaglandin endoperoxide synthase and prostaglandin biosynthesis. *FASEB J* 1999; **13**: 1121-1136 [PMID: 10385604]

33 **Galea E**, Feinstein DL. Regulation of the expression of the inflammatory nitric oxide synthase (NOS2) by cyclic AMP. *FASEB J* 1999; **13**: 2125-2137 [PMID: 10593859]

34 **Tetsuka T**, Daphna-Iken D, Srivastava SK, Baier LD, DuMaine J, Morrison AR. Cross-talk between cyclooxygenase and nitric oxide pathways: prostaglandin E2 negatively modulates induction of nitric oxide synthase by interleukin 1. *Proc Natl Acad Sci USA* 1994; **91**: 12168-12172 [PMID: 7527554 DOI: 10.1073/pnas.91.25.12168]

P- Reviewers Alireza M, Chen XM
S- Editor Jiang L **L- Editor** A **E- Editor** Lu YJ



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i8.1219

World J Gastroenterol 2013 February 28; 19(8): 1219-1229
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

ORIGINAL ARTICLE

NAFLD fibrosis score: A prognostic predictor for mortality and liver complications among NAFLD patients

Sombat Treeprasertsuk, Einar Björnsson, Felicity Enders, Sompongse Suwanwalaikorn, Keith D Lindor

Sombat Treeprasertsuk, Sompongse Suwanwalaikorn, Department of Medicine, Faculty of Medicine, Chulalongkorn University, Bangkok 10330, Thailand
Einar Björnsson, Department of Internal Medicine, Section of Gastroenterology and Hepatology, the National University Hospital of Iceland, IS121 Reykjavik, Iceland
Felicity Enders, Biostatistics and Health Sciences Research, Mayo Clinic, Rochester, MN 55902, United States
Keith D Lindor, Health Solutions, Arizona State University, Tempe, AZ 85004, United States
Author contributions: Treeprasertsuk S designed and performed the experiments, analysed data, wrote the manuscript; Björnsson E critially revised the manuscript; Enders F performed statistical analysis and interpreted data; Suwanwalaikorn S edited the manuscript; Lindor KD suggested the study concept and were also involved in editing the manuscript.
Correspondence to: Sombat Treeprasertsuk, MD, Associate Professor, Department of Medicine, Faculty of Medicine, Chulalongkorn University, 4 Rama Road, Pathumwan District, Bangkok 10330, Thailand. battan5410@gmail.com
Telephone: +66-2-2564265 Fax: +66-2-2527839
Received: September 26, 2012 Revised: November 1, 2012
Accepted: November 21, 2012
Published online: February 28, 2013

Abstract

AIM: To study whether the severity of liver fibrosis estimated by the nonalcoholic fatty liver disease (NAFLD) fibrosis score can predict all-cause mortality, cardiac complications, and/or liver complications of patients with NAFLD over long-term follow-up.

METHODS: A cohort of well-characterized patients with NAFLD diagnosed during the period of 1980-2000 was identified through the Rochester Epidemiology Project. The NAFLD fibrosis score (NFS) was used to separate NAFLD patients with and without advanced liver fibrosis. We used the NFS score to classify the probability of fibrosis as < -1.5 for low probability, > -1.5 to < 0.67 for intermediate probability, and > 0.67 for high probability. Primary endpoints included all-cause death and cardiovascular- and/or liver-related mortality. From the 479 patients with NAFLD assessed, 302 patients (63%) greater than 18 years old were included. All patients were followed, and medical charts were reviewed until August 31, 2009 or the date when the first primary endpoint occurred. By using a standardized case record form, we recorded a detailed history and physical examination and the use of statins and metformin during the follow-up period.

RESULTS: A total of 302/479 (63%) NAFLD patients (mean age: 47 ± 13 year) were included with a follow-up period of 12.0 ± 3.9 year. A low probability of advanced fibrosis (NFS < -1.5 at baseline) was found in 181 patients (60%), while an intermediate or high probability of advanced fibrosis (NSF > -1.5) was found in 121 patients (40%). At the end of the follow-up period, 55 patients (18%) developed primary endpoints. A total of 39 patients (13%) died during the follow-up. The leading causes of death were non-hepatic malignancy (n = 13/39; 33.3%), coronary heart disease (CHD) (n = 8/39; 20.5%), and liver-related mortality (n = 5/39; 12.8%). Thirty patients had new-onset CHD, whereas 8 of 30 patients (27%) died from CHD-related causes during the follow-up. In a multivariate analysis, a higher NFS at baseline and the presence of new-onset CHD were significantly predictive of death (OR = 2.6 and 9.2, respectively; P < 0.0001). Our study showed a significant, graded relationship between the NFS, as classified into 3 subgroups (low, intermediate and high probability of liver fibrosis), and the occurrence of primary endpoints. The use of metformin or simvastatin for at least 3 mo during the follow-up was associated with fewer deaths in patients with NAFLD (OR = 0.2 and 0.03, respectively; P < 0.05). Additionally, the rate of annual NFS change in patients with an intermediate or high probability of advanced liver fibrosis was significantly lower than those patients with a low probability of advanced liver fibrosis (0.06 *vs* 0.09, P = 0.004). The annual NFS change in patients who died was sig-

nificantly higher than those in patients who survived (0.14 *vs* 0.07, *P* = 0.03). At the end of the follow-up, we classified the patients into 3 subgroups according to the progression pattern of liver fibrosis by comparing the NFS at baseline to the NFS at the end of the follow-up period. Most patients were in the stable-fibrosis (60%) and progressive-fibrosis (37%) groups, whereas only 3% were in the regressive fibrosis.

CONCLUSION: A higher NAFLD fibrosis score at baseline and a new onset of CHD were significantly predictive of death in patients with NAFLD.

© 2013 Baishideng. All rights reserved.

Key words: Nonalcoholic fatty liver disease fibrosis score; Prognostic predictor; Mortality; Liver complications

Treeprasertsuk S, Björnsson E, Enders F, Suwanwalaikorn S, Lindor KD. NAFLD fibrosis score: A prognostic predictor for mortality and liver complications among NAFLD patients. *World J Gastroenterol* 2013; 19(8): 1219-1229 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i8/1219.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i8.1219

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease in Western countries[1]. The prevalence of NAFLD is increasing and varies significantly with ethnicity, ranging from 24% in blacks to 33% in whites and 45% in Hispanics according to a recent study from the United States[2]. The mortality rate of NAFLD patients in the community was found to be higher than that of the general population in both the US and Sweden[3]. During an average 7.6 years of follow-up, 13% of the patients died, mainly from malignancy, coronary heart disease (CHD) and liver-related mortality[4]. Another study revealed that the survival of patients with nonalcoholic steatohepatitis (NASH) was reduced and that these patients died significantly more often from CHD and liver-related causes[3]. Patients with more advanced liver fibrosis tend to have more liver complications than those without liver fibrosis[4]. Liver biopsy is considered the gold standard for the diagnosis and assessment of fibrosis severity but has several limitations, such as sampling variability, invasiveness and expense[5]. Patients with NASH can have a significant progression of fibrosis within a few years[6-8]. Recently, a simple, noninvasive tool used for liver fibrosis assessment has been developed[9]. This new scoring system, the NAFLD fibrosis score (NFS), is a composite score of age, hyperglycemia, body mass index, platelet count, albumin, and aspartate aminotransferase and alanine aminotransferase (AST/ALT) ratio[9] and was found to independently identify NAFLD patients with and without advanced fibrosis at initial NAFLD diagnosis. Another clinical score composed of body mass index (BMI) $\geqslant$ 28 kg/m^2, AST/ALT ratio $\geqslant$ 0.8 and diabetes mellitus (the BARD score) has also been used to predict an increased chance of liver fibrosis[10]. However, the BARD score does not have the capacity to differentiate the severity of liver fibrosis among patients with a higher BMI or a higher ratio of AST/ALT, whereas the NFS takes into consideration the different ranges of BMI or AST/ALT ratios[9]. A study from Japan validated the NFS and found it to have an acceptable sensitivity, specificity, and positive and negative predictive values for advanced liver fibrosis of 100%, 83%, 63%, and 100%, respectively[11]. A longitudinal study of 103 NAFLD patients showed that the fibrosis stage progressed in 37%, remained stable in 34% and regressed in 29% of patients with a mean interval between liver biopsies of approximately 3 years[12]. The rate of fibrosis change ranged from -2.1 to 1.7 stages per year. The prognostic predictors for a higher rate of liver fibrosis were diabetes, a low initial fibrosis stage and a higher body mass index. In the current study, we aimed to determine whether the severity of liver fibrosis estimated by the NFS can predict a higher risk of overall mortality, cardiac complications, and/or liver complications among patients with NAFLD. Additionally, we aimed to determine the annual rate of the NFS change from the baseline to the end of the follow-up among NAFLD patients.

MATERIALS AND METHODS

Study subjects

In this a historical cohort study, patients residing in Olmsted County, Rochester, Minnesota, United States who had been diagnosed with NAFLD-fatty liver (HICDA Code 05710420), fatty liver hypertrophy (HICDA Code 05710421), fatty liver cirrhosis (HICDA Code 05710422), fatty liver steatohepatitis (HICDA Code 05710423) or NASH (HICDA Code 05710431), or fatty liver steatohepatitis (HICDA Code 05710-42-43) or steatosis (HICDA Code 02790-44-1) between January 1, 1980 and January 1, 2000 were drawn from the Rochester Epidemiology Project (REP) master diagnostic index. The REP index is a unique database system of medical diagnoses of the population living in Olmsted County, Minnesota[13]. Although fatty liver was recognized prior to 1980, this liver condition was better characterized in 1980[14]; therefore, we chose to identify patients after this date.

Inclusion criteria

From the 479 patients with NAFLD assessed, 302 patients (63%) greater than 18 years old were included. All of these patients were followed, and their medical charts were reviewed, until August 31, 2009 or the date when the first primary endpoint occurred. By using a standardized case record form, we recorded a detailed history and physical examination and use of statins and metformin during the follow-up period.

Exclusion criteria

We excluded NAFLD patients who lacked the data

needed for the NFS calculation, patients with pre-existing poor outcomes including overt CHD or overt liver complications at the time of NAFLD diagnosis and patients with duration of follow-up of less than 5 years. One hundred seventy-seven NAFLD patients were excluded due to missing the data needed for the NFS calculation (n = 95), overt CHD confirmed at baseline (n = 63), liver cirrhosis with complications confirmed at baseline (n = 11) and duration of follow-up of less than 5 years (n = 8).

Definitions

The diagnosis of NAFLD was based on a liver biopsy showing steatosis in at least 5% of hepatocytes or fatty infiltration of the liver confirmed by imaging study (ultrasound, computed tomography, or magnetic resonance imaging) and the exclusion of liver disease of other etiologies, including alcohol-induced liver disease (history of excessive alcohol consumption greater than 20 gm/d), drug-induced liver disease, autoimmune or viral hepatitis and cholestatic or metabolic/genetic liver disease[1]. Cirrhosis was defined based on the pathological term for the chronic liver diseases[15]. In our study, 85% of patients (n = 256) were diagnosed by liver imaging. Liver biopsy was performed in 46 patients (15% of 302 patients). The staging of fibrosis was divided into fibrosis stage 0 to stage 4 using the Brunt criteria[16]. The NAFLD patients with a histological liver fibrosis stage of 1-2 were classified as "mild liver fibrosis," and those with a histological fibrosis stage of 3-4 were classified as "advanced liver fibrosis"[16].

The primary endpoints were all-cause mortality, cardiac complications, and/or liver complications. Cardiac complications included new-onset CHD events as recorded in the medical records, defined as congestive heart failure, unstable angina, myocardial infarction, flow-limiting stenosis from angiography or angina requiring revascularization during the follow-up period and need for hospitalization[17]. Liver complications were diagnosed by clinical signs and symptoms[18], including the presence of ascites, variceal bleeding, a severe grade of hepatic encephalopathy, liver failure or hepatocellular carcinoma, that occurred during the follow-up and required hospitalization with or without death[19]. All causes of death listed on the death certificates or pathological findings (underlying, intermediate, immediate and other major conditions) were recorded using the 10th revision of the International Classification of Diseases (ICD-10). The presence of metabolic syndrome (MetS) was defined by using the 2001 National Cholesterol Education Program Adult Treatment Panel Ⅲ (NCEP-ATPⅢ) criteria and the new definition, which requires the presence of at least three of the five features[20,21]. The NFS is composed of 6 variables, including age, hyperglycemia, BMI, platelet count, albumin, and AST/ALT ratio as independent indicators of advanced liver fibrosis[9]. NAFLD fibrosis score = -1.675 + 0.037 × age (year) + 0.094 × BMI (kg/m^2) + 1.13 × IFG/diabetes (yes = 1, no = 0) + 0.99 × AST/ALT ratio - 0.013 × platelet count (×10^9/L) - 0.66 × albumin (g/dL)[9].

In this study, the NFS was used to classify advanced liver fibrosis into 2 categories[9]. NAFLD patients with a score less than -1.5 were classified as "low probability of advanced liver fibrosis," and those patients with a score of at least -1.5 were classified as "intermediate or high probability of advanced liver fibrosis"[9]. Because the information required for determination of the NFS was not always available on the same day, the scores were calculated at the time of NAFLD diagnosis using data from the medical records from visits within 3 mo of the "true" NAFLD diagnosis date and the last follow-up date. If more than one assessment for a given variable was available in the medical records during this time period, the value closest to the "true" follow-up date was used for the NFS calculation[9]. According to the 3 subgroups of low, intermediate and high probability of fibrosis, we used parameters of NFS < -1.5 for low, NFS ⩾ -1.5 to NFS < 0.67 for intermediate and NFS ⩾ 0.67 for high probability of fibrosis at baseline.

At the end of the follow-up period, we classified the patients into 3 subgroups according to the progression pattern of the liver fibrosis by comparing the NFS at baseline to the end of follow-up. The first group had stable fibrosis, defined as stability of the NFS during the follow-up. The second group had regression of fibrosis, defined as a reduction of the NFS to a milder stage of fibrosis during the follow-up. Lastly, the third group had fibrosis progression, defined as an increase of the NFS to a more advanced stage during the follow-up.

Sample-size/statistical power considerations

We assumed that the overall mortality rate in NAFLD would be 12%, with liver-related complications being observed in at least 3% and cardiac complications in 11% of patients based on results from a previous study[4]. We anticipated that at least 150 patients, or 50% of the total cohort, would be classified as "low probability of advanced liver fibrosis" using the NFS, and at least 150 would be classified as "intermediate or high probability of advanced liver fibrosis"[12]. However, a portion of patients were expected to develop new CHD events, liver complications and death. We anticipated that 15% of patients would be identified as having experienced a primary endpoint. We assumed that, at most, 10% of patients with a low probability of advanced liver fibrosis would experience events within 5 years and at least 20% of patients with an intermediate or high probability of advanced liver fibrosis would experience events within 5 years to obtain a statistical power of at least 82%.

Statistical analysis

Patients were categorized by the NFS into 2 groups of probability of advanced liver fibrosis. Differences between the primary endpoints of the two groups of low and high NFS were compared by using the χ^2 test. Differences between NAFLD patients with and without primary endpoints were tested by independent t tests for

Table 1 Demographic data of 302 patients by nonalcoholic fatty liver disease fibrosis score at baseline *n* (%) (mean ± SD)

Variable at baseline	Total (*n* = 302)	Patients with a low probability of advanced liver fibrosis (NFS < -1.5) (*n* = 181)	Patients with an intermediate or high probability of advanced liver fibrosis (NFS ⩾ -1.5) (*n* = 121)	*P* value
Age (yr)	47.3 ± 12.9	42.9 ± 11.1	53.8 ± 12.8	< 0.0001
Sex (% male)	132 (44)	92 (51)	40 (33)	0.002
Race, number (% White)	288 (95)	170 (94)	119 (97.5)	0.150
History of diabetes	48 (16)	5 (2.8)	43 (35.5)	< 0.0001
History of hypertension	125 (41)	55 (30.4)	70 (58)	< 0.0001
BMI (kg/m^2)	33.6 ± 6.2	32.0 ± 5.2	36.0 ± 6.9	< 0.0001
Presence of obesity (BMI > 30 kg/m^2)	221 (73)	121 (67)	100 (82.6)	0.002
Systolic blood pressure (mmHg)	136 ± 18	133 ± 17	139 ± 18	0.003
Diastolic blood pressure (mmHg)	83 ± 9	84 ± 8	81 ± 9	0.010
Cholesterol (mg/dL)	214 ± 48	215 ± 46	214 ± 50	0.780
Triglycerides (mg/dL)	221 ± 167	208 ± 123	242 ± 218	0.150
Glucose (mg/dL)	115 ± 41	103 ± 25	132 ± 54	< 0.0001
AST (U/L)	41.4 ± 21.9	40.8 ± 21.8	42.2 ± 28.9	0.620
ALT (U/L)	61.5 ± 43.3	69.7 ± 46	49.4 ± 35.7	< 0.0001
AST/ALT ratio	0.8 ± 0.4	0.7 ± 0.3	1.0 ± 0.6	< 0.0001
GGT (U/L)	131.9 ± 39.8	129.9 ± 32.9	134.2 ± 46.7	0.560
Platelets (× 10^9/L)	240 ± 62	259 ± 60	212 ± 53	< 0.0001
Albumin (g/dL)	4.3 ± 0.4	4.4 ± 0.3	4.1 ± 0.3	< 0.0001
Alkaline phosphatase (U/L)	196 ± 88	186 ± 68	211 ± 111	0.030
Framingham Risk Score	8.4 ± 6.2	6.9 ± 6.4	10.5 ± 5.2	< 0.0001
Calculated CHD risk (%)	16.2 ± 14.6	14.1 ± 13.8	19.3 ± 15.2	0.003
NFS	-1.7 ± 1.4	-2.6 ± 0.8	-0.4 ± 0.9	< 0.0001

NAFLD: Nonalcoholic fatty liver disease; BMI: Body mass index; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; GGT: Glutamyltransferase; CHD: Coronary heart disease; NFS: NAFLD fibrosis score.

continuous variables and were tested by the χ^2 test for proportions. Continuous outcomes were presented as the mean ± SD, and categorical data were presented as numbers (percentage). Logistic regression analysis was used to identify the factors significantly associated with death among NAFLD patients. Only those variables with a *P* value < 0.1 by univariate analysis were included in multivariate analysis. To avoid overestimation of the model, we excluded those variables used as a part of the NFS calculation. We estimated receiver operating characteristics of related variables for the predicting of death to maximize the area under the curve (AUC). Two-sided *P* values < 0.05 were considered statistically significance. The rate of NFS change in each patient was calculated by the difference of NFS at the end of follow-up and at baseline divided by the duration of the follow-up time (Δ NFS/Δ time). Both phases of the study used the SPSS statistical software package (SPSS Version 15.0.1.1, Windows VISTA, July 3, 2007) for analysis. The study was approved by the Institutional Review Board (IRB) of the Mayo Clinic, Rochester, MN, United States, and all participants provided permission for their medical information to be used for research.

RESULTS

Baseline characteristic data of 302 patients with NAFLD

This study included three hundred two NAFLD patients aged 47 ± 13 year (range 21-86 year), of which 95% were white, and 44% were male. Obesity was present in 73%, and a history of diabetes mellitus type Ⅱ and hypertension was observed in 16% and 41%, respectively. Eighty-five percent of patients (*n* = 256) were diagnosed by liver imaging. Liver biopsy was performed in 46 patients (15%). Mild liver fibrosis (stage F 0-2) was found in 34 patients (74%), while advanced fibrosis (stage F 3-4) was found in 12 patients (26%). NAFLD patients who underwent a liver biopsy had a significantly lower diastolic blood pressure, a lower BMI and a higher AST level than those without a liver biopsy (*P* < 0.05). The characteristics of the 302 patients based on the degree of advanced liver fibrosis estimated by the NFS at baseline are shown in Table 1. A low probability of advanced liver fibrosis (NFS < -1.5) was found in 60% of patients, while an intermediate or high probability of advanced liver fibrosis (NFS ⩾ -1.5) was found in 40%. The mean ± SD value of NFS in patients with a low probability of advanced liver fibrosis was -2.6 ± 0.8 and was lower than in patients with an intermediate or high probability of advanced liver fibrosis (-0.4 ± 0.9, *P* < 0.0001). Patients with a low probability of advanced liver fibrosis had a lower CHD risk at baseline, as estimated by the Framingham risk score (FRS) calculation, compared to patients with an intermediate or high probability of advanced liver fibrosis (14% *vs* 19%, *P* = 0.003).

Clinical outcomes of long-term follow-up

The mean follow-up of the total cohort was 11.9 ± 3.9 years for a total of 3594 person-years. Approximately 47% of patients with a low probability of advanced liver fibrosis at baseline progressed to an intermediate or high probability of advanced liver fibrosis at the end of follow-up, while 94% of patients with an intermediate

Table 2 Clinical parameters, laboratory features and clinical outcomes at the end of follow-up by nonalcoholic fatty liver disease fibrosis score at baseline *n* (%) (mean ± SD)

Variable at the end of follow-up	Patients with a low probability of advanced liver fibrosis (NFS < -1.5) (*n* = 181)	Patients with an intermediate or high probability of advanced liver fibrosis (NFS > -1.5) (*n* = 121)	*P* value
Clinical findings			
BMI (kg/m^2)	32.9 ± 6.6	34.9 ± 7.6	0.02
Obesity (BMI > 30 kg/m^2)	119 (65.8)	91 (75.2)	0.08
NFS	-1.4 ± 1.3	0.4 ± 1.4	< 0.0001
NFS of intermediate or high probability of advanced liver fibrosis	85 (47)	114 (94)	< 0.0001
History of diabetes	54 (29.8)	83 (68.6)	< 0.0001
Use of metformin	32 (17.7)	48 (39.7)	< 0.0001
Use of glitazones	10 (5.5)	19 (15.7)	0.003
Use of aspirin	84 (46)	83 (69)	0.0001
History of hypothyroidism	19 (10.5)	31 (25.6)	0.0005
History of cholecystectomy	27 (15)	33 (27.3)	0.008
History of obstructive sleep apnea	33 (18.2)	34 (28.1)	0.04
Laboratory findings			
AST (U/L)	38.9 ± 30.6	33.2 ± 17.8	0.04
ALT (U/L)	53.9 ± 49.7	38.9 ± 21	0.0004
AST/ALT ratio	0.8 ± 0.5	1.0 ± 0.8	0.03
Hematocrit (%)	40.4 ± 4.4	38.6 ± 5.3	0.003
Platelets (× 10^9/L)	259 ± 67	217 ± 74	< 0.0001
Albumin (g/dL)	4.1 ± 0.4	3.9 ± 0.6	< 0.0001
Cholesterol (mg/dL)	193 ± 40	178 ± 43	0.005
LDL-cholesterol (mg/dL)	109 ± 34	92 ± 30	< 0.0001
Glucose (mg/dL)	119 ± 42	131 ± 42	0.02
Clinical outcomes at the end of follow-up			
Lost to follow up	27 (15)	8 (7)	
Alive with continued follow-up	131 (72)	81 (67)	
Presence of primary endpoints	23 (13)	32 (26)	0.002
All-cause death	12 (6.6)	27 (22.3)	< 0.0001
New events of coronary heart disease	15 (8.3)	15 (12.4)	0.24
Liver complications	1 (0.6)	5 (4.1)	0.03

NAFLD: Nonalcoholic fatty liver disease; BMI: Body mass index; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; NFS: NAFLD fibrosis score; LDL: Low-density lipoprotein.

or high probability of advanced liver fibrosis remained in the same group. At the end of the follow-up, patients with an intermediate or high probability of advanced liver fibrosis had a significantly higher BMI, more frequent diabetes, and more frequent histories of hypothyroidism, cholecystectomy and obstructive sleep apnea. Furthermore, the patients with an intermediate or high probability of advanced liver fibrosis were more likely to use metformin, glitazones and aspirin compared to those with a low probability of advanced liver fibrosis ($P < 0.05$).

A history of diabetes at baseline was found in 16%, while the proportion of patients with NAFLD who developed impaired fasting glycemia or diabetes during the follow-up was 89 patients (29%) with an average interval of 6.3 ± 4.2 year (range 0.2-17.2) from the diagnosis of NAFLD. Patients who developed impaired fasting glycemia or diabetes during the follow-up were more frequent male, had less hypertension, and had a lower NAFLD Fibrosis Score at baseline compared to those with diabetes at baseline ($P < 0.05$). The primary endpoints of both groups were not significantly different.

Patients with an intermediate or high probability of advanced liver fibrosis had a significantly higher glucose and a higher AST/ALT ratio at the end of follow-up compared to those with a low probability of advanced liver fibrosis. Hematocrit, platelet count, AST, ALT, albumin, cholesterol and low-density lipoprotein (LDL)-cholesterol level were significantly lower in patients with an intermediate or high probability of advanced liver fibrosis compared to those with a low probability of advanced liver fibrosis (Table 2). At the end of follow-up, 55 patients developed primary endpoints, including death or the presence of CHD or liver complications. Thirty of these patients had new-onset CHD, whereas 8 of 30 patients (27%) died from CHD-related complications during the follow-up period (Table 2). Patients with new CHD events ($n = 30$) were significantly older and had a higher systolic blood pressure (SBP), FRS at baseline, calculated % CHD risk at baseline, and NFS at the end of follow-up and exhibited a lower ALT than those patients without new CHD events ($n = 272$) ($P < 0.05$). In addition, the NFS at baseline was similar in patients with and without new CHD events (-1.2 ± 1.6 *vs* -1.8 ± 1.4, $P = 0.07$). Liver complications occurred in 6 patients, 5 of whom (83%) died during the follow-up. The liver complications included massive ascites requiring abdomi-

Table 3 Causes of mortality in 39 patients with nonalcoholic fatty liver disease

Causes of death	All causes mortality *n* (% of death)	All causes mortality (% of 302 patients)
Non-liver cancer	13 (33.3)	4.3%
Coronary heart disease	8 (20.5)	2.6%
Liver-related mortality (including hepatocellular carcinoma)	5 (12.8)	1.7%
Infection (including sepsis)	4 (10.3)	1.3%
Stroke	3 (7.7)	1.0%
Cardiac arrhythmia	2 (5.1)	0.7%
COPD and/or respiratory failure	2 (5.1)	0.7%
Other causes of death (GI bleeding, renal failure)	2 (5.1)	0.7%
Total	39 (100)	12.9%

COPD: Chronic obstructive lung disease; GI: Gastrointestinal.

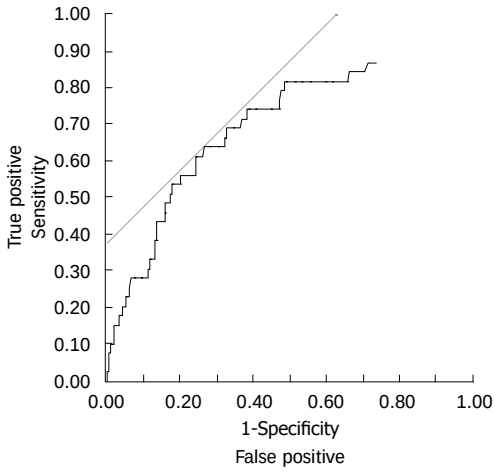


Figure 1 Presence of death estimated by the nonalcoholic fatty liver disease fibrosis score at baseline. Using the receiver operating characteristics curve, the nonalcoholic fatty liver disease fibrosis score at baseline of -0.9 was the best cutoff value for predicting death based on a sensitivity of 62%, specificity of 76%, positive predictive value of 28%, negative predictive value of 93% and area under the curve of 0.7.

nal paracentesis (n = 3), hepatopulmonary syndrome and hepatocellular carcinoma (n = 1 each).

A total of 39/302 (13%) patients died during the follow-up period. The leading causes of death were non-hepatic malignancy (n = 13/39; 33.3%), CHD (n = 8/39; 20.5%), and liver related mortality (n = 5/39; 12.8%). The other 13 patients (33.3%) died from various causes (Table 3). The primary types of cancers were gastric cancer (n = 2), colon cancer (n = 2), pancreatic cancer (n = 2), breast cancer (n = 2), leiomyosarcoma of uterus (n = 1), diffuse B cell lymphoma (n = 1), endometrial cancer (n = 1), lung cancer (n = 1) and unknown primary cancer with liver metastasis (n = 1). Additionally, our study showed a significant, graded relationship between the NAFLD fibrosis score, classified into 3 subgroups (low, intermediate and high probability of liver fibrosis), and the occurrence of primary endpoints, as shown in Table 4.

Predicting mortality

Patients who died (n = 39) were significantly older with more frequent diabetes and had a higher SBP, NFS at baseline, FRS, glucose, and a lower diastolic blood pressure, ALT and albumin (Table 5). Moreover, they had greater NFS changes per year than those who survived (n = 263, P <0.05). Three models of multivariate analysis were used to identify the best fit model for predictors of death and are illustrated in Table 6. In model 1, we added 9 variables, including gender, systolic blood pressure, diastolic blood pressure, NFS at baseline, use of metformin, use of simvastatin, use of aspirin, presence of new-onset CHD and new-onset liver complications, without interaction among these variables. Model 2 included 10 variables, gender, systolic blood pressure, diastolic blood pressure, NFS at baseline, NFS changes per year, use of metformin, use of simvastatin, use of aspirin, presence of new-onset of CHD and new-onset of liver complications without interaction among these variables. Finally, model 3 added the interaction between NFS at baseline and NAFLD NFS changes per year with the use of aspirin, metformin, and simvastatin into model 2. We did not add the FRS into these models due to the repetition of several variables in the NFS and the FRS. Model 3 was the best fit model, which found that a higher NFS at baseline and more frequent new-onset CHD were significantly predictive of death (OR = 2.6 and 9.2, respectively; P < 0.0001). The use of metformin or simvastatin for at least 3 mo during the follow-up were associated with fewer deaths in patients with NAFLD (OR = 0.2 and 0.03, respectively; P < 0.05).

Table 7 showed the results of the comparison of NAFLD patients with and without death after excluding those with established type 2 diabetes at baseline (n = 254). After excluding patients with established type 2 diabetes at baseline, we found that a higher NFS at baseline and higher alkaline phosphatase remained significantly predictive of death (OR = 1.9 and 1.006; P <0.0001 and 0.012, respectively) as shown in Table 8. Additionally, Table 9 showed that non-diabetic patients with intermediate or high probability of liver fibrosis had a significantly higher rate of primary end point and all-cause death than those patients with low probability of liver fibrosis.

Using the ROC curves to predict death, we found that a baseline NFS of -0.9 was the best cutoff value with a sensitivity of 62%, specificity of 76%, positive predictive value of 28%, negative predictive value of 93% and AUC of 0.7 (Figure 1).

The median rate of annual NFS change for all patients was 0.1 (IQR of 0.02, 0.13). The rate of annual NFS change in patients with an intermediate or high probability of advanced liver fibrosis was significantly lower than in patients with a low probability of advanced liver fibrosis (0.06 *vs* 0.09, P = 0.004). The annual NFS change in patients who died was significantly higher than those in patients who survived (0.14 *vs* 0.07, P = 0.03). At the end of the follow-up, we classified the patients into 3 subgroups according to the progression pattern of the

Table 4 Association between the primary endpoint and the grading of the nonalcoholic fatty liver disease fibrosis score, classified into 3 subgroups (*n* = 302) *n* (%)

Grading of the NAFLD fibrosis score (*n* = 302)	Low prob. of advanced liver fibrosis (*n* = 181)	Intermediate prob. of advanced liver fibrosis (*n* = 108)	High prob. of advanced liver fibrosis (*n* = 13)	*P* value
Presence of primary endpoint (*n* = 55, 18.2%)	23/181 (12.7)	24/108 (22.2)	8/13 (61.5)	< 0.0001
All-cause death (*n* = 39, 12.9%)	12/181 (6.6)	21/108 (19.4)	6/13 (46.2)	< 0.0001

NAFLD: Nonalcoholic fatty liver disease.

Table 5 Comparison of nonalcoholic fatty liver disease patients alive *vs* deceased *n* (%) (mean ± SD)

Variables	NAFLD patients alive (*n* = 263)	NAFLD patients deceased (*n* = 39)	*P* value
At baseline			
Age (yr)	45.2 ± 11.5	61.1 ± 13.8	< 0.0001
Sex (% male)	120 (45.6)	12 (30.8)	0.08
History of diabetes	37 (14.1)	11 (28.2)	0.02
Systolic blood pressure (mmHg)	134 ± 17	143 ± 21	0.02
Diastolic blood pressure (mmHg)	83 ± 8	79 ± 10	0.03
Glucose (mg/dL)	112 ± 38.6	132.7 ± 54.3	0.03
AST (U/L)	42.2 ± 25.5	35.5 ± 20.0	0.06
ALT (U/L)	64.2 ± 44.6	43.6 ± 27.4	0.000
AST/ALT ratio	0.8 ± 0.4	1.0 ± 0.7	0.06
Albumin (g/dL)	4.3 ± 0.3	4.0 ± 0.4	< 0.0001
FRS	7.9 ± 6.2	11.4 ± 5.2	0.000
Calculated CHD risk (%)	15.3 ± 14.0	22.2 ± 17.1	0.02
NFS	-1.9 ± 1.3	-0.8 ± 1.7	0.0004
NFS of intermediate or high probability of advanced liver fibrosis (%)	94 (35.7)	27 (69.2)	< 0.0001
Presence of histologically advanced liver fibrosis	7/35 (20.0)	5/11 (45.5)	0.09
During the follow-up period	77 (29.3)	3 (7.7)	0.004
Use of metformin			
Use of aspirin	151 (57.4)	16 (41.0)	0.05
Use of simvastatin	107 (40.7)	3 (7.9)	< 0.0001
New events of coronary heart disease	16 (6.1)	14 (35.9)	< 0.0001
Liver complications	1 (0.4)	5 (12.8)	< 0.0001
At the end of follow-up			
BMI (kg/m^2)	33.9 ± 6.9	31.8 ± 8.2	0.1
Hematocrit (%)	40.4 ± 4.1	34.5 ± 6.3	< 0.0001
Glucose (mg/dL)	122.0 ± 38.6	139.0 ± 62.3	0.12
AST/ALT ratio	0.9 ± 0.5	1.3 ± 1.0	0.01
Albumin (g/dL)	4.1 ± 0.3	3.3 ± 0.7	< 0.0001
Creatinine (mg/dL)	1.0 ± 0.5	1.7 ± 1.3	0.004
NFS	-0.9 ± 1.4	0.7 ± 2.3	< 0.0001
NFS change per year (Median; IQR)	0.07 (0.02, 0.12)	0.14 (0.01, 0.31)	0.03
NFS of intermediate to high probability of advanced liver fibrosis (%)	168 (63.9)	31 (79.5)	0.05

To avoid overestimation of the model, we excluded those variables used as a part of NAFLD fibrosis score calculation (age, history of diabetes, aspartate aminotransferase/alanine aminotransferase ratio, platelet count, albumin, body mass index and Framingham risk score). NAFLD: Nonalcoholic fatty liver disease; BMI: Body mass index; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; FRS: Framingham risk score; CHD: Coronary heart disease; NFS: NAFLD fibrosis score.

liver fibrosis by comparing the NFS at baseline to that at the end of follow-up. Most patients were in the stable-fibrosis (60%) and progressive-fibrosis (37%) groups, whereas only 3% were in the regressive-fibrosis group. The annual NFS change in the progressive-fibrosis group and the stable-disease group was 0.20 ± 0.02 and 0.05 ± 0.08, respectively (*P* = not significant).

DISCUSSION

In the current study, we found that a higher NFS at baseline and the presence of new-onset CHD were significantly predictive of death. The use of noninvasive methods to predict poor clinical outcomes in NAFLD patients during follow-up is urgently needed. Studies of noninvasive markers used to identify steatohepatitis patients from NAFLD patients require validation before being widely used, as previously suggested[22]. Although liver biopsy is the currently recommended practice for identifying liver fibrosis, in NAFLD patients with risk factors that include older age, diabetes, severe obesity and metabolic syndrome, serial liver biopsies are invasive and are not applicable in clinical practice. Recently, Rafiq *et al*[22] showed that at least 3 risk factors, including type

Table 6 Multivariate logistic regression model showing OR (95%CI) for predictors for death in 302 patients with non-alcoholic fatty liver disease

Multivariate analysis	*P* value	OR	95%CI
Model 1			
Presence of new-onset CHD	< 0.0001	9.0	2.9-28.4
NFS at baseline	< 0.0001	1.9	1.4-2.6
Use of metformin	0.02	0.2	0.04-0.8
Use of simvastatin	0.001	0.05	0.01-0.3
Model 2			
NFS changes per year	0.04	14.9	1.1-206.4
Presence of new onset of CHD	0.001	8.0	2.4-26.1
NFS at baseline	< 0.0001	2.1	1.5-2.9
Use of metformin	0.02	0.2	0.04-0.7
Use of simvastatin	0.001	0.06	0.01-0.3
Model 3			
Presence of new onset of CHD	< 0.0001	9.2	2.6-32.2
NFS at baseline	< 0.0001	2.6	1.7-3.9
Use of metformin	0.03	0.2	0.04-0.8
Use of simvastatin	0.001	0.03	0.003-0.2
Interaction between NFS at baseline and NFS change per year	0.004	0.06	0.008-0.4
NFS changes per year	0.6	2.2	0.07-67.8

Model 1 without interaction among 9 included variables: gender, systolic blood pressure, diastolic blood pressure, nonalcoholic fatty liver disease (NAFLD) fibrosis score at baseline, use of metformin, use of simvastatin, use of aspirin, presence of new-onset of coronary heart disease (CHD) and new onset of liver complications; Model 2 without interaction among 10 included variables: gender, systolic blood pressure, diastolic blood pressure, NAFLD fibrosis score (NFS) at baseline, NFS changes per year, use of metformin, use of simvastatin, use of aspirin, presence of new-onset coronary heart disease (CHD) and new onset of liver complications; Model 3 added variables of interaction between NFS at baseline and NFS changes per year, interaction among use of aspirin, metformin, aspirin and simvastatin into model 2.

2 diabetes, older age and low albumin level, were predictors of mortality and liver-related mortality. Most of these risk factors are components of the NFS[9]. Thus, a benefit of the current study is an extension of the clinical use of the NFS system for predicting death in patients with NAFLD.

Our results show that the annual NFS change in patients who died was two times higher than in survivors during the follow-up. Thus, it would be valuable to calculate NFS in newly diagnosed patients.

The annual rate of NFS change in the progressive-fibrosis group was approximately 4 times higher than that in the stable-disease group. Therefore, the median value of the annual rate of NFS change might be used as a surrogate marker for progression of liver fibrosis, but this measure requires further study to validate its benefit.

The long-term outcomes of patients with NAFLD are not uniform across the spectrum of the disease[3,22,23]. Poor outcomes are more frequent in patients with NASH, which was confirmed by our results, which showed patients with an intermediate or high probability of advanced liver fibrosis had an increased frequency of primary endpoints, all-cause deaths, and liver complications compared to those with a low probability of advanced liver fibrosis.

Several previous studies have demonstrated a higher mortality rate (30%-45%) than observed in the current study (13%). This difference may be at least partly explained by a difference in patient selection. For instance, the prevalence of diabetes or impaired glucose tolerance, a well-known risk factor for increased mortality, was three times higher in a Swedish study than in the current study (53% *vs* 16%)[3]. In addition, previous studies did not exclude patients with known CHD or known liver complications at baseline[3,22,23].

The use of metformin or simvastatin was found to be a protective factor against death in the present study. This finding is in line with a study by Ekstedt *et al*[24], who showed a significant reduction in liver steatosis in NAFLD patients on statins *vs* those not on statins. A recent study found diabetes mellitus to be one of the important predictors for developing moderate to severe liver fibrosis[25], and treatment with metformin improved liver histology and ALT levels in one-third of patients with NASH[26]. Two other studies suggested that metformin improved only the insulin sensitivity but did not improve liver histology in NASH patients[26,27]. Limited data exist concerning the efficacy of metformin in patients with NAFLD, and the result of the current study does not necessarily imply causality. Our results show that the use of simvastatin seems to improve the prognosis in patients with NAFLD. This improvement may relate to the effect on prevention of new-onset CHD, which accounted for 20% of deaths in our study. Recent data showed that the statins are safe and well-tolerated in patients with NAFLD[28-32]. Moreover, the use of statins was associated with a reduction of hepatic steatosis in NAFLD patients[24,32,33]. No study has assessed the efficacy of statins to reduce CHD mortality in NAFLD patients, although the benefits are well-recognized for both the primary and secondary prevention of CHD and the reduction of overall mortality in the general population[34,35]. Thus, the benefit of statins for CHD prevention in NAFLD patients with dyslipidemia and/or a high calculated risk of coronary heart disease by the FRS should be considered[36,37].

The main strengths of our study are the inclusion of NAFLD patients from the community along with the long-term follow-up. All patients had complete data for calculation of the NFS at the time of NAFLD diagnosis and at the end of the follow-up. The exclusion of known CHD or liver cirrhosis with complications at baseline is important to reduce the overestimation of the incidence of primary endpoints or mortality rate during the follow-up period.

Our study has several limitations. First, only 6.6% of the patients with a low probability of advanced liver fibrosis died, which was less than expected (10%) by a sample size calculation and may affect the power of the study. Second, most of our patients in Olmsted County are white, and recent data showed that non-Caucasian

Table 7 Comparison of nonalcoholic fatty liver disease patients with and without death after excluding those with established type 2 diabetes at baseline (n = 254) n (%) (mean ± SD)

Variables	NAFLD patients without death (n = 226)	NAFLD patients with death (n = 28)	P value
Age (yr)	44.5 ± 11.5	59.2 ± 14.3	< 0.0001
Sex (% male)	113 (50)	10 (35.7)	0.11
History of hypercholesterolemia	56 (24.8)	9 (32.1)	< 0.0001
Obesity	164 (72.6)	22 (78.6)	0.34
BMI (kg/m^2)	33.5 ± 6.2	35.0 ± 6.6	0.25
Platelet count (× 10^9/mm^3)	241 ± 58	242 ± 87	0.98
Glucose (mg/dL)	215.7 ± 46.6	215.7 ± 46.6	
Cholesterol (mg/dL)	215 ± 48	219 ± 56	0.72
HDL-cholesterol (mg/dL)	42 ± 12	43 ± 20	0.76
AST (U/L)	42.0 ± 23.0	39.7 ± 21.0	0.62
ALT (U/L)	65.7 ± 45.0	50 ± 29	0.02
AST/ALT ratio	0.76 ± 0.38	0.93 ± 0.57	0.14
ALP(U/L)	184 ± 66	246 ± 179	0.09
GGT (U/L)	132 ± 41	142 ± 36	0.32
Albumin (g/dL)	4.3 ± 0.3	4.2 ± 0.4	< 0.0001
FRS	7.6 ± 6.2	10.5 ± 5.6	0.02
Calculated CHD risk (%)	15.3 ± 14.4	22.0 ± 18.8	0.076
NFS	-2.11 ± 1.15	-1.09 ± 1.61	0.003
NFS of intermediate or high probability of advanced liver fibrosis (%)	59 (26.1)	19 (67.9)	< 0.0001
B. During the follow-up periods			
Use of metformin	52 (23.0)	1 (3.6)	0.009
Use of aspirin	119 (52.7)	9 (32.1)	0.03
Use of simvastatin	90 (39.8)	3 (10.7)	< 0.0001
NFS change per year (median; IQR)	0.08 ± 0.08	0.12 ± 0.36	0.61

In order to avoid overestimation of the model, we excluded those variables used as a part of nonalcoholic fatty liver disease fibrosis score calculation (age, history of diabetes, aspartate aminotransferase/alanine aminotransferase ratio, platelet counts, albumin, body mass index and Framingham risk score). NAFLD: Nonalcoholic fatty liver disease; BMI: Body mass index; ALT: Alanine aminotransferase; AST: Aspartate aminotransferase; FRS: Framingham risk score; CHD: Coronary heart disease; NFS: NAFLD fibrosis score; ALP: Alkaline phosphatase; GGT: Glutamyltransferase; HDL: High-density lipoprotein.

Table 8 Multivariate logistic regression model showing OR (95%CI) of predictors for death in 254 patients with nonalcoholic fatty liver disease after excluding those with established type 2 diabetes at baseline

Multivariate analysis	P value	OR	95%CI
NAFLD fibrosis score at baseline	< 0.0001	1.92	1.4-2.7
Alkaline phosphatase	0.012	1.006	1.001-1.010

Model without interaction among 8 included variables; gender, nonalcoholic fatty liver disease fibrosis score at baseline, use of metformin, use of simvastatin, use of aspirin, history of hypercholesterolemia, alanine aminotransferase and alkaline phosphatase. NAFLD: Nonalcoholic fatty liver disease.

race was an important predictor of decreased survival[22]. With the relatively small sample size and the represented local population in the United States, our results are not necessarily applicable in other ethnic groups.

The current study is important because we extended the clinical use of the NFS system for predicting death or liver complications in NAFLD patients. A higher NFS at baseline and the presence of new-onset CHD can be used as prognostic predictors for mortality and liver complications among NAFLD patients. Further research is needed to validate the benefit of the NFS for predicting death or liver complications in NAFLD patients with other ethnic groups. The NFS is simpler and less invasive than liver biopsy for the initial evaluation of the degree of liver fibrosis in patients with NAFLD. The NFS should be calculated for all patients with NAFLD at initial consultation to estimate the probability of advanced liver fibrosis.

COMMENTS

Background

Nonalcoholic fatty liver disease (NAFLD) is one of the most common causes of chronic liver disease in Western countries. During an average 7.6 years of follow-up, 13% of the patients died, mainly from malignancy, coronary heart disease (CHD) and liver-related mortality.

Research frontiers

Another study revealed that the survival of patients with nonalcoholic steatohepatitis was reduced and that these patients died significantly more often from CHD and liver-related causes. Patients with more advanced liver fibrosis tend to have more liver complications than those without liver fibrosis.

Innovations and breakthroughs

A study from Japan validated the NAFLD Fibrosis Score and found it to have an acceptable sensitivity, specificity, and positive and negative predictive values for advanced liver fibrosis of 100%, 83%, 63%, and 100%, respectively.

Peer review

In this manuscript, the authors intend to evaluate whether the NAFLD fibrosis score could act as a prognostic predictor for mortality and liver complications among NAFLD patients. It is suggested that the NAFLD fibrosis score is a simple, non-invasive method for predicting prognosis in patients with NAFLD. In general, this paper is significant and of clinical importance.

Table 9 The association between the primary end point and the grading of the nonalcoholic fatty liver disease fibrosis score, classified into 2 subgroups, after excluding those with established type 2 diabetes at baseline *n* (%)

Grading of the NAFLD fibrosis score (*n* = 254)	Low prob. of advanced liver fibrosis (*n* = 176)	Intermediate prob. or High prob. of advanced liver fibrosis (*n* = 78)	*P* value
Presence of primary end point (*n* = 43; 17%)	20/176 (11.4)	23/78 (29.5)	< 0.0001
All-cause death (*n* = 28; 11%)	9/176 (5.1)	19/78 (24.4)	< 0.0001

NAFLD: Nonalcoholic fatty liver disease.

REFERENCES

1 **Adams LA**, Lindor KD. Nonalcoholic fatty liver disease. *Ann Epidemiol* 2007; **17**: 863-869 [PMID: 17728149 DOI: 10.1016/j.annepidem.2007.05.013]
2 **Browning JD**, Szczepaniak LS, Dobbins R, Nuremberg P, Horton JD, Cohen JC, Grundy SM, Hobbs HH. Prevalence of hepatic steatosis in an urban population in the United States: impact of ethnicity. *Hepatology* 2004; **40**: 1387-1395 [PMID: 15565570 DOI: 10.1002/hep.20466]
3 **Ekstedt M**, Franzén LE, Mathiesen UL, Thorelius L, Holmqvist M, Bodemar G, Kechagias S. Long-term follow-up of patients with NAFLD and elevated liver enzymes. *Hepatology* 2006; **44**: 865-873 [PMID: 17006923 DOI: 10.1002/hep.21327]
4 **Adams LA**, Lymp JF, St Sauver J, Sanderson SO, Lindor KD, Feldstein A, Angulo P. The natural history of nonalcoholic fatty liver disease: a population-based cohort study. *Gastroenterology* 2005; **129**: 113-121 [PMID: 16012941 DOI: 10.1053/j.gastro.2005.04.014]
5 **Oh MK**, Winn J, Poordad F. Review article: diagnosis and treatment of non-alcoholic fatty liver disease. *Aliment Pharmacol Ther* 2008; **28**: 503-522 [PMID: 18532991 DOI: 10.1111/j.1365-2036.2008.03752.x]
6 **Powell EE**, Cooksley WG, Hanson R, Searle J, Halliday JW, Powell LW. The natural history of nonalcoholic steatohepatitis: a follow-up study of forty-two patients for up to 21 years. *Hepatology* 1990; **11**: 74-80 [PMID: 2295475 DOI: 10.1002/hep.1840110114]
7 **Harrison SA**, Torgerson S, Hayashi PH. The natural history of nonalcoholic fatty liver disease: a clinical histopathological study. *Am J Gastroenterol* 2003; **98**: 2042-2047 [PMID: 14499785 DOI: 10.1111/j.1572-0241.2003.07659.x]
8 **Fassio E**, Alvarez E, Domínguez N, Landeira G, Longo C. Natural history of nonalcoholic steatohepatitis: a longitudinal study of repeat liver biopsies. *Hepatology* 2004; **40**: 820-826 [PMID: 15382171]
9 **Angulo P**, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, Enders F, Saksena S, Burt AD, Bida JP, Lindor K, Sanderson SO, Lenzi M, Adams LA, Kench J, Therneau TM, Day CP. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. *Hepatology* 2007; **45**: 846-854 [PMID: 17393509 DOI: 10.1002/hep.21496]
10 **Harrison SA**, Oliver D, Arnold HL, Gogia S, Neuschwander-Tetri BA. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. *Gut* 2008; **57**: 1441-1447 [PMID: 18390575 DOI: 10.1136/gut.2007.146019]
11 **Nakano M**, Murohisa T, Imai Y, Hiraishi H. [Validity of the NAFLD fibrosis score in a Japanese population]. *Nihon Shokakibyo Gakkai Zasshi* 2012; **109**: 751-759 [PMID: 22688100]
12 **Adams LA**, Sanderson S, Lindor KD, Angulo P. The histological course of nonalcoholic fatty liver disease: a longitudinal study of 103 patients with sequential liver biopsies. *J Hepatol* 2005; **42**: 132-138 [PMID: 15629518 DOI: 10.1016/j.jhep.2004.09.012]
13 **Melton LJ**. History of the Rochester Epidemiology Project. *Mayo Clin Proc* 1996; **71**: 266-274 [PMID: 8594285]
14 **Ludwig J**, Viggiano TR, McGill DB, Oh BJ. Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. *Mayo Clin Proc* 1980; **55**: 434-438 [PMID: 7382552]
15 **Ginés P**, Quintero E, Arroyo V, Terés J, Bruguera M, Rimola A, Caballería J, Rodés J, Rozman C. Compensated cirrhosis: natural history and prognostic factors. *Hepatology* 1987; **7**: 122-128 [PMID: 3804191 DOI: 10.1002/hep.1840070124]
16 **Brunt EM**. Pathology of nonalcoholic steatohepatitis. *Hepatol Res* 2005; **33**: 68-71 [PMID: 16214395 DOI: 10.1016/j.hepres.2005.09.006]
17 **Wilson PW**, D'Agostino RB, Levy D, Belanger AM, Silbershatz H, Kannel WB. Prediction of coronary heart disease using risk factor categories. *Circulation* 1998; **97**: 1837-1847 [PMID: 9603539 DOI: 10.1161/01.CIR.97.18.1837]
18 **European Association for the Study of the Liver.** EASL clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis. *J Hepatol* 2010; **53**: 397-417 [PMID: 20633946 DOI: 10.1016/j.jhep.2010.05.004]
19 **Lebrec D**, Vinel JP, Dupas JL. Complications of portal hypertension in adults: a French consensus. *Eur J Gastroenterol Hepatol* 2005; **17**: 403-410 [PMID: 15756092 DOI: 10.1097/00042737-200504000-00003]
20 **National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III).** Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III) final report. *Circulation* 2002; **106**: 3143-3421 [PMID: 12485966]
21 **Alberti KG**, Zimmet P, Shaw J. The metabolic syndrome--a new worldwide definition. *Lancet* 2005; **366**: 1059-1062 [PMID: 16182882 DOI: 10.1016/S0140-6736(05)67402-8]
22 **Rafiq N**, Bai C, Fang Y, Srishord M, McCullough A, Gramlich T, Younossi ZM. Long-term follow-up of patients with nonalcoholic fatty liver. *Clin Gastroenterol Hepatol* 2009; **7**: 234-238 [PMID: 19049831 DOI: 10.1016/j.cgh.2008.11.005]
23 **Matteoni CA**, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 1999; **116**: 1413-1419 [PMID: 10348825 DOI: 10.1016/S0016-5085(99)70506-8]
24 **Ekstedt M**, Franzén LE, Mathiesen UL, Holmqvist M, Bodemar G, Kechagias S. Statins in non-alcoholic fatty liver disease and chronically elevated liver enzymes: a histopathological follow-up study. *J Hepatol* 2007; **47**: 135-141 [PMID: 17400325 DOI: 10.1016/j.jhep.2007.02.013]
25 **Hossain N**, Afendy A, Stepanova M, Nader F, Srishord M, Rafiq N, Goodman Z, Younossi Z. Independent predictors of fibrosis in patients with nonalcoholic fatty liver disease. *Clin Gastroenterol Hepatol* 2009; **7**: 1224-1229, 1224-1229, [PMID: 19559819 DOI: 10.1111/j.1365-2036.2004.01888.x]
26 **Uygun A**, Kadayifci A, Isik AT, Ozgurtas T, Deveci S, Tuzun A, Yesilova Z, Gulsen M, Dagalp K. Metformin in the treatment of patients with non-alcoholic steatohepatitis. *Ali-*

ment Pharmacol Ther 2004; **19**: 537-544 [PMID: 14987322]
27 **Angelico F**, Burattin M, Alessandri C, Del Ben M, Lirussi F. Drugs improving insulin resistance for non-alcoholic fatty liver disease and/or non-alcoholic steatohepatitis. *Cochrane Database Syst Rev* 2007; (1): CD005166 [PMID: 17253544]
28 **Chalasani N**, Aljadhey H, Kesterson J, Murray MD, Hall SD. Patients with elevated liver enzymes are not at higher risk for statin hepatotoxicity. *Gastroenterology* 2004; **126**: 1287-1292 [PMID: 15131789 DOI: 10.1053/j.gastro.2004.02.015]
29 **Chalasani N**. Statins and hepatotoxicity: focus on patients with fatty liver. *Hepatology* 2005; **41**: 690-695 [PMID: 15789367 DOI: 10.1002/hep.20671]
30 **Tandra S**, Vuppalanchi R. Use of statins in patients with liver disease. *Curr Treat Options Cardiovasc Med* 2009; **11**: 272-278 [PMID: 19627660]
31 **Nakahara T**, Hyogo H, Kimura Y, Ishitobi T, Arihiro K, Aikata H, Takahashi S, Chayama K. Efficacy of rosuvastatin for the treatment of non-alcoholic steatohepatitis with dyslipidemia: An open-label, pilot study. *Hepatol Res* 2012; **42**: 1065-1072 [PMID: 22583925 DOI: 10.1111/j.1872-034X.2012.01034.x]
32 **Nseir W**, Mograbi J, Ghali M. Lipid-lowering agents in nonalcoholic fatty liver disease and steatohepatitis: human studies. *Dig Dis Sci* 2012; **57**: 1773-1781 [PMID: 22419057 DOI: 10.1007/s10620-012-2118-3]
33 **Ajamieh H**, Farrell G, Wong HJ, Yu J, Chu E, Chen J, Teoh N. Atorvastatin protects obese mice against hepatic ischemia-reperfusion injury by Toll-like receptor-4 suppression and endothelial nitric oxide synthase activation. *J Gastroenterol Hepatol* 2012; **27**: 1353-1361 [PMID: 22432744 DOI: 10.1111/j.1440-1746.2012.07123.]
34 **Gould AL**, Rossouw JE, Santanello NC, Heyse JF, Furberg CD. Cholesterol reduction yields clinical benefit: impact of statin trials. *Circulation* 1998; **97**: 946-952 [PMID: 9529261]
35 **Hebert PR**, Gaziano JM, Chan KS, Hennekens CH. Cholesterol lowering with statin drugs, risk of stroke, and total mortality. An overview of randomized trials. *JAMA* 1997; **278**: 313-321 [PMID: 9228438]
36 **Treeprasertsuk S**, Lopez-Jimenez F, Lindor KD. Nonalcoholic fatty liver disease and the coronary artery disease. *Dig Dis Sci* 2011; **56**: 35-45 [PMID: 20464495 DOI: 10.1007/s10620-010-1241-2]
37 **Treeprasertsuk S**, Leverage S, Adams LA, Lindor KD, St Sauver J, Angulo P. The Framingham risk score and heart disease in nonalcoholic fatty liver disease. *Liver Int* 2012; **32**: 945-950 [PMID: 22299674 DOI: 10.1111/j.1478-3231.2011.02753.x]

P- Reviewers Targher G, Hsieh PS
S- Editor Zhai HH **L- Editor** A **E- Editor** Lu YJ

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i8.1230

World J Gastroenterol 2013 February 28; 19(8): 1230-1238
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

ORIGINAL ARTICLE

Schistosoma japonicum egg antigen up-regulates fibrogenesis and inhibits proliferation in primary hepatic stellate cells in a concentration-dependent manner

Ping Liu, Mi Wang, Xiao-Dan Lu, Shu-Juan Zhang, Wang-Xian Tang

Ping Liu, Mi Wang, Xiao-Dan Lu, Shu-Juan Zhang, Wang-Xian Tang, Institute of Liver Diseases, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, Hubei Province, China
Author contributions: Liu P performed the majority of the experiments; Wang M, Lu XD and Zhang SJ coordinated the study and provided analytical tools; Liu P and Wang M designed the experiments; Liu P and Tang WX wrote the manuscript.
Supported by Natural Science Foundation of China, No. 81071381
Correspondence to: Wang-Xian Tang, Professor, Institute of Liver Diseases, Tongji Hospital, Tongji Medical College, Huazhong University of Science and Technology, 1095 Jie-Fang Avenue, Wuhan 430030, Hubei Province, China. tangwx@tjh.tjmu.edu.cn
Telephone: +86-27-83662873 Fax: +86-27-83662640
Received: September 14, 2012 Revised: December 27, 2012
Accepted: January 23, 2013
Published online: February 28, 2013

Abstract

AIM: To investigate the effects of different concentrations of *Schistosoma japonicum* (*S. japonicum*) egg antigen on fibrogenesis and apoptosis in primary hepatic stellate cells (HSCs).

METHODS: A mouse model of schistosomiasis-associated liver fibrosis (SSLF) was established by infecting mice with schistosomal cercaria *via* the abdomen. HSCs were isolated from SSLF mice by discontinuous density gradient centrifugation, and their identity was confirmed by immunofluorescence double staining of α-smooth muscle actin (α-SMA) and desmin. The growth inhibitory effect and 50% inhibitory concentration (IC50) of *S. japonicum* egg antigen for primary HSCs (24 h) were determined using a cell counting kit-8 (CCK-8) assay. The expression levels of α-SMA, matrix metalloproteinase-9 (MMOL/LP-9) and tissue inhibitor of metalloproteinases-1 (TIMP-1) in HSCs in response to different concentrations of *S. japonicum* egg antigen were detected by Western blotting and real-time reverse transcription-polymerase chain reaction. The levels of phospho-P38 (P-P38), phospho-Jun N-terminal kinase (P-JNK) and phospho-Akt (P-AKT) in HSCs were detected by Western blotting.

RESULTS: An SSLF mouse model was established, and primary HSCs were successfully isolated and cultured. *S. japonicum* egg antigen inhibited HSC proliferation in a concentration-dependent manner. The IC50 of the *S. japonicum* egg antigen was 244.53 ± 35.26 μg/mL. *S. japonicum* egg antigen enhanced α-SMA expression at both the mRNA and protein levels and enhanced TIMP-1 expression at the mRNA level in HSCs ($P < 0.05$), whereas the expression of MMOL/LP-9 was attenuated at both the mRNA and protein levels in a concentration-dependent manner ($P < 0.05$). A high concentration of *S. japonicum* egg antigen enhanced P-P38, P-JNK and P-AKT activation ($P < 0.05$). The changes in α-SMA and MMOL/LP-9 expression induced by *S. japonicum* egg antigen were closely correlated with P-P38 and P-JNK activation ($P < 0.05$). The attenuation of MMOL/LP-9 was also correlated with P-AKT activation ($P < 0.05$), but the increase in α-SMA expression was not. TIMP-1 expression was not correlated with P-P38, P-JNK or P-AKT activation.

CONCLUSION: *S. japonicum* egg antigen promotes fibrogenesis, activates the P38/JNK mitogen-activated protein kinase and AKT/PI3K signaling pathways and inhibits proliferation in primary HSCs isolated from SSLF mice in a concentration-dependent manner.

© 2013 Baishideng. All rights reserved.

Key words: Schistosomiasis; Liver fibrosis; Hepatic stellate cells; Mitogen-activated protein kinase; Akt

Liu P, Wang M, Lu XD, Zhang SJ, Tang WX. *Schistosoma japonicum* egg antigen up-regulates fibrogenesis and inhibits proliferation in primary hepatic stellate cells in a concentration-dependent manner. *World J Gastroenterol* 2013; 19(8): 1230-1238 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i8/1230.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i8.1230

INTRODUCTION

Schistosomiasis is a water-borne parasitic disease that plagues many tropical and subtropical regions. At least 200 million people in 76 countries are currently afflicted by this disease, and a further 500-600 million people are at risk of infection[1]. *Schistosoma japonicum* (*S. japonicum*), the Asian schistosome, causes schistosomiasis in China, Japan, the Philippines and Indonesia. A nationwide schistosomiasis survey carried out in 2003 indicated that there were still more than 800 000 people infected with *S. japonicum* in China[2].

Despite recent progress in anti-schistosomal strategies, clinical management remains a challenge because schistosomiasis-associated liver fibrosis (SSLF) is a complex, multi-step and often fatal disease. Cercaria that are transmitted through the skin can lay a large number of eggs, which then pass through the sinusoidal endothelial vascular system, settle in the liver, release immol/Lunocompetent products, interact with various liver cells and finally lead to liver fibrosis[3]. This liver fibrosis could develop into an irreversible advanced stage upon repeated exposure to the causative agents (*i.e.*, *S. japonicum* eggs). Primary hepatic stellate cells (HSCs) are believed to be the crucial contributors to the fibrotic process by producing extracellular matrix and interrupting the balance of fiber generation *vs* degradation[4]. Some studies have investigated the mechanism underlying the pathogenesis of SSLF. *S. mansoni* eggs can stimulate hepatic endothelial cell proliferation[5] and migration, promote angiogenesis[6], induce fibroblast proliferation[7] and collagen synthesis[8] and down-regulate LX-2 activation and fibrogenesis[9]. However, little is known about the mechanisms that are active in HSCs during the development and progression of SSL[3,10].

In this study, primary HSCs isolated from SSLF mice were exposed to different concentrations of *S. japonicum* egg antigen and then analyzed to better understand the interaction between HSCs and SSLF.

MATERIALS AND METHODS

Animals

Healthy 4- to 6-wk-old male BALB/C mice were obtained from a schistosomiasis control station in Hubei province, China.

Reagents

S. japonicum egg antigen [0.01 g/mL in phosphate-buffered saline (PBS)] was obtained from the Hubei schistosomiasis control station and diluted to the working concentration in Dulbecco's modified Eagle's medium (DMEM) supplemented with 2% fetal bovine serum (FBS) before use.

Animal model development

The SSLF model was established by abdominal infection with schistosomal cercaria according to the method used in a previous study[11]. In brief, mice in the model group were percutaneously infected with *S. japonicum* by placing a glass slide carrying 20 ± 2 cercariae in non-chlorinated water on the abdomen of each mouse for 15 min. Mice in the control group were treated with non-chlorinated water containing no cercariae. All mice were raised for 6 wk under pathogen-free conditions with free access to food and water. All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals of the Chinese Council on Animal Care. Liver samples were taken from 10 infected and 10 normal mice and fixed in 4% (v/v) paraformaldehyde (PFA) in PBS.

Masson staining

Masson staining was performed using standard methods to observe collagen fiber deposition. About 5 middle-power fields were randomly selected from each sample for analysis, and the ratio of the area occupied by collagen fibers to the total area was quantified using Image Pro Plus 6.0 software (Media Cybernetics Inc., United States).

Hepatic stellate cell isolation and culture

HSCs were prepared by the discontinuous density gradient centrifugation technique previously described by Schafer *et al*[12], with minor modifications. Briefly, the liver was perfused with Solution I [137 mmol/L NaCl, 5.4 mmol/L KCl, 0.6 mmol/L NaH_2PO_4•$2H_2O$, 0.8 mmol/L Na_2HPO_4•$12H_2O$, 10 mmol/L HEPES, 0.5 mmol/L EGTA, 4.2 mmol/L $NaHCO_3$, 5 mmol/L glucose, 100 U/mL penicillin and 100 U/mL streptomycin (Gibco, United States), pH = 7.4] and Solution II [137 mmol/L NaCl, 5.4 mmol/L KCl, 0.6 mmol/L NaH_2PO_4•$2H_2O$, 0.8 mmol/L Na_2HPO_4•$12H_2O$, 10 mmol/L HEPES, 3.8 mmol/L $CaCl_2$•$2H_2O$, 24.2 mmol/L $NaHCO_3$, 5 mmol/L glucose, 600 mg/L collagenase IV (Gibco, United States), 400 mg/L pronase (Sigma, United States), 20 mg/L DNAse (Gibco, United States), pH = 7.4] through the hepatic portal vein. The livers were removed and forced through a 200 gauge mesh. Parenchymal cells were separated by centrifugation at 20 *g* for 5 min. The supernatant was transferred to a 50 mL centrifuge tube and centrifuged at 500 *g* for 7 min. The pellet was resuspended in 15% OptiPrep (Sigma, United States), and 11% OptiPrep and 5 mL GBSS (120 mmol/L NaCl, 5 mmol/L KCl, 0.84 mmol/L Na_2HPO_4•$2H_2O$, 0.22 mmol/L KH_2PO_4, 1.9 mmol/L $MgCl_2$•$6H_2O$, 1.5 mmol/L $CaCl_2$•$2H_2O$, 27 mmol/L $NaHCO_3$, 5 mmol/L glucose, pH = 7.4) were

then layered on the top of the 15% OptiPrep, and the gradient was centrifuged at 1400 *g* for 17 min. The interface between the 11% OptiPrep and the GBSS was collected and washed twice with GBSS. The collected cells were cultured in DMEM containing 10% FBS (Gibco, United States). The cell viability, as measured by a Trypan Blue exclusion assay, was approximately 90%. Primary HSCs from passages 7-8 were used in this study.

Immunofluorescence staining

Immunofluorescence double staining of α-smooth muscle actin (α-SMA) and desmin was used to identify activated HSCs. Briefly, cell slides were fixed in 4% PFA and incubated with a blocking solution containing 0.1% Triton X-100 and 5% bovine serum albumin (BSA) in PBS, followed by incubation with anti-desmin (Abcam, United Kingdom) and anti-α-SMA (Boster, China) primary antibodies at 4 ℃ overnight. After three washes with PBS, the slides were incubated with secondary FITC-conjugated antibodies and Cy3-conjugated antibodies (Proteintech, United States) for 1 h at 37 ℃ and then with Hoechst 33258 for 5 min. The cells were observed and imaged using a TE-2000 Nikon Inverted fluorescence microscope, and the number of positive cells per 100 cells was analyzed.

Cell counting Kit-8

The cell growth inhibitory effect and 50% inhibitory concentration (IC50) of *S. japonicum* egg antigen for primary HSCs were determined using a cell counting kit-8 (CCK-8) assay. HSCs were treated as follows: cultured cells (1×10^4/well) in a 96-well plate were exposed to a range of concentrations of *S. japonicum* egg antigen (1, 5, 25, 125, 250, 625, 1250, 2500 and 3125 μg/mL) for 24 h, and 10 μL of water soluble tetrazolium-8 (WST-8) (Promoter, China) was then added to the medium containing the *S. japonicum* egg antigen. The cells were incubated then for 2 h. The absorbance of each well was read at 450 nm, and a blank well that contained only culture medium and was used for background correction. The percent inhibition was calculated according to the following formula: Inhibition (%) = [1 - (treated/control)] × 100[13,14]. The IC50 was determined using Statistical Product and Service Solutions (SPSS) 16.0 software (SPSS Inc., United States).

Real-time reverse transcription-polymerase chain reaction

Cultured cells were incubated with various concentrations of *S. japonicum* egg antigen (0, 5, 10, 15, 50, 250 μg/mL) for 24 h. Total RNA was isolated from HSCs using the TRIzol reagent (Invitrogen, United States) according to the protocol described by the manufacturer. RNA samples were quantified by measuring the absorbance at 260 nm and 280 nm using a spectrophotometer, and all samples had an *A*260/*A*280 ratio between 1.8 and 2.0, which indicated a high purity of the extracted RNA. The RNA concentrations were calculated based on the absorbance at 260 nm. Aliquots of total RNA (0.5 μg) from each sample were reverse transcribed into cDNA according to the instructions provided with the first-strand cDNA synthesis kit (TaKaRa, Japan). Equal amounts of the reverse transcription products were subjected to polymerase chain reaction amplification using SYBR Green as a fluorescent indicator on an AB iCycler system (AB, United States). The levels of α-SMA, matrix metalloproteinase-9 (MMOL/LP-9) and tissue inhibitor of metalloproteinases-1 (TIMP-1) mRNAs were normalized to the level of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA. The fold change in the expression of target genes between the experimental and control samples was calculated using the $2^{-\Delta\Delta CT}$ method, as previously described[15]. The primers used in this study were synthesized by Invitrogen Ltd. (Invitrogen, United States): α-SMA sense CGGGAGAAAATGACCCAGATT, α-SMA antisense GGACAGCACAGCCTGAATAGC, MMOL/LP-9 sense ACAGCCAACTATGACCAGGAT, MMOL/LP-9 antisense CAGGAAGACGAAGGGGAAGAC, TIMP-1 sense CTTGGTTCCCTGGCGTACTC, TIMP-1 antisense ACCTGATCCGTCCACAAACAG, GAPDH sense GGTTGTCTCCTGCGACTTCA and GAPDH antisense GGGTGGTCCAGGGTTTCTTA.

Western blotting

Western blot analysis was performed as described previously[16]. In brief, proteins from HSCs were extracted using RIPA lysis buffer (50 mmol/L Tris, 150 mmol/L NaCl, 1% Triton X-100, 1% sodium deoxycholate, 0.1% SDS, pH = 7.4) containing protease inhibitors. After the samples were boiled for 5 min at 95 ℃ in 5 × loading buffer, equal amounts (50 μg) of cell homogenates were separated by 12% SDS-PAGE. The proteins were then electrophoretically transferred at 250 mAH onto polyvinylidene fluoride membranes. The membranes were blocked with 5% nonfat dry milk or BSA in Tris-buffered saline-Tween 20 (TBST) and probed at 4 ℃ overnight with primary antibodies against α-SMA (1:200, Boster, China), MMOL/LP-9 (1: 200, Boster, China), TIMP-1and β-actin (1:500, Santa Cruz, United States), phospho-P38 (P-P38) and total P38 (T-P38) (1:1000, Cell Signaling Technology, United States), phospho-JNK (P-JNK) and total-JNK (T-JNK) (1:1000, Cell Signaling Technology, United States), phospho-AKT (P-AKT) (1: 1000, Cell Signaling Technology, United States) and total-AKT (T-AKT) (1:400, Santa Cruz, United States). The membranes were then washed and incubated for 1 h with horseradish peroxidase-conjugated secondary antibodies diluted 1:5000 or 1:2000. The membranes were washed, and all blots were visualized using an ECL detection system (Biouniquer, China). The bands were quantitated in grayscale using Image J software (NIH, United States).

Statistical analysis

The results of multiple observations are presented as the means ± SD of at least 3 independent experiments.

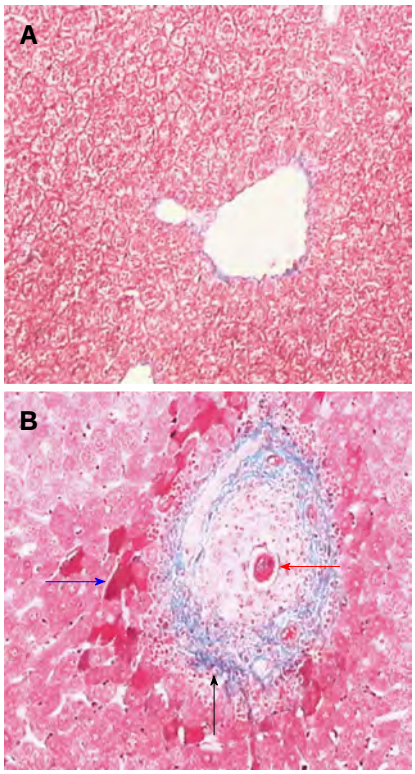


Figure 1 Masson staining of 6 wk post-infect mice livers (× 200). A: Normal mice; B: Infected mice, the red arrow indicated the eggs deposited in the vein, the blue represented acidophilic necrosis, and the black was the collagen fiber deposited around the vein.

Table 1 Ratio of collagen fiber deposited area to the totalin the normal and infected mice livers (mean ± SD)

Group	*n*	Ratio of deposited collagen fiber area against the total
Normal	10	5.18% ± 1.88%
Infected	10	14.53% ± 2.90%[a]

[a]$P < 0.05$ *vs* Normal.

Student's *t* test was used to evaluate the difference in the ratio of the collagen fiber-deposited area to the total area between infected and normal mouse livers. ANOVA was used to evaluate the differences between the *S. japonicum* egg antigen-treated group and the control group. Person linear correlation analysis was used to analyze the correlations between the α-SMA, MMOL/LP-9 and TIMP-1 expression levels and the P-P38, P-JNK and P-AKT levels. All *P* values were two sided, and $P < 0.05$ was considered statistically significant. Statistical analysis was performed using SPSS 16.0 software.

RESULTS

Establishment of the mouse model of SSLF

Masson staining showed that collagen fibers were deposited at the periphery of the eosinophilic granuloma and that eggs were deposited in the venae of mouse liver tissues at 6 wk post-infection; in contrast, there were no collagen fibers deposited in the normal mouse livers except in the vessels (Figure 1). In addition, the ratio of the collagen fiber-deposited area to the total area was significantly greater in the SSLF livers than in the normal livers (Table 1). The above results suggest that the SSLF mouse model was established successfully.

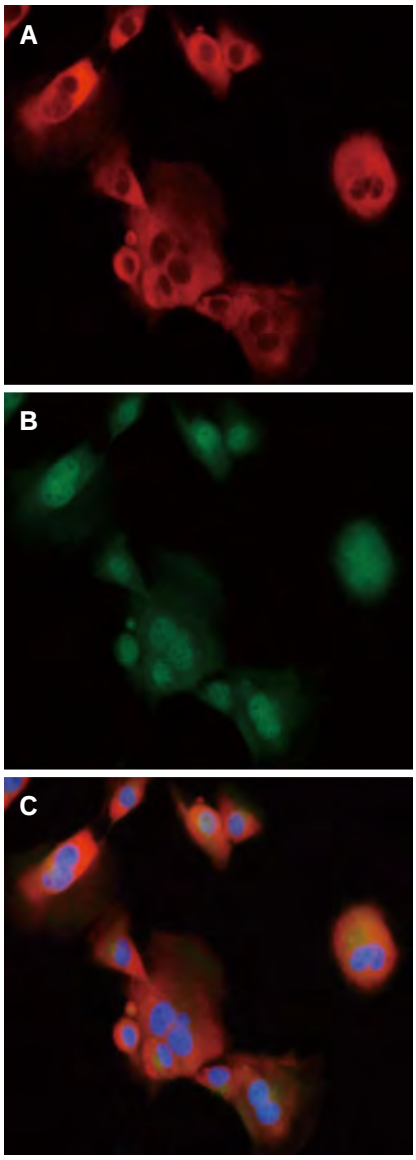


Figure 2 Immunofluorescence staining in primary primary hepatic stellate cells (× 400). A: α-Smooth muscle actin (red hue); B: Desmin (green hue); C: Overlay.

Identification of isolated HSCs

Primary HSCs isolated from SSLF mice displayed a quiescent phenotype. When cultured in plastic culture dishes, these cells began to exhibit an activated phenotype. The expression levels of α-SMA and desmin increased, and this feature was used to identify activated HSCs. Immunofluorescence double staining showed that α-SMA and desmin were expressed in the cytoplasm of HSCs (Figure 2). Approximately 95% of the cells expressed both α-SMA and desmin, suggesting that primary HSCs were

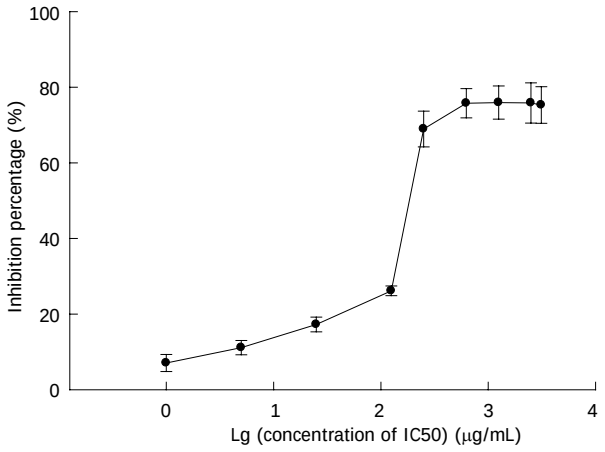


Figure 3 **Inhibitory proliferation of *Schistosoma japonicum* egg antigen in primary hepatic stellate cells.** The black dots were quantification of 3 independent experiments.

isolated successfully.

Inhibitory effect and IC50 of S. japonicum egg antigen for primary HSCs

Figure 3 shows that *S. japonicum* egg antigen inhibited the proliferation of primary HSC in a concentration-dependent manner. The percent inhibition values for *S. japonicum* egg antigen were 7.06% ± 2.26% for 1 μg/mL, 11.13% ± 1.90% for 5 μg/mL, 17.25% ± 1.95% for 25 μg/mL, 26.16% ± 1.28% for 125 μg/mL, 68.96% ± 4.73% for 250 μg/mL, 75.77% ± 3.87% for 625 μg/mL, 75.95% ± 4.40% for 1250 μg/mL, 75.84% ± 5.33% for 2500 μg/mL and 75.30% ± 4.84% for 3125 μg/mL. The IC50, defined as the concentration of a substance that reduces cell survival by 50%, is a useful parameter to quantify the effect of a substance on cell survival. In this study, the IC50 of *S. japonicum* egg antigen was calculated to be 244.53 ± 35.26 μg/mL.

Expression of α-SMA, MMP-9 and TIMP-1 in response to S. japonicum egg antigen in vitro

As shown in Figure 4, *S. japonicum* egg antigen at 50 or 250 μg/mL enhanced α-SMA expression in HSCs at both the mRNA and protein levels. At the mRNA level, the fold change in α-SMA expression was 1.97 ± 0.18 in response to 50 μg/mL and 2.18 ± 0.22 in response to 250 μg/mL relative to the control (1.00 ± 0.10, $P < 0.05$) (Figure 4C). At the protein level, the α-SMA/β-actin ratio was 0.94 ± 0.03 at 50 μg/mL and 1.06 ± 0.04 at 250 μg/mL, compared with 0.63 ± 0.05 for the control ($P < 0.05$, Figure 4A and B).

However, at the mRNA level, 3.45 ± 0.36-fold reduction in response to 50 μg/mL and 4.49 ± 0.28-fold reduction in response to 250 μg/mL in MMOL/LP-9 expression were observed after 24h with egg antigen treatment, compared with 1.05 ± 0.12 for the control ($P < 0.05$, Figure 4C). At the protein level, the MMOL/LP-9/β-actin ratios were 0.59 ± 0.03 at 50 μg/mL and 0.49 ± 0.10 at 250 μg/mL, compared with 0.98 ± 0.08 for the

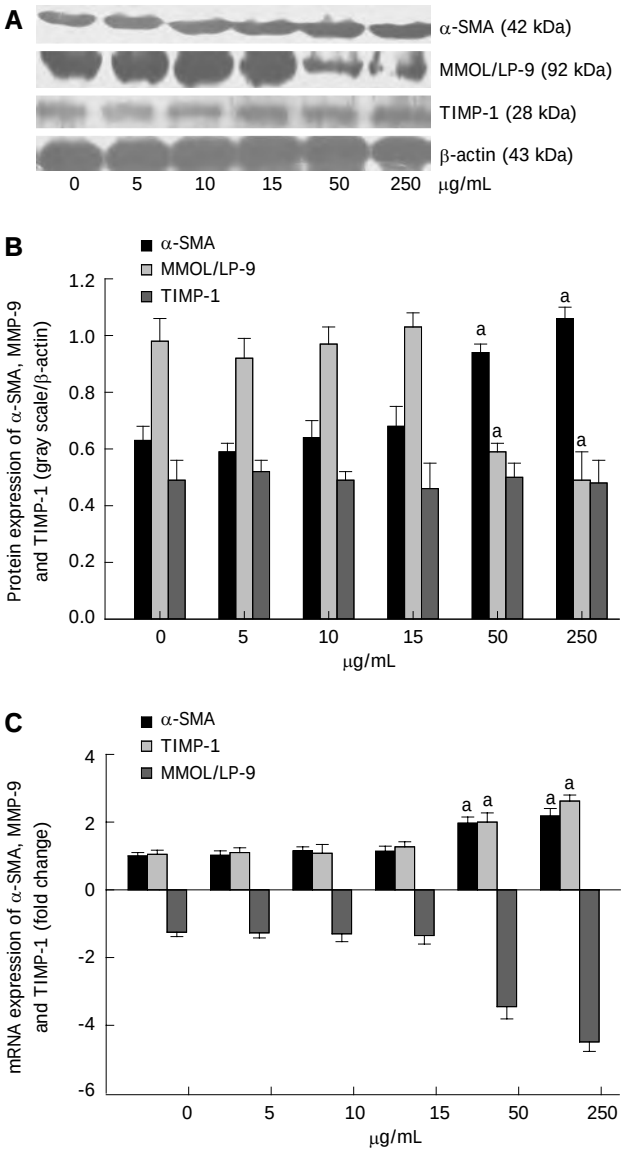


Figure 4 Western blotting (A, B) and real-time polymerase chain reaction (C) of α-smooth muscle actin, matrix metalloproteinase-9 and tissue inhibitor of metalloproteinases-1 induced by *Schistosoma japonicum* egg antigen in primary hepatic stellate cells. A: Western blotting of α-smooth muscle actin (α-SMA), matrix metalloproteinase-9 (MMOL/LP-9) and tissue inhibitor of metalloproteinases-1 (TIMP-1), and representatives of 3 independent experiments; B: The histograms reported as ratio of respective target gray scale to that of β-actin. C: The histograms represented the fold change of target genes normalized to glyceraldehyde-3-phosphate dehydrogenase levels and quantified of 3 independent experiments. [a]$P < 0.05$ *vs* 0 μg/mL.

control ($P < 0.05$, Figure 4A and B). In addition, *S. japonicum* egg antigen also enhanced TIMP-1 expression at the mRNA level. The fold change in TIMP-1 mRNA expression was 2.00 ± 0.27 in response to 50 μg/mL and 2.62 ± 0.18 in response to 250 μg/mL compared with 1.05 ± 0.13 for the control ($P < 0.05$, Figure 4C). However, as shown in Figure 4A and B, *S. japonicum* egg antigen had no effect on TIMP-1 expression at the protein level.

Collectively, the above observations indicate that *S. japonicum* egg antigen promoted fibrogenesis in HSCs and participated in the extracellular matrix remodeling observed in SSLF.

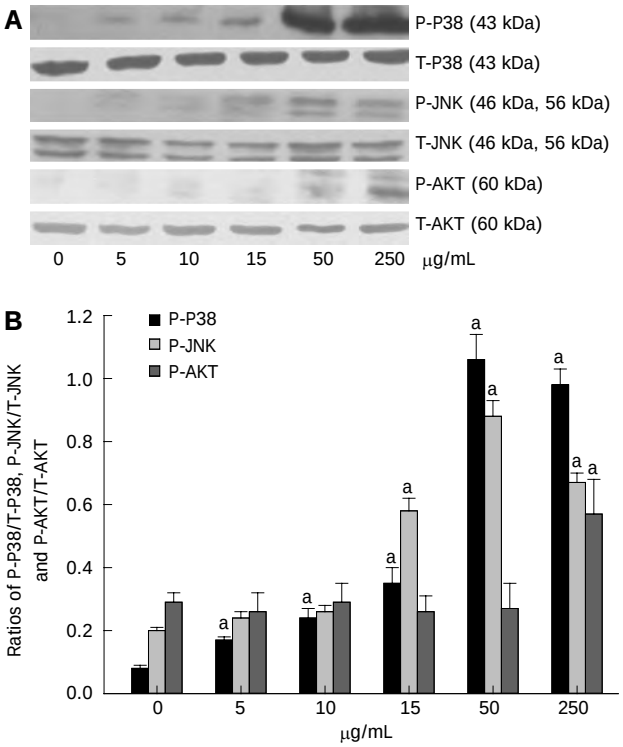


Figure 5 **Western blotting of phospho-P38, phospho-Jun N-terminal kinase and phospho-Akt activation induced by *Schistosoma japonicum* egg antigen in primary hepatic stellate cells.** A: Western blotting of phospho-P38 (P-P38), phospho-Jun N-terminal kinase (P-JNK) and phospho-Akt (P-AKT), and representatives of 3 independent experiments; B: The histograms respectively reported as ratio of P-P38/T-P38, P-JNK/T-JNK, and P-AKT/T-AKT. $^{a}P < 0.05$ *vs* 0 μg/mL.

P38/JNK MAPK and AKT signaling pathway activation by S. japonicum egg antigen in vitro

As shown in Figure 5, the P-P38, P-JNK and P-AKT activation levels were enhanced by stimulation with the *S. japonicum* egg antigen. The P-P38/T-P38 ratio was 1.03 ± 0.08 at 50 μg/mL and 1.05 ± 0.01 at 250 μg/mL *vs* 0.18 ± 0.02 for the control ($P < 0.05$). The P-JNK/T-JNK ratio was 0.58 ± 0.04 at 15 μg/mL, 0.88 ± 0.05 at 50 μg/mL and 0.67 ± 0.03 at 250 μg/mL *vs* 0.20 ± 0.01 for the control ($P < 0.05$). The P-AKT/T-AKT ratio was 0.57 ± 0.11 at 250 μg/mL *v* 0.29 ± 0.03 for the control ($P < 0.05$). The above results indicate that *S. japonicum* egg antigen activated the P38/JNK mitogen-activated protein kinase (MAPK) and AKT signaling pathways in a concentration-dependent manner.

Relationships among the levels of the α-SMA, MMP-9 and TIMP-1 and P-P38, P-JNK and P-AKT in HSCs

α-SMA protein expression was positively correlated with the P-P38 ($r = 0.669$, $P = 0.002$) and P-JNK levels ($r = 0.686$, $P = 0.002$). MMOL/LP-9 expression was negatively correlated with the P-P38 ($r = -0.976$, $P = 0.000$), P-JNK ($r = -0.86$, $P = 0.000$) and P-AKT levels ($r = -0.529$, $P = 0.024$). However, TIMP-1 expression was not correlated with the P-P38, P-JNK or P-AKT level. α-SMA expression was not correlated with the P-AKT level.

DISCUSSION

HSC activation is considered the key step in liver fibrogenesis, representing a transition from the quiescent state into a proliferative, fibrogenic and contractile state. HSCs reside in the space of disse and are recruited to areas of hepatic injury by chemokines, where they become activated[17]. During this process, HSCs are transformed from the quiescent to activated phenotype, a process that is accompanied by an increase in the expression levels of α-SMA and desmin[18,19], which are considered to be markers of HSC activation. The activated HSCs adopt a myofibroblast-like phenotype and secrete collagen-rich matrix into the extracellular space, leading to liver fibrosis[20]. HSCs have been implicated as the effector cells of hepatic fibrosis and cirrhosis associated with hepatitis B virus, hepatitis C virus, genetic hemochromatosis, biliary atresia, cystic fibrosis and alcoholic liver disease in humans[20-23]. HSCs also play a role in eosinophilic granulomatous inflammol/Lation and SSLF[24-26]. Bartley *et al*[25] showed that activated HSCs could be detected at the edges of eosinophilic granulomas in *S. japonicum*-infected mice livers at 6 wk post-infection, and the localization of these cells was coincident with areas of collagen deposition. In this study, collagen fiber deposition was observed at the periphery of the eosinophilic granuloma, consistent with the previous reports[25]. In addition, we also found that *S. japonicum* egg antigen inhibited HSC proliferation in a concentration-dependent manner.

Activated HSC-secreted MMOL/LPs (*e.g.*, MMOL/LP-9) and their inhibitors (*e.g.*, TIMP-1) remodel the tissue matrix[22], and an imbalance in MMOL/LP/TIMP expression has been associated with cumulative fibrosis[27]. The expression of α-SMA is increased in the livers of SSLF patients and infected mice and is closely correlated with the degree of liver fibrosis[25,27]. In our experiment, the *S. japonicum* egg antigen attenuated MMOL/LP-9 expression and enhanced α-SMA expression at both the mRNA and protein levels, and it also enhanced TIMP-1 expression at the mRNA level. However, the TIMP-1 protein level was not affected. The mRNA and protein expression levels are not always proportional due to the activities of various regulatory mechanisms, *e.g.*, post-transcriptional modulation by miRNAs. The above results imply that the *S. japonicum* egg antigen is involved in MMOL/LP-9-mediated matrix remodeling and thus collagen deposition, leading to liver fibrosis. The results of our study partially contradict to the previous report that *S. mansoni* eggs can down-regulate the activation of and fibrogenesis in the human hepatic stellate cell line LX-2[9]. One potential reason for this discrepancy is the different cell models and schistosomal egg antigens used in the two studies. *In vitro* exposure to schistosome eggs induces only limited activation of human dendritic cells (DCs) but strong activation of murine DCs[28]. Similarly, in this study, the same concentrations of egg antigen (5 μg/mL,

10 μg/mL, and 15 μg/mL) that attenuated α-SMA expression in human LX-2 cells had no effect on primary mouse HSCs. In addition, *S. japonicum* and *S. mansoni* eggs release different antigens[29], which may play a role in the discrepancy.

MAPKs are pivotal transmitters of extracellular signals such as hormones, cytokines, growth factors, and various environmental stress signals[30]. The MAPK family has three major subfamilies: ERK, P38, and JNK, and the P38 and JNK pathways are involved in HSC transformation[31] and in the regulation of the complex life cycle and host-parasite interactions of *S. japonicum*[32]. Many studies have shown that the regulation of MMOL/LP-9[33-36] and α-SMA[37-39] in various cell models is dependent on the activation of the P38 and (or) JNK signaling pathways. In addition, AKT/PI3K activation contributes to liver[40], hear[41] and pulmonar[42-44] fibrosis. To gain insight into the potential signaling mechanisms mediating the HSC responses induced by the *S. japonicum* egg antigen, we investigated the roles of the P38/JNK MAPK and AKT signaling pathways by assessing their phosphorylation status in primary HSCs after stimulation by the *S. japonicum* egg antigen. In this study, the P-P38 and P-JNK levels were significantly enhanced after the treatment. The increase in α-SMA expression that was stimulated by the *S. japonicum* egg antigen was positively correlated with the P-P38 and P-JNK levels but not the P-AKT level. Attenuated MMOL/LP-9 expression was negatively correlated with the P-P38, P-JNK and P-AKT levels. TIMP-1 expression was not correlated with the P-P38, P-JNK or P-AKT level. We also noted that only *S. japonicum* egg antigen concentrations of 250 μg/mL or greater were associated with AKT activation, suggesting that AKT signaling may have a less important role than P38/JNK MAPK signaling in the induction of fibrogenesis by the *S. japonicum* egg antigen.

In conclusion, the results of this study suggest that the *S. japonicum* egg antigen promotes fibrogenesis and inhibits the proliferation of primary HSCs in a concentration-dependent manner. These effects of the *S. japonicum* egg antigen may be attributed to the activation of the P38/JNK signaling pathways in HSCs. Thus, inhibitors of the P38/JNK signaling pathways may serve as potential therapeutic treatments for SSLF.

ACKNOWLEDGMENTS

The authors thank Doctor Ying Chang for her revision of the manuscript. The authors would also like to acknowledge Doctor Zhi-Jun Wang for providing technical assistance during preliminary experiments.

COMMENTS

Background

Under the action of stimulus, hepatic stellate cells (HSCs) are activated and secreted extracellular matrix (ECM) into extracellular space, which leads to liver fibrosis. HSCs have been attributed as the effector cells for schistosomiasis-associated liver fibrosis (SSLF). Continuous schistosome eggs deposition in liver tissue was the chief factors leading to SSLF. So the study on the interaction between schistosome eggs antigen and HSCs is very important.

Research frontiers

As the effector cells of liver fibrosis, HSC is usually used as the cell model to study the liver fibrosis *in vitro*. The research hotspot on SSLF is the effects induced by schistosome eggs in HSCs and the involved mechanisms, and finding out the targets of treatment on SSLF for future.

Innovations and breakthroughs

The authors isolated primary HSCs which maintained the original features of HSC genes, and tried to explore the involved signaling pathways. The results of this study showed that *Schistosoma japonicum* (*S. japonicum*) eggs promoted the fibrogenesis and inhibited the proliferation of primary HSCs *in vitro*, P38/JNK MAPK signaling pathways might play an important role in the fibrogenesis induced by *S. japonicum* eggs, however AKT/PI3K signaling pathway might be not involved.

Applications

The results of this study suggest that *S. japonicum* egg antigen induced-fibrogenesis is related with P38/JNK MAPK signaling pathway activation, and the inhibitors of the P38/JNK signaling pathways may serve as potential therapeutic treatments for SSLF.

Terminology

Schistosomiasis is a water-borne parasitic disease that plagues many tropical and subtropical regions in world. There are 6 types schistosome which can infect humans: *Schistosoma haematobium*, *Schistosoma mansoni*, *S. japonicum*, *Schistosoma mekongi*, *Schistosoma intercalatum*, *Schistosoma malayensis*. *S. japonicum*, the Asian schistosome, causes schistosomiasis in China, Japan, the Philippines and Indonesia. Cercariae of schistosome parasitize in oncomelania that live in water and infect humans through bare skins, develop into imaginess in human bodies, and produce a large number of eggs which can deposit in liver tissues. With the eggs continuous deposition, eosinophilic granuloma and liver fibrosis, which are the pathological characteristics of schistosomiasis, are finally induced.

Peer review

This is a good study in which authors describe the fibrogenesis and inhibitory proliferation effects induced by *S. japonicum* in primary HSCs, and analyzed the signaling pathways (*e.g.*, P38/JNK MAPK) which may be involved in the above effects. The results are interesting and suggest that the inhibitors of the P38/JNK signaling pathways may serve as potential therapeutic treatments for SSLF in future.

REFERENCES

1 **Gryseels B**, Polman K, Clerinx J, Kestens L. Human schistosomiasis. *Lancet* 2006; **368**: 1106-1118 [PMID: 16997665 DOI: 10.1016/S0140-6736(06)69440-3]

2 **Xiao DL**, Yu Q, Dang H, Guo JG, Zhou XN, Wang LY. Schistosomiasis situation in People's Republic of China in 2003. *Zhonghua Xue Xi Chong Bing Fangzhi Zazhi* 2004; 16: 401-405

3 **Wilson MS**, Mentink-Kane MM, Pesce JT, Ramalingam TR, Thompson R, Wynn TA. Immunopathology of schistosomiasis. *Immunol Cell Biol* 2007; **85**: 148-154 [PMID: 17160074 DOI: 10.1038/sj.icb.7100014]

4 **Booth M**, Mwatha JK, Joseph S, Jones FM, Kadzo H, Ireri E, Kazibwe F, Kemijumbi J, Kariuki C, Kimani G, Ouma JH, Kabatereine NB, Vennervald BJ, Dunne DW. Periportal fibrosis in human Schistosoma mansoni infection is associated with low IL-10, low IFN-gamma, high TNF-alpha, or low RANTES, depending on age and gender. *J Immunol* 2004; **172**: 1295-1303 [PMID: 14707108]

5 **Freedman DO**, Ottesen EA. Eggs of Schistosoma mansoni stimulate endothelial cell proliferation in vitro. *J Infect Dis* 1988; **158**: 556-562 [PMID: 2457636 DOI: 10.1093/infdis/158.3.556]

6 **Kanse SM**, Liang O, Schubert U, Haas H, Preissner KT, Doenhoff MJ, Dennis RD. Characterisation and partial purification of Schistosoma mansoni egg-derived pro-angiogenic factor. *Mol Biochem Parasitol* 2005; **144**: 76-85 [PMID: 16169609 DOI: 10.1016/j.molbiopara.2005.08.001]

7 **Wyler DJ**, Tracy JW. Direct and indirect effects of soluble extracts of Schistosoma mansoni eggs on fibroblast proliferation in vitro. *Infect Immun* 1982; **38**: 103-108 [PMID: 6890530]

8 **Boros DL**, Lande MA. Induction of collagen synthesis in cultured human fibroblasts by live Schistosoma mansoni eggs and soluble egg antigens (SEA). *Am J Trop Med Hyg* 1983; **32**: 78-82 [PMID: 6681691]

9 **Anthony B**, Mathieson W, de Castro-Borges W, Allen J. Schistosoma mansoni: egg-induced downregulation of hepatic stellate cell activation and fibrogenesis. *Exp Parasitol* 2010; **124**: 409-420 [PMID: 20045695]

10 **Anthony B**, Allen JT, Li YS, McManus DP. Hepatic stellate cells and parasite-induced liver fibrosis. *Parasit Vectors* 2010; **3**: 60 [PMID: 20663176 DOI: 10.1186/1756-3305-3-60]

11 **Chen BL**, Zhang GY, Yuan WJ, Wang SP, Shen YM, Yan L, Gu H, Li J. Osteopontin expression is associated with hepatopathologic changes in Schistosoma japonicum infected mice. *World J Gastroenterol* 2011; **17**: 5075-5082 [PMID: 22171141 DOI: 10.3748/wjg.v17.i46.5075]

12 **Schäfer S**, Zerbe O, Gressner AM. The synthesis of proteoglycans in fat-storing cells of rat liver. *Hepatology* 1987; **7**: 680-687 [PMID: 3111965]

13 **Cuello M**, Ettenberg SA, Nau MM, Lipkowitz S. Synergistic induction of apoptosis by the combination of trail and chemotherapy in chemoresistant ovarian cancer cells. *Gynecol Oncol* 2001; **81**: 380-390 [PMID: 11371126]

14 **Li HN**, Nie FF, Liu W, Dai QS, Lu N, Qi Q, Li ZY, You QD, Guo QL. Apoptosis induction of oroxylin A in human cervical cancer HeLa cell line in vitro and in vivo. *Toxicology* 2009; **257**: 80-85 [PMID: 19135124 DOI: 10.1016/j.tox.2008.12.011]

15 **Livak KJ**, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 2001; **25**: 402-408 [PMID: 11846609 DOI: 10.1006/meth.2001.1262]

16 **Zhang DX**, Zou AP, Li PL. Ceramide-induced activation of NADPH oxidase and endothelial dysfunction in small coronary arteries. *Am J Physiol Heart Circ Physiol* 2003; **284**: H605-H612 [PMID: 12424096]

17 **Cassiman D**, Libbrecht L, Desmet V, Denef C, Roskams T. Hepatic stellate cell/myofibroblast subpopulations in fibrotic human and rat livers. *J Hepatol* 2002; **36**: 200-209 [PMID: 11830331 DOI: 10.1016/S0168-8278(01)00260-4]

18 **Chang D**, Ramalho LN, Ramalho FS, Martinelli AL, Zucoloto S. Hepatic stellate cells in human schistosomiasis mansoni: a comparative immunohistochemical study with liver cirrhosis. *Acta Trop* 2006; **97**: 318-323 [PMID: 16473318 DOI: 10.1016/j.actatropica.2005.12.006]

19 **Yokoi Y**, Namihisa T, Kuroda H, Komatsu I, Miyazaki A, Watanabe S, Usui K. Immunocytochemical detection of desmin in fat-storing cells (Ito cells). *Hepatology* 1984; **4**: 709-714 [PMID: 6204917 DOI: 10.1002/hep.1840040425]

20 **Iredale JP**. Cirrhosis: new research provides a basis for rational and targeted treatments. *BMJ* 2003; **327**: 143-147 [PMID: 12869458]

21 **Ramm GA**, Crawford DH, Powell LW, Walker NI, Fletcher LM, Halliday JW. Hepatic stellate cell activation in genetic haemochromatosis. Lobular distribution, effect of increasing hepatic iron and response to phlebotomy. *J Hepatol* 1997; **26**: 584-592 [PMID: 9075666 DOI: 10.1016/S0168-8278(97)80424-2]

22 **Ramm GA**, Nair VG, Bridle KR, Shepherd RW, Crawford DH. Contribution of hepatic parenchymal and nonparenchymal cells to hepatic fibrogenesis in biliary atresia. *Am J Pathol* 1998; **153**: 527-535 [PMID: 9708812 DOI: 10.1016/S0002-]

23 **Lewindon PJ**, Pereira TN, Hoskins AC, Bridle KR, Williamson RM, Shepherd RW, Ramm GA. The role of hepatic stellate cells and transforming growth factor-beta(1) in cystic fibrosis liver disease. *Am J Pathol* 2002; **160**: 1705-1715 [PMID: 12000722 DOI: 10.1016/S0002-9440(10)61117-0]

24 **Barbosa Júnior Ade A**, Pfeifer U, Andrade ZA. Role of fat-storing cells in schistosomal hepatic fibrosis of mice. *Virchows Arch B Cell Pathol Incl Mol Pathol* 1993; **64**: 91-96 [PMID: 8220823 DOI: 10.1007/BF02915100]

25 **Bartley PB**, Ramm GA, Jones MK, Ruddell RG, Li Y, McManus DP. A contributory role for activated hepatic stellate cells in the dynamics of Schistosoma japonicum egg-induced fibrosis. *Int J Parasitol* 2006; **36**: 993-1001 [PMID: 16806222 DOI: 10.1016/j.ijpara.2006.04.015]

26 **Maksimov VI**, Molodova GA, Bespalova TI, Denisov VM. [Effect of different pH values on the activity and quaternary structure of asparaginase in Escherichia coli extracts]. *Prikl Biokhim Mikrobiol* 1975; **11**: 539-545 [PMID: 1737]

27 **Singh KP**, Gerard HC, Hudson AP, Boros DL. Expression of matrix metalloproteinases and their inhibitors during the resorption of schistosome egg-induced fibrosis in praziquantel-treated mice. *Immunology* 2004; **111**: 343-352 [PMID: 15009436 DOI: 10.1111/j.0019-2805.2004.01817.x]

28 **Perona-Wright G**, Jenkins SJ, MacDonald AS. Dendritic cell activation and function in response to Schistosoma mansoni. *Int J Parasitol* 2006; **36**: 711-721 [PMID: 16696981 DOI: 10.1016/j.ijpara.2006.02.003]

29 **Mathieson W**, Wilson RA. A comparative proteomic study of the undeveloped and developed Schistosoma mansoni egg and its contents: the miracidium, hatch fluid and secretions. *Int J Parasitol* 2010; **40**: 617-628 [PMID: 19917288 DOI: 10.1016/j.ijpara.2009.10.014]

30 **Widmann C**, Gibson S, Jarpe MB, Johnson GL. Mitogen-activated protein kinase: conservation of a three-kinase module from yeast to human. *Physiol Rev* 1999; **79**: 143-180 [PMID: 9922370]

31 **Reeves HL**, Dack CL, Peak M, Burt AD, Day CP. Stress-activated protein kinases in the activation of rat hepatic stellate cells in culture. *J Hepatol* 2000; **32**: 465-472 [PMID: 10735617 DOI: 10.1016/S0168-8278(00)80398-0]

32 **Wang L**, Yang Z, Li Y, Yu F, Brindley PJ, McManus DP, Wei D, Han Z, Feng Z, Li Y, Hu W. Reconstruction and in silico analysis of the MAPK signaling pathways in the human blood fluke, Schistosoma japonicum. *FEBS Lett* 2006; **580**: 3677-3686 [PMID: 16765950 DOI: 10.1016/j.febslet.2006.05.055]

33 **Liu WH**, Chang LS. Caffeine induces matrix metalloproteinase-2 (MMP-2) and MMP-9 down-regulation in human leukemia U937 cells via Ca2+/ROS-mediated suppression of ERK/c-fos pathway and activation of p38 MAPK/c-jun pathway. *J Cell Physiol* 2010; **224**: 775-785 [PMID: 20432471 DOI: 10.1002/jcp.22180]

34 **Cho HJ**, Jeong YJ, Park KK, Park YY, Chung IK, Lee KG, Yeo JH, Han SM, Bae YS, Chang YC. Bee venom suppresses PMA-mediated MMP-9 gene activation via JNK/p38 and NF-kappaB-dependent mechanisms. *J Ethnopharmacol* 2010; **127**: 662-668 [PMID: 19969058 DOI: 10.1016/j.jep.2009.12.007]

35 **Hsu HH**, Liu CJ, Shen CY, Chen YJ, Chen LM, Kuo WH, Lin YM, Chen RJ, Tsai CH, Tsai FJ, Huang CY. p38α MAPK mediates 17β-estradiol inhibition of MMP-2 and -9 expression and cell migration in human lovo colon cancer cells. *J Cell Physiol* 2012; **227**: 3648-3660 [PMID: 22377968 DOI: 10.1002/jcp]

36 **Cheng CY**, Hsieh HL, Hsiao LD, Yang CM. PI3-K/Akt/JNK/NF-κB is essential for MMP-9 expression and outgrowth in human limbal epithelial cells on intact amniotic membrane. *Stem Cell Res* 2012; **9**: 9-23 [PMID: 22459175 DOI: 10.1016/j.scr.2012.02.005]

37 **Hu Y**, Peng J, Feng D, Chu L, Li X, Jin Z, Lin Z, Zeng Q. Role of extracellular signal-regulated kinase, p38 kinase, and activator protein-1 in transforming growth factor-beta1-induced alpha smooth muscle actin expression in human fetal lung fibroblasts in vitro. *Lung* 2006; **184**: 33-42 [PMID: 16598650 DOI: 10.1007/s00408-005-2560-5]

38 **Nomiyama Y**, Tashiro M, Yamaguchi T, Watanabe S, Taguchi M, Asaumi H, Nakamura H, Otsuki M. High glucose

activates rat pancreatic stellate cells through protein kinase C and p38 mitogen-activated protein kinase pathway. *Pancreas* 2007; **34**: 364-372 [PMID: 17414061 DOI: 10.1097/MPA.0b013e31802f0531]

39 **Akamatsu N**, Nakajima H, Ono M, Miura Y. Increase in acetyl CoA synthetase activity after phenobarbital treatment. *Biochem Pharmacol* 1975; **24**: 1725-1727 [PMID: 15 DOI: 10.1152/ajpgi.00339.2004]

40 **Son G**, Hines IN, Lindquist J, Schrum LW, Rippe RA. Inhibition of phosphatidylinositol 3-kinase signaling in hepatic stellate cells blocks the progression of hepatic fibrosis. *Hepatology* 2009; **50**: 1512-1523 [PMID: 19790269 DOI: 10.1002/hep.23186]

41 **Lian H**, Ma Y, Feng J, Dong W, Yang Q, Lu D, Zhang L. Heparin-binding EGF-like growth factor induces heart interstitial fibrosis via an Akt/mTor/p70s6k pathway. *PLoS One* 2012; **7**: e44946 [PMID: 22984591 DOI: 10.1371/jour]

42 **He Z**, Gao Y, Deng Y, Li W, Chen Y, Xing S, Zhao X, Ding J, Wang X. Lipopolysaccharide induces lung fibroblast proliferation through Toll-like receptor 4 signaling and the phosphoinositide3-kinase-Akt pathway. *PLoS One* 2012; **7**: e35926 [PMID: 22563417 DOI: 10.1371/journal.pone.0035926]

43 **Wygrecka M**, Zakrzewicz D, Taborski B, Didiasova M, Kwapiszewska G, Preissner KT, Markart P. TGF-β1 induces tissue factor expression in human lung fibroblasts in a PI3K/JNK/Akt-dependent and AP-1-dependent manner. *Am J Respir Cell Mol Biol* 2012; **47**: 614-627 [PMID: 22771387 DOI: 10.1165/rcmb]

44 **Conte E**, Fruciano M, Fagone E, Gili E, Caraci F, Iemmolo M, Crimi N, Vancheri C. Inhibition of PI3K prevents the proliferation and differentiation of human lung fibroblasts into myofibroblasts: the role of class I P110 isoforms. *PLoS One* 2011; **6**: e24663 [PMID: 21984893 DOI: 10.1371/journal.pone.0024663]

P- Reviewer Venugopal SK **S- Editor** Zhai HH **L- Editor** A **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i8.1239

World J Gastroenterol 2013 February 28; 19(8): 1239-1246
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

ORIGINAL ARTICLE

Effect of silencing of high mobility group A2 gene on gastric cancer MKN-45 cells

Chun-Hui Wei, Li-Xiu Wei, Ming-Yu Lai, Jia-Zhuang Chen, Xi-Jing Mo

Chun-Hui Wei, Li-Xiu Wei, Ming-Yu Lai, Jia-Zhuang Chen, Department of Geriatric Gastroenterology, the First Affiliated Hospital of Guangxi Medical University, Nanning 530021, Guangxi Zhuang Autonomous Region, China
Xi-Jing Mo, Department of Gastroenterology, the First Affiliated Hospital of Guangxi Chinese Medical University, Nanning 530001, Guangxi Zhuang Autonomous Region, China
Author contributions: Wei CH and Lai MY contributed equally to this work; Wei CH, Wei LX, Lai MY, Chen JZ and Mo XJ designed the research; Lai MY was responsible for the whole study; Wei CH, Wei LX, Chen JZ and Mo XJ performed the research and analyzed the data; and Wei CH wrote the paper.
Supported by The Natural Science Foundation of Guangxi, No. 2010GXNSFA013166; and the Key Project of Health Department of Guangxi, No. Zhong 2010021
Correspondence to: Ming-Yu Lai, Professor, Department of Geriatric Gastroenterology, the First Affiliated Hospital of Guangxi Medical University, Nanning 530021, Guangxi Zhuang Autonomous Region, China. laimingyu2005103@yahoo.com.cn
Telephone: +86-771-5356585 Fax: +86-771-5356585
Received: September 3, 2012 Revised: January 3, 2013
Accepted: January 11, 2013
Published online: February 28, 2013

Abstract

AIM: To investigate the effect of high mobility group A2 (*HMGA2*) gene silencing on gastric cancer MKN-45 cells *in vitro*.

METHODS: HMGA2 short hairpin RNA (shRNA) expression plasmids were constructed, including a pair of random scrambled sequences. Human gastric cancer cell line MKN-45 cells were divided into three groups: blank control group (non-transfected cells), transfected group (cells transfected with HMGA2 shRNA recombinant plasmid) and scrambled sequence group (transfected with random scrambled plasmid). Cells were transfected with HMGA2 shRNA recombinant plasmids and scrambled plasmid *in vitro*, and the cells transfection efficiency was assayed by fluorescence microscopy. The HMGA2 messenger RNA (mRNA) expression was detected by reverse transcription polymerase chain reaction, gastric cancer cells apoptosis was detected by flow cytometry, cell proliferation was detected by methyl thiazol tetrazolium, and the protein expression of phosphatidylinositol 3-kinase (PI3K), protein kinase B (Akt), P27, caspase-9 and B-cell leukemia/lymphoma-2 (Bcl-2) were analyzed by Western blotting.

RESULTS: Compared with the blank control group and the scrambled sequence group, the levels of HMGA2 mRNA and protein expression in the transfected group were significantly reduced ($P < 0.05$). The relative HMGA2 mRNA expression levels of the blank control group, transfected group and scrambled sequence group were 0.674 ± 0.129, 0.374 ± 0.048 and 0.689 ± 0.124, respectively. The relative HMGA2 protein expression levels of the blank control group, transfected group and scrambled sequence group were 0.554 ± 0.082, 0.113 ± 0.032 and 0.484 ± 0.123, respectively. Moreover, transfection with the scrambled sequence had no effect on the expression of HMGA2. After being transfected with shRNA for 24, 48 and 72 h, the cell apoptotic rates of the transfected group were 21.65% ± 0.28%, 39.98% ± 1.82% and 24.51% ± 0.93%, respectively, which significantly higher than those of blank control group (4.72% ± 1.34%, 5.83% ± 0.13% and 5.22% ± 1.07%) and scrambled sequence group (4.28% ± 1.33%, 7.87% ± 1.43% and 6.71% ± 0.92%). After 24, 48 and 72 h, the cell proliferation inhibition rates in the transfected group were 31.57% ± 1.17%, 39.45% ± 2.07% and 37.56% ± 2.32%, respectively; the most obvious cell proliferation inhibition appeared at 48 h after transfection. Compared with the blank control group and scrambled sequence group, after transfection of shRNA for 72 h, the protein expression levels of PI3K (0.042 ± 0.005 *vs* 0.069 ± 0.003, 0.067 ± 0.05), Akt (0.248 ± 0.004 *vs* 0.489 ± 0.006, 0.496 ± 0.104) and Bcl-2 (0.295 ± 0.084 *vs* 0.592 ± 0.072, 0.594 ± 0.109) were significantly reduced. The protein expression levels of P27 (0.151 ± 0.010 *vs* 0.068

± 0.014, 0.060 ± 0.013) and caspase-9 (0.136 ± 0.042 *vs* 0.075 ± 0.010, 0.073 ± 0.072) were significantly upregulated.

CONCLUSION: *HMGA2* shRNA gene silencing induces apoptosis and suppresses proliferation of MKN-45 cells.

© 2013 Baishideng. All rights reserved.

Key words: Gastric cancer cells; RNA interference; High mobility group A2; Proliferation; Apoptosis

Wei CH, Wei LX, Lai MY, Chen JZ, Mo XJ. Effect of silencing of high mobility group A2 gene on gastric cancer MKN-45 cells. *World J Gastroenterol* 2013; 19(8): 1239-1246 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i8/1239.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i8.1239

INTRODUCTION

Gastric cancer is one of the most common malignancies, being the fourth most common cancer and the second leading cause of cancer related deaths (740 000 deaths in 2008), with nearly one million newly diagnosed cases each year[1]. Up to now, the exact molecular mechanisms involved in carcinogenesis and progression of gastric cancer have remained unclear. Therefore, investigating the cell proliferation and apoptosis involved in these processes is essential for gastric cancer research.

The high mobility group A2 (*HMGA2*) gene is a member of the high mobility group AT-hook (HMGA) gene family. HMGA2 plays a critical role in several cellular biological processes, including cell transformation, growth, differentiation, senescence and cycle control[2-4]. Normally, HMGA2 is abundantly expressed during embryogenesis, whereas its expression is low or absent in normal adult differentiated tissues[5,6]. Evidence shows that HMGA2 is overexpressed in breast cancer[7], pancreatic cancer[8,9], esophageal squamous cell carcinoma[10], non-small cell lung cancer[11], oral squamous cell cancer[12], bladder cancer[13], ovarian cancer[14,15] and colorectal cancer[16,17]. Its expression is correlated with malignant degree, pathological type and metastasis[18]. Thus, HMGA2 might play a central role in the disease progression of many types of tumors. Overexpression of HMGA2 has also been described in gastric cancer[19]. The study showed that the overexpression of HMGA2 in gastric cancer patients was significantly correlated with poor prognosis and low overall survival. However, the exact mechanism of HMGA2 in gastric cancer is not clear.

The phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) signaling pathway plays a central role in many human cancers. Akt regulates cell proliferation, differentiation and apoptosis *via* activation or inhibition of downstream target proteins, such as P27, caspase-9 and B-cell leukemia/lymphoma-2 (Bcl-2)[20,21].

The aims of the present study were to investigate the effect of HMGA2 expression on gastric cancer cell proliferation and apoptosis. Moreover, the expressions of PI3K, Akt, P27, caspase-9 and Bcl-2 were detected to exploit the possible mechanism.

MATERIALS AND METHODS

Construction of HMGA2- shRNA expression plasmid

The human *HMGA2* was treated as the target gene (Accession Number: NM-003484) and four pairs of short hairpin RNA (shRNA) oligonucleotides were designed and synthesized (Cyagen Biosciences Inc., United States). A pair of random scrambled sequences, which have no homologous relation with any human gene sequences and whose shRNAs would not interfere with human mRNA were also designed (Table 1). The complementary form was obtained by annealing, which was then cloned and inserted into vector pLLU2 to construct a recombinant plasmid. The plasmid was transformed into stb13 strain. Finally, the plasmid was identified by restriction enzyme digestion and sequenced (Cyagen Biosciences Inc., United States). According to the preliminary experimental results, the first pair shRNA oligonucleotides (Table 1) was the best interfering sequence, and the recombinant plasmid harboring it was used for subsequent experiments.

Cell culture and transfection

Gastric cancer MKN-45 cells (ATCC, United States) were cultured in Dulbecco's modified Eagle's medium (DMEM) (Solarbio Science and Technology Co., Ltd., Beijing, China) containing 10% placental bovine serum. After 24 h incubation at 37 ℃ with 5% CO_2, logarithmic growth phase cells in good condition were digest by 0.25% trypsin + ethylenediaminetetraacetic acid. After calculating the cell number, cells were seeded into 6-well plates at 1×10^5 cells per well. When the cells reached more than 80% confluence (usually the next day), plasmid and lipofectamine were added dropwise to form a mixture of 1:1 with mixing. After culturing in serum-free and antibiotic-free DMEM for 6 h, the culture medium was replaced by DMEM and the cultured was continued for 24 h. The cells were then transfected using a Lipofectamine™2000 Transfection Kit (Invitrogen, United States) according to the manufacturer's instructions. Cells were randomly counted in 10 microscope fields at 24, 48 and 72 h after transfection. The ratio of the number of cells with green fluorescent emission and the total cells number indicated the transfection efficiency. Non-transfected cells from the same batch were collected as the blank control group. The cells transfected with the random scrambled sequence plasmid served as the scrambled sequence group.

Reverse transcription polymerase chain reaction analysis

Total RNA was isolated using TRIzol (Sangon Biotech Co., Ltd., Shanghai, China). A Reverse Transcriptase Kit (Fermentas China Co., Ltd.) was used to create cDNA, according to the manufacturer's instructions: 1 μg RNA, Oligo dT_{18} as the primer, and 42 ℃ for 60 min followed by 70 ℃ for 5 min. Reverse transcriptase polymerase

Table 1 Short hairpin RNA oligo sequences targeting high mobility group A2

Oligo name	Oligo sequence 5′ to 3′
shHMGA2-1-F	TAGTCCCTCTAAAGCAGCTCAACTCGAGTTGAGCTGCTTTAGAGGGACTTTTTTC
shHMGA2-1-R	TCGAGAAAAAAGTCCCTCTAAAGCAGCTCAACTCGAGTTGAGCTGCTTTAGAGGGACTA
shHMGA2-2-F	TAGGAGGAAACTGAAGAGACATCTCGAGATGTCTCTTCAGTTTCCTCCTTTTTTC
shHMGA2-2-R	TCGAGAAAAAAGGAGGAAACTGAAGAGACATCTCGAGATGTCTCTTCAGTTTCCTCCTA
shHMGA2-3-F	TCTCACAAGAGTCTGCCGAAGACTCGAG TCTTCGGCAGACTCTTGTGAGTTTTTC
shHMGA2-3-R	TCGAGAAAAACTCACAAGAGTCTGCCGAAGACTCGAGTCTTCGGCAGACTCTTGTGAGA
Scrambled-F	TGCGCGCTTTGTAGGATTCGCTCGAGCGAATCCTACAAAGCGCGCTTTTTC
Scrambled-R	TCGAGAAAAAGCGCGCTTTGTAGGATTCGCTCGAGCGAATCCTACAAAGCGCGCA

shHMGA2: Short hairpin high mobility group A2; F: Forward; R: Reserve.

chain reaction (RT-PCR) assays were carried out using on an ABI 2720 (Applied Biosystems, Foster City, CA, United States). The PCR primers (synthesized by Sangon Biotech Co., Ltd., Shanghai, China) used to detect HMGA2 and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were as follows: HMGA2, upstream primer 5'-TACTCTGTCTCTGCCTGTGC-3', downstream primer 5'-GGAGTGAATTGTGTCCCTTGA-3' (product length 228 bp); GAPDH, upstream primer 5'-GAAGGTGAAGGTCGGAGTC-3', downstream primer 5'-TCACACCCATGACGAACAT -3' (product length 401 bp). The 25 μL PCR amplification reaction mixture contained: cDNA, 1.5 μL; Taq Mix, 12.5 μL; 10 μmoL/L; each primer, 1 μL; RNase-free Water, 9 μL. Amplification reaction program: initial denaturation at 95 ℃ for 5 min; 35 cycles of denaturation at 94 ℃ for 30 s, annealing at 58 ℃ for 35 s, extension at 72 ℃ for 30 s; and final extension at 72 ℃ for 7 min. The PCR products were analyzed on agarose gels; gel analysis software was used to analyze the bands. The gray value ratio of the objective band and the β-actin band indicated the relative expression levels of HMGA2 mRNA.

Western blotting analysis of the protein expression levels of HMGA2, PI3K, Akt, P27, caspase-9 and Bcl-2

Cells were lysed in RIPA cell lysis buffer (Sangon Biotech Co., Ltd., Shanghai, China). The ratio of lysate and loading buffer was 4:1, degeneration and proteins were extracted. About 80 μg of proteins were fractionated by electrophoresis on sodium dodecyl sulfate-polyacrylamide gels and electroblotted onto a polyvinylidene fluoride membrane. The membrane was blocked with 5% nonfat milk for 2 h at room temperature and incubated overnight with the primary antibody (Santa Cruz Biotechnology, inc., United States). The membrane was washed with Tris-Buffered-Saline with Tween (TBST) three times for 10 min each, and then incubated for 2 h at room temperature with the secondary antibody (Zhongshanjinqiao Biotechnology Co., Ltd., Beijing, China). The membranes were washed with TBST 3 times for 10 min each, before being developed and fixed. β-actin (Zhongshanjinqiao Biotechnology Co., Ltd., Beijing, China) was used as an internal control. Gel analysis software was used to scan and analyze the bands. The gray value ratio of objective band and internal loading control band was used to determine the objective proteins expression levels.

Flow cytometric analysis of cell apoptosis

Each group of cells was digested into a single cell suspension using 0.25% trypsin. One hundred μL of buffer from the Annexin v-FITC and PE Apoptosis Detection Kit (BD Co., United States), 5 μL ADD and 5 μL PE were added to the cells, which were incubated without shaking at room temperature for 15 min in the dark. About 400 μL of buffer was then added, then the apoptosis rate of each group of cells was assayed by flow cytometry.

In vitro cell proliferation assay

A suspension of each group of cells was obtained by 0.25% trypsin digestion. The cells were centrifuged (1000 *g*, 5 min), resuspended in 1 mL culture medium and counted. A cell suspension containing 2×10^4/200 μL cells was distributed into 96-well plates: six sets of parallel holes were used for each group. The cells were then incubated at 37 ℃ in 5% CO_2. One 96-well plate was removed daily and 20 μL per well of a fresh new methyl thiazol tetrazolium (MTT) solution (5 mg/mL) was added, and the plate incubated at 37 ℃ for 4 h. The supernatants were removed, and 150 μL of dimethyl sulfoxide was added to each well and the plates were oscillated for 10 min. The optical density value (*A* value) of the cells in each well was measured by an enzyme-labeling instrument at a wavelength of 570 nm, and a growth curve for the cells was drawn. The cell proliferation inhibition rate (CPIR) was calculated according to the formula: CPIR (%) = $(1 - A_{\text{experimental group}} / A_{\text{control group}}) \times 100\%$.

Statistical analysis

SPSS 13.0 software (Chicago, United States) was used to perform the statistical analysis. Data were expressed as mean ± SD. One-way analysis of variance was used to analyze the correlation of data among three or more groups, and an *SNK* test was used for pairwise comparison between groups. $P < 0.05$ was considered significant.

RESULTS

Fluorescence microscopy analysis of cell transfection

The result showed that the transfection efficiency of the

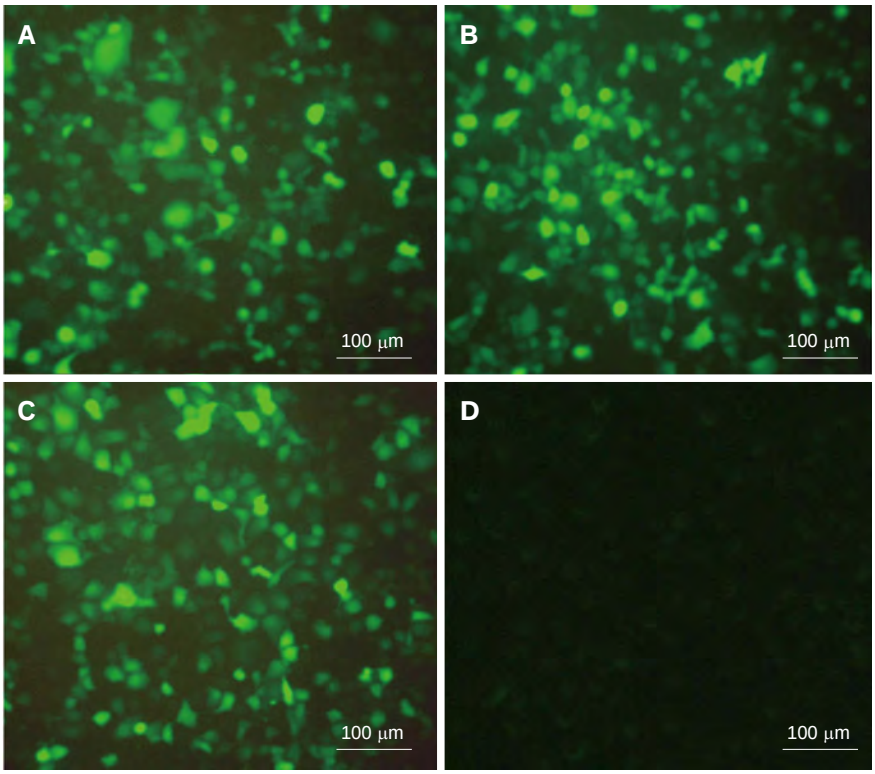


Figure 1 Cell transfection under the fluorescence microscope (magnification, ×400). A: Transfected group 24 h after transfection; B: Transfected group 48 h after transfection; C: Transfected group 72 h after transfection; D: Scrambled sequence group 48 h after transfection.

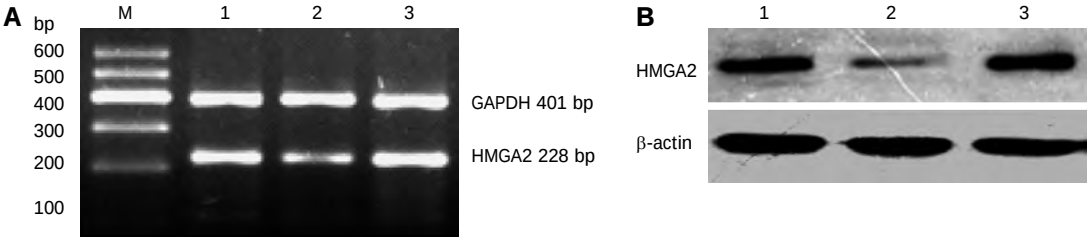


Figure 2 Expression of high mobility group A2 messenger RNA and high mobility group A2 protein. A: Expression of high mobility group A2 (*HMGA2*) messenger RNA (mRNA); B: Expression of HMGA2 protein. M: Marker; 1: Blank control group; 2: Transfected group; 3: Scrambled sequence group. GAPDH: Glyceraldehyde 3-phosphate dehydrogenase.

transfected group cells was 60%-70% after transfection for 48 h and was significantly higher than that at 24 h. Compared with 48 h, there was no significant change at 72 h. The scrambled sequence group cells were not transfected at any time (Figure 1).

Expression of HMGA2 mRNA in each group of cells

Compared with the blank control group and the scrambled sequence group, the expression of HMGA2 mRNA in the transfected group was significantly reduced (0.374 ± 0.048 *vs* 0.674 ± 0.129 and 0.689 ± 0.124, $P < 0.05$); however, the difference between the blank control group and the scrambled sequence group was not statistically significant ($P > 0.05$) (Figure 2). The results showed that the RNA interference had a targeted inhibition affect on the *HMGA2* gene.

Expression of HMGA2 protein in each group of cells

Compared with the blank control group and scrambled sequence group, the HMGA2 the protein expression was significantly decreased, the difference was statistically significant (0.113 ± 0.032 *vs* 0.554 ± 0.082 and 0.484 ± 0.123, $P < 0.05$); however, the difference between the blank control group and the scrambled sequence group was not statistically significant ($P > 0.05$) (Figure 2). The results showed that transfection of HMGA2 shRNA expression vector had significantly inhibited the protein expression from the target gene.

Apoptosis analysis of gastric cancer cells

The cell apoptotic rate of the transfected group was significantly higher than that of the blank control group and scrambled sequence group; the difference was statistically significant ($P < 0.05$). The highest cell apoptotic rate appeared at 48 h after transfection. The difference between the blank control group and the scrambled sequence group was not statistically significant ($P > 0.05$) (Figure 3).

Cell proliferation assay

The MTT assay revealed that there were remarkable dif-

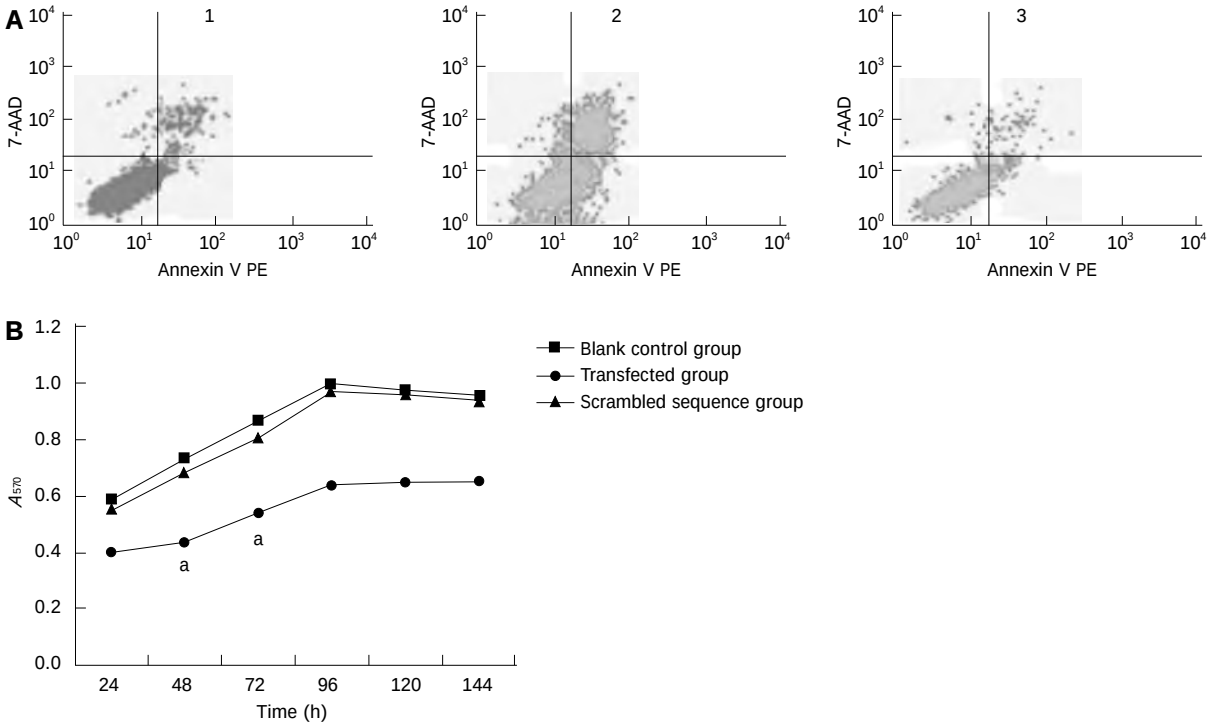


Figure 3 Apoptosis and cell proliferation analysis. A: Flow cytometry. The cell apoptotic rate of the transfected group was significantly higher than those of the blank control group and the scrambled sequence group; B: Cell proliferation analysis. $^{a}P < 0.05$ *vs* black control group. 1: Blank control group; 2: Transfected group; 3: Scrambled sequence group.

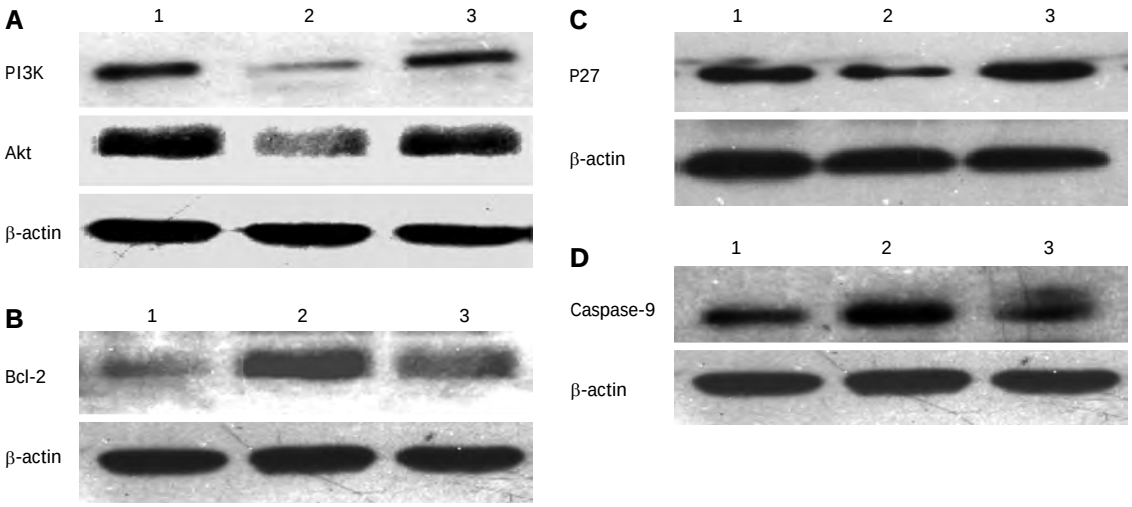


Figure 4 Western blotting analysis of protein expression. A: Phosphatidylinositol 3-kinase (PI3K), protein kinase B (Akt); B: B-cell leukemia/lymphoma-2 (Bcl-2); C: P27; D: Caspase-9. 1: Blank control group; 2: Transfected group; 3: Scrambled sequence group.

ferences in cell proliferation at 24, 48 and 72 h after transfection with shRNA. The most obvious cell proliferation inhibition appeared at 48 h after transfection in the transfected group ($P < 0.05$). There was no significant difference in the inhibition rate of the blank control group and the scrambled sequence group ($P > 0.05$) (Figure 3).

Expression of PI3K, Akt, P27, caspase-9 and Bcl-2 proteins

Compared with the blank control group and scrambled sequence group, after transfection of shRNA for 72 h, the protein expression levels of PI3K (0.042 ± 0.005 *vs* 0.069 ± 0.003 and 0.067 ± 0.05, $P < 0.05$), Akt (0.248 ± 0.004 *vs* 0.489 ± 0.006 and 0.496 ± 0.104, $P < 0.05$) and Bcl-2 (0.295 ± 0.084 *vs* 0.592 ± 0.072 and 0.594 ± 0.109, $P < 0.05$) were significantly reduced. Difference between the blank control group and the scrambled sequence group were statistically significant ($P > 0.05$) (Figure 4A and B). The protein expression levels of P27 (0.151 ± 0.010 *vs* 0.068 ± 0.014 and 0.060 ± 0.013, $P < 0.05$) and caspase-9 (0.136 ± 0.042 *vs* 0.075 ± 0.010 and 0.073 ± 0.072, $P < 0.05$) were significantly upregulated. The difference between the blank control group and the

scrambled sequence group was not statistically significant ($P > 0.05$) (Figure 4C and D).

DISCUSSION

Activation of oncogenes, inactivation of suppressor genes and abnormal expression of apoptosis-related genes are closely related to carcinogenesis and progression of gastric cancer. HMGA2 is a transcription factor that is involved in transcriptional regulation of various tumor-related genes[22]. Several studies[14,15,23] have showed that HMGA2 plays a critical role in the processes of many tumors, including enhancing cell growth and invasion. Moreover, the prognosis of patients with high HMGA2 expression is worse than those with low HMGA2 expression[24,25]. The clinical association of HMGA2 with gastric cancer has been reported[19]. The study showed that overexpression of HMGA2 appeared in human gastric cancer, and might be involved in cancer invasion and metastasis. However, the exact mechanism of HMGA2's involvement in gastric cancer is not clear, especially in relation to signaling pathways and the molecular mechanism.

In the present study, fluorescence microscopy revealed that the transfection efficiency of the transfected group cells can reach about 60%-70%, and that recombinant plasmid was effectively expressed in cells. HMGA2 shRNA transfection could effectively inhibit the expression of *HMGA2* gene. The inhibition was achieved without off-target effects, which was confirmed at the mRNA and protein levels by RT-PCR and Western blotting. According to the results of MTT and flow cytometry, cell proliferation was inhibited and apoptosis was increased after transfection. Therefore, we conclude that HMGA2 shRNA gene silencing can effectively induce proliferation inhibition and apoptosis of MKN-45 cells *in vitro* without off-target effects.

The PI3K/Akt signaling pathway plays important roles in cell survival and resistance to apoptosis[26]; it has been shown to be activated in many cancers and activated Akt acts to phosphorylate P27, caspase-9 and Bcl-2 to promote the resistance of cancer cells to apoptosis[20,21]. A study showed that activation of the PI3K/Akt signaling pathway is induced by overexpression of HMGA2[27]. In this study, western blotting showed that the expressions of PI3K and Akt protein were reduced in the transfected group. Thus, decreased expression of HMGA2 inhibits the activity of thePI3K/Akt signaling pathway.

P27 is a cyclin-dependent kinase (CDK) inhibitor that functions as a fail-safe mechanism in DNA repair and apoptosis[28]. A report showed that the *P27* gene reduced cell mitosis division and inhibited cell generation[29]. Overexpression of P27 inhibits CDK activation and entry into the S phase of the cell cycle[30]. The expression level of P27 was positively correlated with cell differentiation, and its loss of function may subsequently contribute to tumorigenesis[31]. Caspase-9, a member of the protease family, is intimately associated with the initiation of apoptosis, and is thought to be activated, while Akt is inhibited[32]. Activated caspase-9 is able to cleave caspase-3, leading to apoptosis[33]. Bcl-2 is a key anti-apoptotic protein involved in the regulation of apoptosis and is overexpressed in many tumors[34-36]. A previous study showed that Bcl-2 delays cell cycle entry by inhibiting Myc activity through the elevation of p27. Bcl-2 can be induced to express *via* the PI3K/Akt signaling pathway and inhibit cell apoptosis[21,37].

In our study, western blotting revealed that the protein expression levels of Bcl-2 were reduced, whereas the protein expression level of P27 and caspase-9 were upregulated. Thus, decreased expression of HMGA2 inhibits the activity of the PI3K/Akt signaling pathway, and than inhibits cells proliferation and increases apoptosis *via* upregulation of the protein expression of P27 and caspase-9, and decreased protein expression of Bcl-2.

In summary, our results demonstrated that HMGA2 shRNA gene silencing could induce apoptosis and suppress proliferation of MKN-45 cells *in vitro*. The PI3K/Akt signaling pathway, P27, caspase-9 and Bcl-2 may play important roles in this HMGA2-mediated effect. We suggest that HMGA2 shRNA gene silencing may be suitable for gastric cancer therapy by suppressing the proliferation and inducing apoptosis of gastric cancer cells.

COMMENTS

Background

The newly discovered oncogene, high mobility group A2 (*HMGA2*), is an endonuclear chromatin protein, which is highly expressed in many malignant tumors. The mechanism by which HMGA2 causes tumors remains unclear, particularly in gastric cancer.

Research frontiers

RNA interference can specifically mediate degradation of HMGA2 messenger RNA, block the expression of the gene, inhibit cell proliferation and increase apoptosis, which could be exploited for gastric cancer gene therapy.

Innovations and breakthroughs

This study demonstrated that RNA interference (RNAi) specifically downregulated the expression of HMGA2, inhibited cell proliferation and increased apoptosis. The mechanism appears to be that the decreased expression of HMGA2 inhibits the activity of the phosphatidylinositol 3-kinase (PI3K)/protein kinase B (Akt) signaling pathway. Inhibition of cell proliferation and increased apoptosis is associated with upregulated protein expression of P27 and caspase-9, and decreasing expression of B-cell leukemia/lymphoma-2 (Bcl-2).

Applications

The aims of this study were to investigate the effect of HMGA2 expression on gastric cancer cell proliferation and apoptosis. RNAi was used regulate the expression of HMGA2. Moreover, the expression of PI3K, Akt, P27, caspase-9 and Bcl-2 were detected to investigate the possible mechanism. This will help enrich the theoretical research into the pathogenesis of gastric cancer, and provide an experimental basis for clinical diagnosis and gene therapy of gastric cancer.

Peer review

This is a well-written manuscript and the results are interesting. It has potential application in gastric cancer gene therapy.

REFERENCES

1 **Jemal A**, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. *CA Cancer J Clin* 2011; **61**: 69-90 [PMID: 21296855 DOI: 10.3322/caac.20107]
2 **Di Cello F**, Hillion J, Hristov A, Wood LJ, Mukherjee M,

Schuldenfrei A, Kowalski J, Bhattacharya R, Ashfaq R, Resar LM. HMGA2 participates in transformation in human lung cancer. *Mol Cancer Res* 2008; **6**: 743-750 [PMID: 18505920 DOI: 10.1158/1541-7786.mcr-07-0095]

3 **Lanahan A**, Williams JB, Sanders LK, Nathans D. Growth factor-induced delayed early response genes. *Mol Cell Biol* 1992; **12**: 3919-3929 [PMID: 1508193]

4 **Narita M**, Narita M, Krizhanovsky V, Nuñez S, Chicas A, Hearn SA, Myers MP, Lowe SW. A novel role for high-mobility group a proteins in cellular senescence and heterochromatin formation. *Cell* 2006; **126**: 503-514 [PMID: 16901784 DOI: 10.1016/j.cell.2006.05.052]

5 **Rogalla P**, Drechsler K, Frey G, Hennig Y, Helmke B, Bonk U, Bullerdiek J. HMGI-C expression patterns in human tissues. Implications for the genesis of frequent mesenchymal tumors. *Am J Pathol* 1996; **149**: 775-779 [PMID: 8780382]

6 **Gattas GJ**, Quade BJ, Nowak RA, Morton CC. HMGIC expression in human adult and fetal tissues and in uterine leiomyomata. *Genes Chromosomes Cancer* 1999; **25**: 316-322 [PMID: 10398424]

7 **Fabjani G**, Tong D, Wolf A, Roka S, Leodolter S, Hoecker P, Fischer MB, Jakesz R, Zeillinger R. HMGA2 is associated with invasiveness but not a suitable marker for the detection of circulating tumor cells in breast cancer. *Oncol Rep* 2005; **14**: 737-741 [PMID: 16077985]

8 **Hristov AC**, Cope L, Reyes MD, Singh M, Iacobuzio-Donahue C, Maitra A, Resar LM. HMGA2 protein expression correlates with lymph node metastasis and increased tumor grade in pancreatic ductal adenocarcinoma. *Mod Pathol* 2009; **22**: 43-49 [PMID: 18843278 DOI: 10.1038/modpathol.2008.140]

9 **Piscuoglio S**, Zlobec I, Pallante P, Sepe R, Esposito F, Zimmermann A, Diamantis I, Terracciano L, Fusco A, Karamitopoulou E. HMGA1 and HMGA2 protein expression correlates with advanced tumour grade and lymph node metastasis in pancreatic adenocarcinoma. *Histopathology* 2012; **60**: 397-404 [PMID: 22276603 DOI: 10.1111/j.1365-2559.2011.04121.x]

10 **Liu Q**, Lv GD, Qin X, Gen YH, Zheng ST, Liu T, Lu XM. Role of microRNA let-7 and effect to HMGA2 in esophageal squamous cell carcinoma. *Mol Biol Rep* 2012; **39**: 1239-1246 [PMID: 21598109 DOI: 10.1007/s11033-011-0854-7]

11 **Wu Y**, Song Y, Liu H. Expression and its clinical significance of HMGA2 in the patients with non-small cell lung cancer. *Zhongguo Feiai Zazhi* 2008; **11**: 377-381 [PMID: 20731938 DOI: 10.3779/j.issn.1009-3419.2008.03.005]

12 **Miyazawa J**, Mitoro A, Kawashiri S, Chada KK, Imai K. Expression of mesenchyme-specific gene HMGA2 in squamous cell carcinomas of the oral cavity. *Cancer Res* 2004; **64**: 2024-2029 [PMID: 15026339]

13 **Langelotz C**, Schmid P, Jakob C, Heider U, Wernecke KD, Possinger K, Sezer O. Expression of high-mobility-group-protein HMGI-C mRNA in the peripheral blood is an independent poor prognostic indicator for survival in metastatic breast cancer. *Br J Cancer* 2003; **88**: 1406-1410 [PMID: 12778070 DOI: 10.1038/sj.bjc.6600935]

14 **Park SM**, Shell S, Radjabi AR, Schickel R, Feig C, Boyerinas B, Dinulescu DM, Lengyel E, Peter ME. Let-7 prevents early cancer progression by suppressing expression of the embryonic gene HMGA2. *Cell Cycle* 2007; **6**: 2585-2590 [PMID: 17957144]

15 **Malek A**, Bakhidze E, Noske A, Sers C, Aigner A, Schäfer R, Tchernitsa O. HMGA2 gene is a promising target for ovarian cancer silencing therapy. *Int J Cancer* 2008; **123**: 348-356 [PMID: 18452175 DOI: 10.1002/ijc.23491]

16 **Huang ML**, Chen CC, Chang LC. Gene expressions of HMGI-C and HMGI(Y) are associated with stage and metastasis in colorectal cancer. *Int J Colorectal Dis* 2009; **24**: 1281-1286 [PMID: 19609535 DOI: 10.1007/s00384-009-0770-7]

17 **Wang X**, Liu X, Li AY, Chen L, Lai L, Lin HH, Hu S, Yao L, Peng J, Loera S, Xue L, Zhou B, Zhou L, Zheng S, Chu P, Zhang S, Ann DK, Yen Y. Overexpression of HMGA2 promotes metastasis and impacts survival of colorectal cancers. *Clin Cancer Res* 2011; **17**: 2570-2580 [PMID: 21252160 DOI: 10.1158/1078-0432.ccr-10-2542]

18 **Chau K**, Arlotta P, Patel UA, Crane-Robinson C, Manfioletti G, Ono SJ. A novel downstream positive regulatory element mediating transcription of the human high mobility group (HMG) I-C gene. *FEBS Lett* 1999; **457**: 429-436 [PMID: 10471823 DOI: 10.1016/bbr.2011.03.031]

19 **Motoyama K**, Inoue H, Nakamura Y, Uetake H, Sugihara K, Mori M. Clinical significance of high mobility group A2 in human gastric cancer and its relationship to let-7 microRNA family. *Clin Cancer Res* 2008; **14**: 2334-2340 [PMID: 18413822 DOI: 10.1158/1078-0432.ccr-07-4667]

20 **Liang J**, Slingerland JM. Multiple roles of the PI3K/PKB (Akt) pathway in cell cycle progression. *Cell Cycle* 2003; **2**: 339-345 [PMID: 12851486]

21 **Wang XT**, Pei DS, Xu J, Guan QH, Sun YF, Liu XM, Zhang GY. Opposing effects of Bad phosphorylation at two distinct sites by Akt1 and JNK1/2 on ischemic brain injury. *Cell Signal* 2007; **19**: 1844-1856 [PMID: 17555943 DOI: 10.1016/j.cellsig.2007.04.005]

22 **Zhang Q**, Wang Y. Homeodomain-interacting protein kinase-2 (HIPK2) phosphorylates HMGA1a at Ser-35, Thr-52, and Thr-77 and modulates its DNA binding affinity. *J Proteome Res* 2007; **6**: 4711-4719 [PMID: 17960875 DOI: 10.1021/pr700571d]

23 **Watanabe S**, Ueda Y, Akaboshi S, Hino Y, Sekita Y, Nakao M. HMGA2 maintains oncogenic RAS-induced epithelial-mesenchymal transition in human pancreatic cancer cells. *Am J Pathol* 2009; **174**: 854-868 [PMID: 19179606 DOI: 10.2353/ajpath.2009.080523]

24 **Venkatesan N**, Kandalam M, Pasricha G, Sumantran V, Manfioletti G, Ono SJ, Reddy MA, Krishnakumar S. Expression of high mobility group A2 protein in retinoblastoma and its association with clinicopathologic features. *J Pediatr Hematol Oncol* 2009; **31**: 209-214 [PMID: 19262251 DOI: 10.1097/MPH.0b013e318197978d]

25 **Liu X**, Lai L, Wang X, Xue L, Leora S, Wu J, Hu S, Zhang K, Kuo ML, Zhou L, Zhang H, Wang Y, Wang Y, Zhou B, Nelson RA, Zheng S, Zhang S, Chu P, Yen Y. Ribonucleotide reductase small subunit M2B prognoses better survival in colorectal cancer. *Cancer Res* 2011; **71**: 3202-3213 [PMID: 21415168 DOI: 10.1158/0008-5472.CAN-11-0054]

26 **Sos ML**, Fischer S, Ullrich R, Peifer M, Heuckmann JM, Koker M, Heynck S, Stückrath I, Weiss J, Fischer F, Michel K, Goel A, Regales L, Politi KA, Perera S, Getlik M, Heukamp LC, Ansén S, Zander T, Beroukhim R, Kashkar H, Shokat KM, Sellers WR, Rauh D, Orr C, Hoeflich KP, Friedman L, Wong KK, Pao W, Thomas RK. Identifying genotype-dependent efficacy of single and combined PI3K- and MAPK-pathway inhibition in cancer. *Proc Natl Acad Sci USA* 2009; **106**: 18351-18356 [PMID: 19805051 DOI: 10.1073/pnas.0907325106]

27 **Ikeda K**, Mason PJ, Bessler M. 3'UTR-truncated Hmga2 cDNA causes MPN-like hematopoiesis by conferring a clonal growth advantage at the level of HSC in mice. *Blood* 2011; **117**: 5860-5869 [PMID: 21460244 DOI: 10.1182/blood-2011-02-334425]

28 **Sherr CJ**, Roberts JM. CDK inhibitors: positive and negative regulators of G1-phase progression. *Genes Dev* 1999; **13**: 1501-1512 [PMID: 10385618]

29 **Zhang WG**, Yu JP, Wu QM, Tong Q, Li SB, Wang XH, Xie GJ. Inhibitory effect of ubiquitin-proteasome pathway on proliferation of esophageal carcinoma cells. *World J Gastroenterol* 2004; **10**: 2779-2784 [PMID: 15334669]

30 **Polyak K**, Kato JY, Solomon MJ, Sherr CJ, Massague J, Roberts JM, Koff A. p27Kip1, a cyclin-Cdk inhibitor, links transforming growth factor-beta and contact inhibition to

cell cycle arrest. *Genes Dev* 1994; **8**: 9-22 [PMID: 8288131]
31 **Choi HR**, Tucker SA, Huang Z, Gillenwater AM, Luna MA, Batsakis JG, El-Naggar AK. Differential expressions of cyclin-dependent kinase inhibitors (p27 and p21) and their relation to p53 and Ki-67 in oral squamous tumorigenesis. *Int J Oncol* 2003; **22**: 409-414 [PMID: 12527941]
32 **Shultz JC**, Goehe RW, Wijesinghe DS, Murudkar C, Hawkins AJ, Shay JW, Minna JD, Chalfant CE. Alternative splicing of caspase 9 is modulated by the phosphoinositide 3-kinase/Akt pathway via phosphorylation of SRp30a. *Cancer Res* 2010; **70**: 9185-9196 [PMID: 21045158 DOI: 10.1158/0008-5472.can-10-1545]
33 **Yamakawa N**, Takahashi A, Mori E, Imai Y, Furusawa Y, Ohnishi K, Kirita T, Ohnishi T. High LET radiation enhances apoptosis in mutated p53 cancer cells through Caspase-9 activation. *Cancer Sci* 2008; **99**: 1455-1460 [PMID: 18422753 DOI: 10.1111/j.1349-7006.2008.00818.x]
34 **Green DR**, Reed JC. Mitochondria and apoptosis. *Science* 1998; **281**: 1309-1312 [PMID: 9721092]
35 **Azad N**, Vallyathan V, Wang L, Tantishaiyakul V, Stehlik C, Leonard SS, Rojanasakul Y. S-nitrosylation of Bcl-2 inhibits its ubiquitin-proteasomal degradation. A novel antiapoptotic mechanism that suppresses apoptosis. *J Biol Chem* 2006; **281**: 34124-34134 [PMID: 16980304 DOI: 10.1074/jbc.M602551200]
36 **Thomadaki H**, Scorilas A. BCL2 family of apoptosis-related genes: functions and clinical implications in cancer. *Crit Rev Clin Lab Sci* 2006; **43**: 1-67 [PMID: 16531274 DOI: 10.1080/10408360500295626]
37 **Greider C**, Chattopadhyay A, Parkhurst C, Yang E. BCL-x(L) and BCL2 delay Myc-induced cell cycle entry through elevation of p27 and inhibition of G1 cyclin-dependent kinases. *Oncogene* 2002; **21**: 7765-7775 [PMID: 12420213 DOI: 10.1038/sj.onc.1205928]

P- Reviewer Mukherjee SK **S- Editor** Gou SX
L- Editor Stewart GJ **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i8.1247

World J Gastroenterol 2013 February 28; 19(8): 1247-1255
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Predictors of colorectal cancer testing using the California Health Inventory Survey

Alexandra Modiri, Kian Makipour, Javier Gomez, Frank Friedenberg

Alexandra Modiri, Kian Makipour, Javier Gomez, Frank Friedenberg, Section of Gastroenterology, Department of Medicine, Temple University Hospital, Philadelphia, PA 19140, United States
Author contributions: All authors had access to the data and a role in writing the manuscript.
Supported by Grant K24 DK83268 awarded to Friedenberg F
Correspondence to: Frank Friedenberg, MD, MS (Epi), Section of Gastroenterology, Department of Medicine, Temple University Hospital, 3401 North Broad Street, Philadelphia, PA 19140, United States. friedfk@tuhs.temple.edu
Telephone: +1-215-7073431 Fax: +1-215-7072684
Received: August 14, 2012 Revised: November 18, 2012
Accepted: December 25, 2012
Published online: February 28, 2013

Abstract

AIM: To identify key variables associated with colon cancer testing using the 2009 California Health Inventory Survey (CHIS).

METHODS: The CHIS has been conducted biennially since 2001 using a two-stage, geographically stratified random-digit-dial sample design to produce a representative sample of the entire State. For this study we used survey data from 2001-2009 inclusive. We restricted our analysis to White, Black, and Hispanic/Latinos aged 50-80 years. Weighted data was used to calculate the proportion of participants who underwent some form of colon cancer testing (colonoscopy, flexible sigmoidoscopy or fecal occult blood testing) within the previous 5 years stratified by race/ethnicity. For inferential analysis, boot-strapping with replacement was performed on the weighted sample to attain variance estimates at the 95%CI. For mean differences among categories we used *t*-tests and for comparisons of categorical data we used Pearson's χ^2. Binary logistic regression was used to identify independent variables associated with undergoing some form of testing. Trend analysis was performed to determine rates of testing over the study period stratified by race.

RESULTS: The CHIS database for 2009 had 30 857 unique respondents corresponding to a weighted sample size of 10.6 million Californians. Overall, 63.0% (63.0-63.1) underwent a colon cancer test within the previous 5 years; with 70.5% (70.5%-70.6%) of this subset having undergone colonoscopy. That is 44.5% (44.4%-44.5%) of all individuals 50-80 underwent colonoscopy. By multivariable regression, those tested were more likely to be male (OR = 1.06; 95%CI: 1.06-1.06), Black (OR = 1.30; 95%CI: 1.30-1.31), have a family member with colon cancer (OR = 1.71; 95%CI: 1.70-1.72), and have health insurance (OR = 2.71; 95%CI: 2.70-2.72). Progressive levels above the poverty line were also associated with receiving a test (100%-199%: 1.21; 1.20-1.21), (200%-299%:1.41; 1.40-1.42), (> 300:1.69; 1.68-1.70). The strongest variable was physician recommendation (OR = 3.90; 95%CI: 3.88-3.91). For the Hispanic/Latino group, additional variables associated with testing were success of physician-patient communication (OR = 2.44; 95%CI: 2.40-2.48) and naturalized citizenship status (OR = 1.91; 95%CI: 1.89-1.93). Trend analysis demonstrated increased colon cancer testing for all racial/ethnic subgroups from 2001-2009 although the rate remained considerably lower for the Hispanic/Latino subgroup.

CONCLUSION: Using CHIS we identified California citizens most likely to undergo colon cancer testing. The strongest variable associated with testing for all groups was physician recommendation.

© 2013 Baishideng. All rights reserved.

Key words: California Health Inventory Survey; Colon cancer testing; Colonoscopy; Hispanic

Modiri A, Makipour K, Gomez J, Friedenberg F. Predictors of colorectal cancer testing using the California Health Inventory Survey. *World J Gastroenterol* 2013; 19(8): 1247-1255 Available from: URL: http://www.wjgnet.com/1007-9327/

full/v19/i8/1247.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i8.1247

INTRODUCTION

Colorectal cancer is the fourth leading cause of new cancer cases diagnosed in the United States and is the second leading cause of cancer death[1]. Population-based surveys such as the Behavior Risk Factor Surveillance System (BRFSS) and the National Health Interview Survey (NHIS) suggest that colon cancer screening tests are underutilized in the United States[2]. Based on Surveillance, Epidemiology and End Results (SEER) data, adults over the age of 50 demonstrate a low prevalence of colon cancer screening[2-5]. One study reported that only 59% of adults aged ⩾ 50 years were screened for colon cancer[6].

Colon cancer can be prevented in most cases by strict adherence to accepted colon cancer screening guidelines[7]. A position paper co-authored by several specialty societies listed flexible sigmoidoscopy, Computed tomography colonography, barium enema every 5 years, or colonoscopy every 10 years in average risk adults ⩾ 50 years of age as acceptable for the detection of adenomatous polyps and cancer[7-10]. Annual fecal occult blood testing (FOBT), preferably with immunochemical methodology, as an acceptable alternative method for cancer screening[7,8]. Cancer screening using stool DNA remains under investigation[8,10]. Despite a lack of evidence from randomized controlled trials, colonoscopy has been shown to be a more sensitive test for the detection of adenomas and colon cancer than flexible sigmoidoscopy +/- FOBT[11,12].

Factors identified as positive predictors of colon cancer screening participation have included older age, male gender, married status, negative smoking history, higher education, white non-Hispanic race, family history of colon cancer, regular medical care, physician recommendation, participation in other preventive health care services, and health insurance coverage[2,4,13-26].

The California Health Inventory Survey (CHIS) is the nation's largest state health survey. A random-dial telephone survey is conducted every two years on a wide range of health topics. CHIS data gives a detailed picture of the health care needs of California's large and diverse population. More than 50 000 Californians including adults, teenagers and children, are surveyed[27]. The specific aim of this study was to explore the CHIS database to identify demographic, socioeconomic, health and behavioral factors associated with participation in colon cancer testing. In addition, another aim was to perform a detailed analysis of the Latino population.

MATERIALS AND METHODS

CHIS is a collaborative project of the University of California at Los Angeles Center for Health Policy Research, the California Department of Health Services, and the Public Health Institute. (http://www.chis.ucla.edu/methodology.html). The survey has been conducted biennially since 2001 using a two-stage, geographically stratified random-digit-dial sample design to produce a representative sample of the State. At the first stage, telephone numbers are drawn within predefined geographic areas or "strata". Telephone numbers are screened to determine if they are households and thus eligible for the survey. At the second stage, one adult is randomly selected among all adults living in a household.

Questionnaires in CHIS include, among others, health status, health conditions, physical disability, mental health, health behaviors, women's health, cancer history and prevention, food environment, neighborhood and housing, access to and use of health care, health insurance, public program eligibility, interpersonal violence, employment, income and demographics including preferred spoken language, language of TV, radio, and newspaper. Participant citizenship, immigration status, country of birth and English language proficiency is also ascertained.

Data processing

For this study CHIS data from 2001-2009 was downloaded in SPSS v19 (IBM, Armonk, NY, United States) format. Each survey was individually weighted using the final sample weight (RAKEDW0). RAKEDW0 accounts for sample selection probabilities and statistical adjustments for possible under coverage and nonresponse biases. It is essential to weigh the data for valid variance estimation otherwise it will be underestimated.

Statistical analysis

We first analyzed the group aged 50-80 years from the most recent survey, 2009. Racial/ethnic analysis was limited to White, Black, and Hispanic/Latino groups as classified by the UCLA CHPR definition. For descriptive and inferential analyses, boot-strapping with replacement (iterations = 1000) was performed to attain variance estimates at the 95%CI[28,29]. This was performed using the bootstrapping module for SPSS. For mean differences among categories we used *t*-tests and for comparisons of categorical data we used Pearson's χ^2. We performed multivariable logistic regression using colon cancer testing (colonoscopy, flexible sigmoidoscopy or FOBT) as the dependent variable. Independent predictor variables entered into the model were those judged to be of clinical importance in univariate analysis. The output was expressed as odds ratios and their bootstrapped 95% confidence intervals. We subsequently stratified our sample and repeated the regression analysis for those in the Hispanic/Latino sub-group.

We used weighted CHIS data for 2001-2009 to calculate the proportion of participants who underwent some form of colon cancer testing within the previous 5 years stratified by race/ethnicity. The proportion was derived by dividing the number of individuals age 50-80 in each race who underwent testing in a given year by the total number of 50-80 year olds in that particular racial/ethnic

Table 1 Characteristics of California Health Inventory Survey participants ages 50-80 years (bootstrap 95%CI)

	Total *n* = 10 596 208	Colonoscopy/Flexible sigmoidoscopy/ Fecal occult blood test *n* = 6 678 773	Colonoscopy *n* = 4 711 189	No colonoscopy/Flexible sigmoidoscopy/Fecal occult blood test *n* = 3 917 435
Mean age, yr	63.33 (63.32-63.33)	64.19 (64.18-64.20)	64.69 (64.68-64.70)	61.86 (61.85-61.87)
Males, %	46.6 (46.5-46.6)	46.8 (46.7-46.8)	47.2 (47.1-47.2)	46.3 (46.2-46.3)
Body mass index, kg/m^2, %				
< 18.5	1.7 (1.6-1.7)	1.5 (1.5-1.5)	1.5 (1.5-1.6)	2.0 (1.9-2.0)
18.5-24.99	36.1 (36.0-36.1)	35.1 (35.0-35.1)	36.3 (36.3-36.4)	37.8 (37.8-37.9)
25.0-29.99	37.2 (37.1-37.2)	38.6 (38.6-38.7)	37.8 (37.8-37.9)	34.7 (34.7-34.8)
> 30.0	25.1 (25.1-25.1)	24.8 (24.8-24.9)	24.3 (24.3-24.3)	25.5 (25.5-25.6)
Ethnicity[1], %				
White	60.8 (60.8-60.9)	63.7 (63.7-63.8)	66.1 (66.1-66.2)	55.9 (55.8-56.0)
African American	6.1 (6.1-6.1)	6.3 (6.3-6.3)	6.0 (6.0-6.0)	5.8 (5.8-5.8)
Latino	14.8 (14.8-14.9)	12.2 (12.2-12.3)	10.6 (10.6-10.7)	19.2 (19.2-19.3)
Asian	11.3 (11.2-11.3)	10.7 (10.7-10.7)	10.5 (10.4-10.5)	12.2 (12.2-12.2)
Other	7.0 (7.0-7.0)	7.0 (7.0-7.0)	6.7 (6.6-6.7)	5.8 (5.7-5.8)
Smoking status, %				
Current	11.1 (11.1-11.2)	8.2 (8.2-8.3)	8.0 (8.0-8.0)	16.1 (16.1-16.1)
Former	35.9 (35.8-35.9)	37.8 (37.8-37.9)	38.4 (38.3-38.4)	32.5 (32.5-32.6)
Never	53.0 (52.9-53.0)	53.9 (53.9-54.0)	53.6 (53.6-53.7)	51.3 (51.3-51.4)
Did not complete high school, %	15.7 (15.7-15.8)	12.9 (12.9-13.0)	10.7 (10.7-10.8)	20.4 (20.4-20.5)
Marital status, %				
Married	65.0 (65.0-65.0)	67.9 (67.8-67.9)	68.8 (68.7-68.8)	60.1 (60.0-60.1)
Living w/partner	4.1 (4.1-4.1)	3.7 (3.7-3.8)	3.6 (3.6-3.6)	4.6 (4.6-4.7)
Widow/separated/divorced	25.1 (25.1-25.2)	23.7 (23.6-23.7)	23.2 (23.2-23.2)	27.7 (27.6-27.7)
Never married	5.8 (5.8-5.8)	4.7 (4.7-4.7)	4.4 (4.4-4.4)	7.6 (7.6-7.7)
Household income	73 671	79 191	81 798	64 260
(United States $)	(73 630-73 720)	(79 151-79 238)	(81 728-81 876)	(64 157-64 336)
Income relative to poverty level, %				
0-99	10.8 (10.8-10.8)	7.7 (7.7-7.8)	7.1 (7.1-7.1)	16.5 (16.4-16.5)
100-199	17.1 (17.1-17.2)	14.9 (14.8-14.9)	14.3 (14.3-14.3)	21.3 (21.2-21.3)
200-299	13.7 (13.6-13.7)	13.2 (13.2-13.3)	13.5 (13.5-13.5)	14.4 (14.4-14.5)
> 300	58.4 (58.4-58.4)	64.2 (64.1-64.2)	65.1 (65.1-65.2)	47.8 (47.6-47.9)
Dwelling[2], %				
Urban	39.7 (39.6-39.7)	39.5 (39.4-39.5)	37.6 (37.6-37.7)	40 (40.0-40.1)
Secondary city	20.3 (20.2-20.3)	19.5 (19.5-19.6)	19.4 (19.3-19.4)	21.4 (21.4-21.5)
Suburban	25.1 (25.1-25.1)	26.9 (26.9-27.0)	28.2 (28.2-28.3)	22.1 (22.0-22.1)
Rural	15.0 (14.9-15.0)	14.1 (14.0-14.1)	14.8 (14.8-14.8)	16.4 (16.4-16.5)
Insurance status, %				
Uninsured	9.5 (9.5-9.5)	4.6 (4.6-4.6)	3.3 (3.3-3.3)	17.8 (17.8-17.9)
Medicare and medicaid	8.9 (8.9-9.0)	8.0 (8.0-8.0)	8.5 (8.5-8.6)	10.5 (10.5-10.5)
Medicare and others	27.2 (27.2-27.2)	31.8 (31.8-31.9)	33.9 (33.9-34.0)	19.3 (19.3-19.4)
Medicare only	3.4 (3.4-3.4)	3.1 (3.1-3.1)	3.4 (3.4-3.4)	3.8 (3.8-3.8)
Medicaid	3.6 (3.6-3.6)	2.3 (2.3-2.3)	2.1 (2.0-2.1)	5.8 (5.7-5.8)
Employer-based	41.1 (41.0-41.1)	43.6 (43.6-43.6)	42.2 (42.1-42.2)	36.7 (36.7-36.7)
Privately purchased	4.6 (4.6-4.6)	4.6 (4.6-4.6)	4.6 (4.6-4.6)	4.6 (4.5-4.6)
Other	1.8 (1.8-1.8)	2.0 (2.0-2.0)	2.1 (2.0-2.1)	1.5 (1.5-1.5)
Alcohol use, %				
Never	93.3 (93.2-93.4)	93.5 (93.4-93.6)	93.5 (93.4-93.6)	93.0 (93.0-93.1)
Yes	6.7 (6.2-6.8)	6.6 (6.5-6.9)	6.5 (6.5-6.8)	6.9 (6.8-7.0)
Working status, %				
Employed full time	41.4 (41.4-41.4)	39.9 (39.8-39.9)	38.1 (38.1-38.2)	44.0 (43.9-44.0)
Employed part time	7.1 (7.1-7.1)	7.0 (7.0-7.1)	7.2 (7.2-7.2)	7.1 (7.1-7.2)
Mod-Severe work impaired	6.1 (6.1-6.1)	5.4 (5.4-5.4)	5.3 (5.3-5.4)	7.4 (7.4-7.5)
Spouse Employed F/P Time	32.6 (32.4-32.6)	33.6 (33.5-33.6)	33.4 (33.4-33.6)	30.8 (30.7-30.8)

[1]UCLA CHPR Definition; [2] By census tract.

category for that year. A two-tailed *P* value < 0.05 was considered significant for hypothesis testing.

RESULTS

Patients

There were 47 614 individual participants in the 2009 CHIS survey. This corresponded to a full weighted sample size of 27.6 million individuals from the State of California. There were 30 857 unique respondents between the ages of 50-80 years corresponding to a weighted sample of 10.6 million citizens. Table 1 categorizes these participants as to whether they received at least one colon cancer test within the previous 5 years and further identifies those who received colonoscopy. Overall, 63.0% (63.0-63.1) underwent a colon cancer test; with 70.5%

Table 2 Health screening issues and compliance as related to receiving a colonoscopy for California Health Inventory Survey participants ≥ 50 years old (bootstrap 95%CI)

	Colonoscopy *n* = 4 711 189	No colonoscopy *n* = 5 885 019
Number doctor visits in past year, %	4.22 (4.22-4.22)	3.28 (3.28-3.28)
Site of health provider, %		
Doctor's office	78.1 (78.1-78.2)	65.1 (65.0-65.1)
Community/government clinic	18.1 (18.1-18.2)	21.4 (21.3-21.4)
Emergency room	0.2 (0.2-0.2)	0.8 (0.8-0.8)
Random source/none	0.5 (0.5-0.5)	12.7 (12.6-12.7)
General health, %		
Excellent	17.8 (17.7-17.7)	16.7 (16.6-16.7)
Very good	31.0 (31.0-31.1)	28.2 (28.2-28.3)
Good	29.0 (28.9-29.1)	29.0 (28.9-29.0)
Fair	16.3 (16.3-16.4)	19.2 (19.2-19.3)
Poor	5.8 (5.8-5.8)	6.9 (6.9-6.9)
Medical health, %		
Elevated cholesterol	12.1 (12.1-12.2)	10.0 (9.9-10.0)
Hypertension	43.8 (43.8-43.9)	35.9 (35.8-35.9)
Diabetes	15.9 (15.9-15.9)	15.0 (14.9-15.0)
Heart disease	14.2 (14.1-14.2)	10.8 (10.8-10.8)
Mammograms in past 6 yr, *n*	2.23 (2.23-2.34)	1.64 (1.63-1.64)
PSA ever tested, %	34.3 (34.3-34.4)	22.1 (22.0-22.1)
Physician recommended "colon test", %	67.3 (67.3-67.4)	39.6 (39.5-39.6)
Reason for colonoscopy, %		
Routine screening	75.4 (75.3-75.4)	
Because of a problem	16.8 (16.7-16.8)	
Other	7.9 (7.9-7.9)	
Reason no colonoscopy in 10 yr, %		
Never thought about it		11.9 (11.9-12.0)
Doctor did not recommend		10.0 (10.0-10.1)
No problems/not necessary		9.6 (9.5-9.6)
Too expensive/no insurance		4.5 (4.5-4.6)
Procrastination		3.8 (3.8-3.8)
Too painful/embarrassing		2.9 (2.9-2.9)
Other		57.3 (57.3-57.4)
Family history of CRC, %		
Sibling	2.8 (2.8-2.8)	1.6 (1.6-1.6)
Father	4.1 (4.1-4.1)	1.8 (1.8-1.8)
Mother	3.8 (3.8-3.8)	1.4 (1.4-1.4)
Total	10.7 (10.7-10.7)	4.8 (4.8-4.8)

CRC: Colorectal cancer; PSA: Prostate specific antigen.

(70.5-70.6) of this subset having undergone colonoscopy. That is 44.5% (44.4-44.5) of all individuals 50-80 underwent colonoscopy. The mean age of CHIS participants between the ages of 50-80 years was 63.33 (63.32-63.33). Those who underwent colonoscopy were approximately three years older. Whites comprised the largest racial group at 60.8% (60.8-60.9) followed by Hispanic/Latinos 14.8% (CI 14.8-14.9). Hispanic/Latinos were least likely to undergo a colon cancer test. There were minimal differences in body weight among the test categories; however current smokers were far less likely to undergo a colon cancer test. There appeared to be no association between alcohol use and testing status.

There was an association between educational attainment and colon cancer testing. Those who underwent a colonoscopy were nearly twice as likely [20.4% (20.4-20.5) *vs* 10.7% (10.7-10.8)] to have graduated high school. Marital status and household income also appeared to be associated with testing. Those tested were far more likely to be living above the poverty line threshold as defined by the United States Department of Labor. Among those who underwent a test, 4.6% (4.6-4.6) were uninsured. In contrast, among those who did not undergo a test, 17.8% (17.8-17.9) were uninsured. Factors associated with colonoscopy (Table 2).Respondents who underwent colonoscopy reported more frequent doctor visits in the year prior to the survey. They also reported receiving most of their medical care in a doctor's office setting compared to those who did not [78.1 (78.1-78.2) *vs* 65.1 (65.0-65.1)]. Those who underwent a colonoscopy had a higher prevalence of medical problems such as diabetes and were more likely to participate in health screening tests such as mammograms for females and prostate specific antigen (PSA) tests for males. The prevalence of having a family history of colorectal cancer (CRC) in a first degree relative, usually a parent, was two-fold higher in respondents who underwent colonoscopy [10.7 (10.7-10.7) *vs* 4.8 (4.8-4.8)]. Participants who underwent a colonoscopy were far more likely to receive a recommendation for this test from their physician. A substantial majority were performed for "routine screening". Characteristics of Hispanic/Latino CHIS Participants (Table 3).

The largest number of Hispanic/Latino CHIS participants were of Mexican origin. The prevalence of testing was higher for naturalized citizens compared to those born in the United States. Non-citizens were least likely to undergo testing. Those who did not undergo testing lived within the United States longer than those who were tested.

The ability to speak English and the language spoken at home was associated with testing status. The prevalence of testing in those who spoke "very good" or "good" English compared to their less proficient counterparts was [37.2% (37.0-37.3) *vs* 26.2% (26.1-26.4)]. Those tested were more likely to speak English in their home. In addition, the language of TV/radio/newspaper was also associated with testing. Among Hispanic/Latino respondents who underwent colon testing, rates were higher for those who listened and watched media in English. Testing rates were lowest for those who listened and watched only in Spanish. Respondents were considerably more likely to have undergone a colon test if their doctor conducted the interview in English [41.0% (40.8-41.1) *vs* 21.5% (21.4-21.6)]. Lastly, participants who did not have a test were twice as likely to have "Had hard time understanding their doctor at the last visit" [7.3 (7.2-7.4) *vs* 3.5 (3.5-3.6)]. Multivariable analysis (Table 4).

The output from the regression model demonstrated that Blacks had an odds ratio 30% higher than Whites for undergoing a colon cancer test. Male gender, family history of colon cancer, and progressive levels above the poverty line were also factors independently associated with testing. Having health insurance and a physicians' recommendation were the strongest predictors of testing. For the Hispanic/Latino group, additional independent factors associated with testing included citizenship sta-

Table 3 Language and demographic characteristics of Latino/Hispanic California Health Inventory Survey Participants ages 50-80 years related to colon cancer screening status (bootstrap 95%CI)

	Total	Colonoscopy/Flexible sigmoidoscopy/ Fecal occult blood test	Colonoscopy	No Colonoscopy/Flexible sigmoidoscopy/Fecal occult blood test
	Weighted *n* = 1 487 447	Weighted *n* = 801 832	Weighted *n* = 492 646	Weighted *n* = 685 615
Latino/Hispanic sub-type, %				
Mexican	80.6 (80.5-80.6)	79.8 (79.7-79.9)	79.2 (79.1-79.4)	81.4 (81.3-81.5)
Salvadoran	8.2 (8.1-8.2)	6.4 (6.4-6.5)	8.0 (7.9-8.1)	10.3 (10.3-10.4)
Guatemalan	2.4 (2.3-2.4)	2.0 (2.0-2.0)	1.6 (1.6-1.7)	2.7 (2.7-2.8)
Central American	2.8 (2.7-2.8)	3.5 (3.4-3.5)	3.0 (2.9-3.0)	1.9 (1.9-1.9)
South American	2.1 (2.0-2.1)	2.7 (2.7-2.8)	2.3 (2.3-2.3)	1.4 (1.3-1.4)
Other Latino	3.9 (3.8-3.9)	5.6 (5.5-5.6)	5.9 (5.9-6.0)	2.3 (2.3-2.4)
Citizen status, %				
United states born citizen	31.2 (31.1-31.2)	37.1 (37.0-37.2)	39.3 (39.1-39.4)	24.2 (24.1-24.3)
Naturalized citizen	43.5 (43.4-43.6)	47.4 (47.3-47.6)	50.2 (50.1-50.3)	38.9 (38.8-39.0)
Non-citizen	25.3 (25.2-25.4)	15.4 (15.4-15.5)	10.5 (10.4-10.6)	36.9 (36.8-37.0)
Year lived in United States, %				
≤ 1	0.2 (0.2-0.2)	0.0 (0.0-0.1)	0.0 (0.0-0.2)	0.5 (0.4-0.5)
2-4	1.4 (1.3-1.4)	1.1 (1.1-1.1)	0.8 (0.8-0.8)	1.6 (1.6-1.7)
5-9	1.0 (1.0-1.1)	0.5 (0.5-0.5)	0.3 (0.2-0.3)	1.6 (1.6-1.7)
10-14	3.9 (3.8-3.9)	3.1 (3.1-3.2)	1.3 (1.2-1.3)	4.8 (4.7-4.8)
≥ 15	62.4 (62.3-62.4)	58.1 (58.1-58.3)	58.4 (58.3-58.6)	67.3 (67.2-67.5)
Not ascertained	31.2 (31.2-31.3)	37.2 (37.2-37.3)	39.2 (39.1-39.4)	24.2 (24.1-24.5)
Ability to speak English, %				
Very good	13.0 (12.9-13.1)	15.5 (15.4-15.5)	17.7 (16.9-17.1)	10.1 (10.1-10.2)
Good	19.1 (19.0-19.1)	21.7 (21.6-21.8)	26.9 (26.7-27.0)	16.1 (16.0-16.2)
Not good	33.4 (33.3-33.5)	29.6 (29.5-29.8)	23.1 (23.0-23.3)	37.8 (37.7-37.9)
Not at all	18.8 (18.7-18.8)	14.6 (14.5-14.7)	15.3 (15.2-15.)	23.6 (23.5-23.7)
Language of TV/radio/newspaper, %				
English	17.2 (17.1-17.3)	19.9 (19.8-20.0)	24.3 (24.2-24.5)	14.0 (14.0-14.2)
English and Spanish	33.0 (32.9-33.1)	32.9 (32.7-33.0)	31.1 (30.9-31.2)	33.2 (33.1-33.3)
Only Spanish	34.1 (34.0-34.2)	28.7 (28.6-28.8)	27.6 (27.5-27.8)	40.4 (40.3-40.6)
Not ascertained	15.7 (15.7-15.7)	18.6 (18.5-18.7)	17.0 (16.9-17.1)	12.3 (12.2-12.4)
Language spoken at home, %				
English	15.7 (15.7-15.8)	18.6 (18.5-18.7)	17.0 (16.9-17.1)	12.3 (12.2-12.4)
English and Spanish	46.6 (46.5-46.7)	48.4 (48.3-48.5)	54.0 (53.9-54.3)	44.5 (44.4-44.7)
Only Spanish	37.0 (36.9-37.1)	32.2 (32.2-32.3)	28.1 (27.9-28.2)	42.6 (42.5-42.8)
Other	0.7 (0.7-0.7)	0.8 (0.8-0.9)	0.9 (0.8-0.9)	0.6 (0.6-0.7)
Doctor conducted last interview in, %				
English	32.0 (31.9-32.1)	41.0 (40.8-41.1)	46.4 (46.2-46.5)	21.5 (21.4-21.6)
Spanish	39.7 (39.6-39.8)	34.5 (34.4-34.6)	29.6 (29.4-29.7)	45.7 (45.6-45.9)
Not ascertained	28.3 (28.2-28.4)	24.5 (24.4-24.6)	24.0 (23.9-24.3)	32.8 (32.7-32.9)
Hard time understanding your doctor, %				
Yes	5.3 (5.2-5.3)	3.5 (3.5-3.6)	4.0 (3.9-4.1)	7.3 (7.2-7.4)
No	87.0 (87.0-87.1)	94.1 (94.0-94.2)	92.4 (92.3-92.5)	78.8 (78.7-78.9)
Not ascertained	7.7 (7.7-7.8)	2.4 (2.2-2.6)	3.6 (3.5-3.7)	13.9 (13.8-14.0)

tus and ability to understand their physician during their most recent office visit.

Colon Cancer Testing Trend Data (Figure 1). Figure 1 displays the proportion of CHIS participants age 50-80 who underwent colon cancer testing (colonoscopy, flexible sigmoidoscopy or FOBT) within the previous 5 years stratified by race/ethnicity. The proportion tested increased in all groups between 2001 and 2007 and then appeared to plateau. Overall, the prevalence of testing was nearly identical among Whites and Blacks. The proportion of Hispanic/Latinos who underwent a test rose from approximately 35% in 2001 to 50% in 2009.

DISCUSSION

According to the 2009 CHIS survey, approximately 63.0% of participants aged 50-80 had a colon cancer test such as FOBT, flexible sigmoidoscopy or colonoscopy within the previous 5 years. Of these, approximately 70% underwent colonoscopy as the colon cancer test. Regression analysis demonstrated that those who underwent at least one colon cancer test were more likely to be male, Black, have a family history of CRC, and have health insurance. Progressive levels above the poverty line were also associated with receiving a test. The strongest variable associated with undergoing a test was physician recommendation. For the Hispanic/Latino group, those at increased risk for no testing were those born in the United States and those who "Had hard time understanding their doctor at the last visit".

Trend analysis demonstrated that the proportion of Blacks in California undergoing a colon cancer test is in-

Table 4 Multivariable logistic regression (dependent variable is colon cancer test in previous 5 years)

Variable	*n*	All participants adjusted OR[1] (95%CI)	White adjusted OR[1] (95%CI)	Black adjusted OR[1] (95%CI)	Hispanic adjusted OR[1] (95%CI)
Gender					
Female	5 658 375	1.00	1.00	1.00	1.00
Male	4 937 833	1.06 (1.06-1.06)	1.03 (1.03-1.04)	1.07 (1.06-1.08)	1.25 (1.24-1.26)
High school educated					
No	1 663 605	1.00	1.00	1.00	1.00
Yes	8 932 603	1.01 (1.00-1.01)	0.96 (0.95-0.99)	1.03 (0.98-1.05)	1.28 (1.27-1.29)
Family history of colon cancer					
No	9 809 630	1.00	1.00	1.00	1.00
Yes	786 578	1.71 (1.70-1.72)	1.88 (1.87-1.89)	1.54 (1.53-1.56)	1.29 (1.26-1.33)
Income relative to poverty level					
0%-99%	1 144 390	1.00	1.00	1.00	1.00
100%-199%	1 811 952	1.21 (1.20-1.21)	1.33 (1.30-1.36)	1.14 (1.12-1.15)	0.88 (0.87-0.89)
200%-299%	1 451 680	1.41 (1.40-1.42)	1.44 (1.40-1.48)	1.31 (1.27-1.40)	1.50 (1.49-1.52)
> 300%	6 188 185	1.69 (1.68-1.70)	1.77 (1.72-1.86)	1.66 (1.64-1.69)	1.48 (1.46-1.50)
Health insured					
No	1 006 640	1.00	1.00	1.00	1.00
Yes	9 589 568	2.71 (2.70-2.72)	2.88 (2.87-2.89)	2.14 (2.11-2.23)	3.52 (3.48-3.56)
Physician Recommended colon test					
No	5 095 110	1.00	1.00	1.00	1.00
Yes	5 501 098	3.90 (3.88-3.91)	3.77 (3.74-3.80)	3.99 (3.96-4.02)	4.37 (4.32-4.42)
Citizenship status1					
United States born citizen	464 083				1.00
Naturalized citizen	647 039				1.91 (1.89-1.93)
Years lived in United States[1]					
≤ 1	29 749				1.00
2-4	20 824				0.98 (0.96-1.02)
5-9	14 874				1.38 (1.33-1.44)
10-14	58 010				0.78 (0.75-0.82)
≥ 15	928 167				1.57 (1.54-1.62)
Hard time understanding physician[1]					
Yes	78 835				1.00
No	1 294 079				2.44 (2.40-2.48)

[1]Numbers for Hispanic/Latino only. OR: Odds ratio.

creasing in parallel with other racial/ethnic groups. This is encouraging as prior studies had identified white, non-Hispanic race as a predictor of colon cancer testing[29]. The increased participation by Blacks may be due to the more widespread use of colonoscopy as a colon cancer test. One survey revealed that this is the preferred colon cancer test by this group[13]. We were surprised to find no association between colon cancer testing and educational level. Prior studies suggested educational attainment was a positive predictor of colon cancer screening[27,30]. Similar to prior large population-based studies, our study also identified males, a family history of colon cancer, having health insurance, progressive levels above the poverty line, and having undergone screening with PSA or mammography as predictors of adherence to colon cancer testing[21]. Most importantly, our study confirms physician recommendation as the strongest variable associated with testing.

Several population-based studies looking at predictors of colorectal cancer screening participation have been performed[30]. BRFSS is a nationwide population-based telephone survey designed to measure preventive health practices, such as adherence to colon cancer testing and risk factors for adherence or non-adherence. A study of 52 754 respondents over age 50 in 1997 demonstrated low participation in colon cancer screening protocols with 19.8% for FOBT, 30.5% for sigmoidoscopy and 41.1% for either[21]. Respondents who underwent other preventive testing such as mammography or PAP smear demonstrated a higher adherence to screening[21]. Similarly, responses of 64 084 BRFSS participants over the age of 50 in 1999 demonstrated that colon cancer screening was reported by 43.4%, with 22.8% undergoing colonoscopy or sigmoidoscopy and 9.9% undergoing FOBT[30]. Negative predictors of screening included < college education and lack of health care coverage[30]. An examination of the 2002 BRFSS demonstrated a disparity in colon cancer testing between Hispanic and non-Hispanic Whites. Only, 41.9% of Hispanic respondents aged > 50 underwent colon cancer testing in the past year by either FOBT or colonoscopy/sigmoidoscopy compared to 55.2% of non-Hispanic whites[19].

The NHIS is an in-person survey conducted by the Centers for Disease Control and Prevention. The 2000 NHIS survey revealed that less than half of the United States population > 50 years underwent colon cancer screening at the recommended time intervals[23]. The strongest predictor of adherence was a physician visit within the previous year. A usual source of care, having undergone recent health preventive services such as a

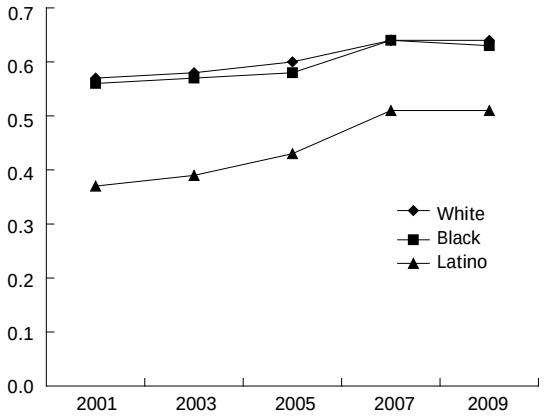


Figure 1 Proportion of individuals aged 50-80 undergoing a colon cancer test in the previous 5 years stratified by race/ethnicity.

mammogram or PAP smear, and physician recommendation were also important predictors[23].

An analysis of health behaviors of Medicare enrollees living in the United States was conducted using the SEER database[3]. A random sample of Medicare claims from 14 states for the period 1995 to 2003 were reviewed and demonstrated increased use of colonoscopy for colon cancer testing as well as a decline in the use of FOBT and sigmoidoscopy. The transition to use of colonoscopy was more rapid in Whites than non-Whites[3]. A similar study of Medicare claims in SEER regions from 1996-2005 showed a persistence of racial and ethnic disparities in the use of colonoscopy for screening as Whites were more likely to undergo this test as the physician preferred modality than Blacks or Hispanics[29]. Data collected in 2003 from 11 SEER regions demonstrated racial/ethnic disparities in colon cancer screening that varied significantly by geography[5]. A telephone survey of Medicare consumers living in three urban counties in North Carolina and 17 urban and rural counties in South Carolina was performed in 2001[17]. Overall, 56.8% of Whites adhered to Medicare-covered intervals for colon cancer testing compared to only 39.1% of Blacks[17]. Blacks preferred to undergo a colonoscopy rather than FOBT.

A major strength of our study is the sampling methodology incorporated into CHIS. Once weighted, the sample is representative of the entire population of the State of California including recent immigrants. Bootstrap methodology with resampling allows for accurate variance estimates. The repeated nature of the survey allows for the identification of trends in the use of health care services for the population. The incorporation of health behaviors, income, education, insurance coverage and other integral variables allows for adjusted estimates of healthcare utilization.

There are important weaknesses of our results. First, the indication for colon cancer testing is not available for every biennial survey. Other than 2009 (Table 2), we were not able to determine whether testing was performed for screening or was indicated for a sign/symptom of colonic disease. Therefore we were careful to use the terms "testing" and "screening" separately in our analysis and discussion. The dependent variable in this manuscript is clearly colon cancer testing. Erroneous conclusions about the presented trend data may be drawn if this is not kept in mind. For example, Blacks may be undergoing more colon tests over time because they are more willing to see their doctor for blood in their stools.

Another important weakness is that medical records are unavailable to confirm testing. Without records, we are left with the possibility of recall bias by the participants which could lead to misclassification. Examples include inability to remember that a doctor recommended a colon cancer test, or even that the participant had a test. Another shortcoming without records is that we are unable to separate whether patients underwent a sigmoidoscopy or FOBT as these results are reported in aggregate. Finally, without personal knowledge of the physician-patient interaction, we cannot further explain the variable "Had hard time understanding their doctor at the last visit". According to CHIS documentation for 2009, only 1.67% of those responding yes to this question answered that this was due to language differences. Other possibilities may include visual, auditory, or intellectual disabilities.

In conclusion, approximately 63% of California citizens between the ages of 50 and 80 have some form of colon cancer testing. Males, Blacks, insured, those with a family history of colorectal cancer, and those living above the poverty line were more likely to undergo a test. The strongest variable associated with testing was physician recommendation. For Hispanic/Latinos, those born in the United States and those with difficulty understanding their doctor are at increased risk for no testing. Our data is encouraging in that the level of participation in colon cancer testing appears to be increasing over the past decade for all studied racial/ethnic groups.

COMMENTS

Background

Colorectal cancer is the fourth leading cause of new cancer cases diagnosed in the United States and is the second leading cause of cancer death. Population-based surveys such as the Behavior Risk Factor Surveillance System (BRFSS) and the National Health Interview Survey (NHIS) suggest that colon cancer screening tests are underutilized in the United States. Colon cancer can be prevented in most cases by strict adherence to accepted colon cancer screening guidelines. Despite a lack of evidence from randomized controlled trials, colonoscopy has been shown to be a more sensitive test for the detection of adenomas and colon cancer than flexible sigmoidoscopy +/- fecal occult blood test (FOBT). The California Health Inventory Survey (CHIS) is the nation's largest state health survey. CHIS data gives a detailed picture of the health care needs of California' s large and diverse population. The specific aim of this study was to explore the CHIS database to identify demographic, socioeconomic, health and behavioral factors associated with participation in colon cancer testing. In addition, another aim was to perform a detailed analysis of the Latino population.

Research frontiers

Colorectal cancer is one of the leading causes of cancer diagnosed in the United States. Prior population-based studies reported positive and negative predictors of adherence to colon cancer testing with mixed results. This large population-based study seeks to clarify these predictors and to provide a detailed analysis of the Latino population.

Innovations and breakthroughs

A major strength of our study is the sampling methodology incorporated into

CHIS. Once weighted, the sample is representative of the entire population of the State of California including recent immigrants. Bootstrap methodology with resampling allows for accurate variance estimates. The repeated nature of the survey allows for the identification of trends in the use of health care services for the population. The incorporation of health behaviors, income, education, insurance coverage and other integral variables allows for adjusted estimates of healthcare utilization.

Applications

The study indicates approximately 63% of California citizens between the ages of 50 and 80 have some form of colon cancer testing. Males, Blacks, insured, those with a family history of colorectal cancer, and those living above the poverty line were more likely to undergo a test. The strongest variable associated with testing was physician recommendation. For Hispanic/Latinos, those born in the United States and those with difficulty understanding their doctor are at increased risk for no testing. Author's data is encouraging in that the level of participation in colon cancer testing appears to be increasing over the past decade for all studied racial/ethnic groups. Most importantly, the data can be used to remove barriers to colon cancer testing amongst the general population and minorities such as the Latino population.

Terminology

CHIS is the nation's largest state health survey. A random-dial telephone survey is conducted every two years on a wide range of health topics. CHIS data gives a detailed picture of the health care needs of California's large and diverse population. Colon cancer testing includes annual FOBT, flexible sigmoidoscopy +/- FOBT, and/or colonoscopy.

Peer review

This large population-based study provides insight into predictors of undergoing colon cancer testing. This data can be used to reduce the prevalence of colorectal cancer in the United States by removing barriers to colon cancer testing in the general population which includes minorities.

REFERENCES

1 **American Cancer Society**. Cancer Facts and Figures 2010. Atlanta, Georgia: American Cancer Society, 2010

2 **Beydoun HA**, Beydoun MA. Predictors of colorectal cancer screening behaviors among average-risk older adults in the United States. *Cancer Causes Control* 2008; **19**: 339-359 [PMID: 18085415 DOI: 10.1007/s10552-007-9100-y]

3 **Fenton JJ**, Cai Y, Green P, Beckett LA, Franks P, Baldwin LM. Trends in colorectal cancer testing among Medicare subpopulations. *Am J Prev Med* 2008; **35**: 194-202 [PMID: 18619761 DOI: 10.1016/j.amepre.2008.05.029]

4 **Fenton JJ**, Tancredi DJ, Green P, Franks P, Baldwin LM. Persistent racial and ethnic disparities in up-to-date colorectal cancer testing in medicare enrollees. *J Am Geriatr Soc* 2009; **57**: 412-418 [PMID: 19175435]

5 **Semrad TJ**, Tancredi DJ, Baldwin LM, Green P, Fenton JJ. Geographic variation of racial/ethnic disparities in colorectal cancer testing among medicare enrollees. *Cancer* 2011; Epub ahead of print [PMID: 21225598 DOI: 10.1002/cncr.25668]

6 2005 National Health Interview Survey. Available from: URL: http://www.cdc.gov/nchs/nhis.htm.

7 **Winawer SJ**, Zauber AG, Ho MN, O'Brien MJ, Gottlieb LS, Sternberg SS, Waye JD, Schapiro M, Bond JH, Panish JF. Prevention of colorectal cancer by colonoscopic polypectomy. The National Polyp Study Workgroup. *N Engl J Med* 1993; **329**: 1977-1981 [PMID: 8247072 DOI: 10.1056/NEJM199312303292701]

8 **Levin B**, Lieberman DA, McFarland B, Smith RA, Brooks D, Andrews KS, Dash C, Giardiello FM, Glick S, Levin TR, Pickhardt P, Rex DK, Thorson A, Winawer SJ. Screening and surveillance for the early detection of colorectal cancer and adenomatous polyps, 2008: a joint guideline from the American Cancer Society, the US Multi-Society Task Force on Colorectal Cancer, and the American College of Radiology. *CA Cancer J Clin* 2008; **58**: 130-160 [PMID: 18322143 DOI: 10.1053/j.gastro.2008.02.002]

9 **Rex DK**, Johnson DA, Anderson JC, Schoenfeld PS, Burke CA, Inadomi JM. American College of Gastroenterology guidelines for colorectal cancer screening 2009 [corrected]. *Am J Gastroenterol* 2009; **104**: 739-750 [PMID: 19240699 DOI: 10.1038/ajg.2009.104]

10 **Selby JV**, Friedman GD, Quesenberry CP, Weiss NS. Effect of fecal occult blood testing on mortality from colorectal cancer. A case-control study. *Ann Intern Med* 1993; **118**: 1-6 [PMID: 8416152]

11 **Imperiale TF**, Wagner DR, Lin CY, Larkin GN, Rogge JD, Ransohoff DF. Risk of advanced proximal neoplasms in asymptomatic adults according to the distal colorectal findings. *N Engl J Med* 2000; **343**: 169-174 [PMID: 10900275 DOI: 10.1056/NEJM200007203430302]

12 **Lieberman DA**, Weiss DG, Bond JH, Ahnen DJ, Garewal H, Chejfec G. Use of colonoscopy to screen asymptomatic adults for colorectal cancer. Veterans Affairs Cooperative Study Group 380. *N Engl J Med* 2000; **343**: 162-168 [PMID: 10900274 DOI: 10.1056/NEJM200007203430301]

13 **Ata A**, Elzey JD, Insaf TZ, Grau AM, Stain SC, Ahmed NU. Colorectal cancer prevention: adherence patterns and correlates of tests done for screening purposes within United States populations. *Cancer Detect Prev* 2006; **30**: 134-143 [PMID: 16638628 DOI: 10.1016/j.cdp.2006.02.003]

14 **Denberg TD**, Melhado TV, Coombes JM, Beaty BL, Berman K, Byers TE, Marcus AC, Steiner JF, Ahnen DJ. Predictors of nonadherence to screening colonoscopy. *J Gen Intern Med* 2005; **20**: 989-995 [PMID: 16307622 DOI: 10.1111/j.1525-1497.2005.00164.x]

15 **Green AR**, Peters-Lewis A, Percac-Lima S, Betancourt JR, Richter JM, Janairo MP, Gamba GB, Atlas SJ. Barriers to screening colonoscopy for low-income Latino and white patients in an urban community health center. *J Gen Intern Med* 2008; **23**: 834-840 [PMID: 18350339 DOI: 10.1007/s11606-008-0572-6]

16 **Jerant AF**, Fenton JJ, Franks P. Determinants of racial/ethnic colorectal cancer screening disparities. *Arch Intern Med* 2008; **168**: 1317-1324 [PMID: 18574089 DOI: 10.1001/archinte.168.12.1317]

17 **Klabunde CN**, Schenck AP, Davis WW. Barriers to colorectal cancer screening among Medicare consumers. *Am J Prev Med* 2006; **30**: 313-319 [PMID: 16530618 DOI: 10.1016/j.amepre.2005.11.006]

18 **Heo M**, Allison DB, Fontaine KR. Overweight, obesity, and colorectal cancer screening: disparity between men and women. *BMC Public Health* 2004; **4**: 53 [PMID: 15533259 DOI: 10.1186/1471-2458-4-53]

19 **Pollack LA**, Blackman DK, Wilson KM, Seeff LC, Nadel MR. Colorectal cancer test use among Hispanic and non-Hispanic U.S. populations. *Prev Chronic Dis* 2006; **3**: A50 [PMID: 16539791]

20 **Rosen AB**, Schneider EC. Colorectal cancer screening disparities related to obesity and gender. *J Gen Intern Med* 2004; **19**: 332-338 [PMID: 15061742 DOI: 10.1111/j.1525-1497.2004.30339.x]

21 **Shapiro JA**, Seeff LC, Nadel MR. Colorectal cancer-screening tests and associated health behaviors. *Am J Prev Med* 2001; **21**: 132-137 [PMID: 11457633 DOI: 10.1016/S0749-3797(01)00329-4]

22 **Schenck AP**, Klabunde CN, Davis WW. Racial differences in colorectal cancer test use by Medicare consumers. *Am J Prev Med* 2006; **30**: 320-326 [PMID: 16530619 DOI: 10.1016/j.amepre.2005.11.005]

23 **Seeff LC**, Nadel MR, Klabunde CN, Thompson T, Shapiro JA, Vernon SW, Coates RJ. Patterns and predictors of colorectal cancer test use in the adult U.S. population. *Cancer* 2004; **100**: 2093-2103 [PMID: 15139050 DOI: 10.1002/cncr.20276]

24 **Shelton RC**, Jandorf L, Ellison J, Villagra C, DuHamel KN. The influence of sociocultural factors on colonoscopy and FOBT screening adherence among low-income Hispanics. *J Health Care Poor Underserved* 2011; **22**: 925-944 [PMID: 21841288 DOI: 10.1353/hpu.2011.0074]

25 **Thompson B**, Coronado G, Neuhouser M, Chen L. Colorec-

tal carcinoma screening among Hispanics and non-Hispanic whites in a rural setting. *Cancer* 2005; **103**: 2491-2498 [PMID: 15880744 DOI: 10.1002/cncr.21124]
26 **Vernon SW**. Participation in colorectal cancer screening: a review. *J Natl Cancer Inst* 1997; **89**: 1406-1422 [PMID: 9326910 DOI: 10.1093/jnci/89.19.1406]
27 Available from: URL: http://www.chis.ucla.edu/about.html
28 **Krewski D**, Rao JNK. Inference From Stratified Samples: Properties of the Linearization, Jackknife and Balanced Repeated Replication Methods. *Ann Statist* 1981; **9**: 1010-1019 [DOI: 10.1214/aos/1176345580]
29 **White A**, Vernon SW, Franzini L, Du XL. Racial and ethnic disparities in colorectal cancer screening persisted despite expansion of Medicare's screening reimbursement. *Cancer Epidemiol Biomarkers Prev* 2011; **20**: 811-817 [PMID: 21546366 DOI: 10.1158/1055-9965.EPI-09-0963]
30 **Ioannou GN**, Chapko MK, Dominitz JA. Predictors of colorectal cancer screening participation in the United States. *Am J Gastroenterol* 2003; **98**: 2082-2091 [PMID: 14499792 DOI: 10.1111/j.1572-0241.2003.07574.x]

P- Reviewers Leitman M, Coleman H, Tanaka T
S- Editor Wen LL **L- Editor** A **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i8.1256

World J Gastroenterol 2013 February 28; 19(8): 1256-1263
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Magnetic resonance imaging: Is there a role in clinical management for acute ischemic colitis?

Maria Antonietta Mazzei, Susanna Guerrini, Nevada Cioffi Squitieri, Giusi Imbriaco, Raffaele Chieca, Serenella Civitelli, Vinno Savelli, Francesco Giuseppe Mazzei, Luca Volterrani

Maria Antonietta Mazzei, Susanna Guerrini, Nevada Cioffi Squitieri, Giusi Imbriaco, Luca Volterrani, Department of Human Pathology and Oncology, Section of Radiological Sciences, University of Siena, 53100 Siena, Italy
Raffaele Chieca, Oncologic Department, UOC Gastroenterology and Digestive Endoscopy, Azienda Ospedaliera Universitaria Senese, 53100 Siena, Italy
Serenella Civitelli, Division of Surgery, Azienda Ospedaliera Universitaria Senese, University of Siena, 53100 Siena, Italy
Vinno Savelli, Department of Surgery and Bioengineering, Section of Surgery, University of Siena, 53100 Siena, Italy
Francesco Giuseppe Mazzei, Department of Diagnostic Imaging, Azienda Ospedaliera Universitaria Senese, 53100 Siena, Italy
Author contributions: Mazzei MA, Guerrini S, Cioffi Squitieri N, Imbriaco G, Chieca R, Civitelli S, Savelli V, and Mazzei FG substantial contributions to conception and design acquisition of data, analysis and interpretation of data; Mazzei MA and Volterrani L contributions to drafting the article, revising it critically for important intellectual content and final approval of the version to be published.
Correspondence to: Maria Antonietta Mazzei, MD, Assistant Professor of Radiology, Department of Human Pathology and Oncology, Section of Radiological Sciences, University of Siena, Viale Bracci 10, 53100 Siena,
Italy. mariaantonietta.mazzei@unisi.it
Telephone: +39-577-585700 Fax: +39-577-44496
Received: August 9, 2012 Revised: November 26, 2012
Accepted: December 15, 2012
Published online: February 28, 2013

Abstract

AIM: To validate the utility of magnetic resonance imaging (MRI) for the clinical management of acute ischemic colitis (IC).

METHODS: This is a magnetic resonance (MR) prospective evaluation of 7 patients who were proved to have acute IC on the basis of clinical, endoscopic and computed tomography (CT) findings and who were imaged in our institution between February 2011 and July 2012. The mean age of the patients was 72.28 years. Abdominal CTs were obtained using a 64-detector row configuration for all patients with un-enhanced and contrast-enhanced scans, in the late arterial phase (start delay 45-50 s) and in the portal venous phase (start delay 70-80 s). The MR examinations were performed using a 1.5T superconducting magnet, using Fast Imaging Employing Steady State Acquisition and T2-weighted fast-recovery fast-spin echo sequences in axial and coronal plane. CT and MRI examinations were analysed for the presence of colonic abnormalities and associated findings.

RESULTS: Segmental involvement was seen in 6 patients (85.71%), with a mean length of involvement of 412 mm (range 145.5-1000 mm). Wall thickness varied between 6 mm and 17.5 mm (mean 10.52 mm) upon CT examinations and from 5 to 15 mm (mean 8.8 mm) upon MR examinations. The MRI appearance of the colonic wall varied over the time: Type Ⅰ appearance with a 3 layer sandwich sign was seen in 5 out of 12 examinations (41.66%), patients underwent MR within a mean of 36 h (ranging from 1 to 54 h) after the CT examination. Type Ⅱ and Ⅲ appearance with a 2 layer sign, was seen in 4 examinations (33.33%), patients underwent MR within a mean of 420.5 h (ranging from 121 to 720 h) after the CT examination. In the remaining three MRI examinations, performed within a mean of 410 h (ranging from 99.5 to 720 h) the colonic wall appeared normal.

CONCLUSION: MRI, only using precontrast images, may be used as a substitute for invasive procedures in diagnosis and follow-up of acute IC.

© 2013 Baishideng. All rights reserved.

Key words: Ischemic colitis; Magnetic resonance imaging; Medical management; Colon; Computed tomography

Mazzei MA, Guerrini S, Cioffi Squitieri N, Imbriaco G, Chieca R, Civitelli S, Savelli V, Mazzei FG, Volterrani L. Magnetic resonance imaging: Is there a role in clinical management for acute ischemic colitis? *World J Gastroenterol* 2013; 19(8): 1256-1263 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i8/1256.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i8.1256

INTRODUCTION

Ischemic colitis (IC) is a relatively common disease[1] and it is considered the most frequent form of intestinal ischemia and the second most frequent cause of lower gastrointestinal bleeding[2]. It represents the consequence of an acute or, more commonly, chronic decrease or blockage in the colonic blood supply, which may be either occlusive or non- occlusive in origin[3]. The original insult causing the ischemic event can rarely be established, but frequently occurs in elderly patients with diffuse disease in small segmental vessels and various co-morbidities. Today, with the introduction of new therapies, pharmacological causes could also be considered[4,5]. The anatomic damage results in ischemic necrosis of variable severity that can range from superficial mucosal involvement to full-thickness transmural necrosis[6]. The treatment depends on the acuteness and severity of the presentation[3]. Most cases of IC are transient and resolve spontaneously and such patients do not require specific therapy, instead very mild cases can be managed on an outpatient basis with a liquid diet, close observation and antibiotics[7]. IC rarely presents itself in a gangrenous form (acute fulminant IC).

The incidence of IC is underestimated because it often has a mild transient nature, clinical presentation can be nonspecific and highly variable, therefore, the diagnosis largely depends on clinical suspicion. In this context the role of imaging techniques remains controversial[8]. Standard radiology yields non-specific and late findings, while computed tomography (CT), the main technique for the noninvasive diagnosis of mesenteric ischemia, is well suited to confirm the clinical suspicion of IC, to suggest IC when it is unsuspected and to diagnose complications, however it requires the use of radiation and an iodinates contrast agent, limiting the possibility to use this technique in a short term follow-up[9-14]. Recently Iacobellis *et al*[15] has proposed magnetic resonance imaging (MRI) as a substitute for invasive procedures in diagnosing and grading acute IC, allowing for the early identification of pathological findings and by defining the evolution of ischemic lesions with 7T magnetic resonance imaging (7T-MRI) on an animal model with acute IC. The purpose of this study was to validate the utility of MRI in the clinical management of acute IC. In particular to show our experience in daily practice, focusing the attention both on the diagnosis and follow-up of this pathological condition.

MATERIALS AND METHODS

Patients population

All human procedures were approved by our Institutional Care Committee.

This is a magnetic resonance (MR) prospective evaluation of 11 patients who were proved to have acute intestinal ischemia on the basis of clinical, endoscopic and CT findings and who were imaged in our institution between February 2011 and July 2012. Among the initial 11 patients, 7 were included in the study because they were identified as having IC. Two patients with extensive small intestinal infarction because of the occlusion of superior mesenteric artery and 2 patients with the occlusion of superior mesenteric vein were not included.

Of the 7 patients, 2 were men and 5 women, with a mean age of 72.28 years (range, 54-97 years). Clinical charts, CT and MR examinations were reviewed. Imaging evaluation was requested because of the following clinical findings: sudden-onset of abdominal pain in all the patients (n = 7) (100%), bloody diarrhoea or bright red rectal blood in 4 patients (57.14%), nausea and vomiting in 1 patient (14.29%), elevated white blood cells (WBC) in 5 patients (71.42%), elevated Lactate dehydrogenase (LDH) levels in 6 patients (85.71%).

Five (71.42%) of these patients had arteriosclerotic cardiovascular disease on the basis of the findings from a clinical evaluation or cardiac work-up. Their mean age was 77 years. One patient (14.29%, 54 years old), during treatment of Lenalidomide for a relapsed multiple myeloma and one patient (14.29%, 80 years old) had hypotensive episodes associated with a recent exacerbation of chronic pancreatitis.

The diagnosis of IC was suspected on the basis of the clinical presentation and CT findings and was confirmed in all the patients: in 5 patients, an endoscopic procedure alone (sigmoidoscopy or colonoscopy) was performed, alternatively an endoscopic procedure and biopsy was performed on 1 patient; and 1 patient, underwent endoscopy and surgery with histological specimen evaluation.

Multidetector-row computed tomography imaging protocol

Abdominal CTs were obtained using a 64-detector row configuration for all patients (Discovery CT 750HD, General Electric Healthcare, Milwaukee, WI, United States). In all patients the examination was performed with a spiral technique in a cranio-caudal direction (from the base of the lungs to the pelvic brim) and supine position. All patients underwent un-enhanced and contrast-enhanced CT, in the late arterial phase (start delay 45-50 s) and in the portal venous phase (start delay 70-80 s) with an intravenous injection of 2 mL/kg of non-ionic contrast material (Iopamiro 370; Bracco Diagnostics, Milan, Italy), followed by 40 mL of saline solution using a peristaltic semiautomated power injector (4 mL/s flow

rate, SIAS 757, Bologna, Italy) with an 18-gauge needle in the antecubital vein. Oral medium contrast was not given to any of the patients; rectal air or rectal contrast material was not administered. The following technical parameters were used: effective slice thickness of 3.75 mm for plain acquisition, 1.25 mm in the late arterial phase and 2.5 mm in the portal venous phase; beam pitch of 0.938, reconstruction interval of 0.8 mm, tube voltage of 120-140 KVp and reference mAs of 250/700 mA. Automatic tube current modulation was used to minimise radiation exposure. Standard reconstruction algorithm was used. Patients were instructed not to breath during helical imaging to avoid motion artefacts.

MRI techniques

The MR examination was performed using a 1.5T superconducting magnet (SIGNA HD 1.5, General Electric Healthcare, Milwaukee, WI, United States), using the following sequences: Fast Imaging Employing Steady State Acquisition (FIESTA, TR/TE, 3.8/1.6; matrix size, 192 × 320; section thickness, 6 mm; intersection gap, 1 mm; field of view, 480 mm × 480 mm; NEX, 1; breath-hold) on coronal plane and T2-weighted fast-recovery fast-spin echo sequence (FRFSE: TR/TE, 6000/70, matrix size, 320 × 256; section thickness, 4mm; intersection gap, 0.4 mm; field of view, 480 mm × 480 mm; NEX, 4; respiratory triggering) in axial and coronal plane. An eight channel body phase-array surface coil was employed. Oral and intravenous medium contrast were not given to any of the patients.

Image analysis and comparison

CT scans and MR images were evaluated by two Radiologists (Mazzei MA and Mazzei FG) experienced in gastrointestinal imaging, reaching a consensus agreement. The following were assessed: the location and length of the colonic segment involved; the appearance and degree of wall thickening; the presence of a double-halo or target configuration (two or three concentric rings); pericolic streakiness, peritoneal fluid or blood, presence of intramural, mesenteric, or portal venous gas, and free intraperitoneal air or other relevant abdominal findings were also recorded.

The bowel wall was considered thickened if it measured more than 3 mm in diameter. Right side colonic involvement was defined as abnormalities affecting a segment or the entire ascending colon including the hepatic flexure. Left-side involvement was defined as abnormalities starting at or distal to the splenic flexure. Finally, the gross appearance of the affected colonic wall at CT was divided into three morphologic groups according to Balthazar *et al*[14]: (1) Type I CT (acute IC), wall thickening with heterogeneous enhancement and zones of low attenuation compatible with severe colonic edema; there was enhancement of the mucosa consistent with an acute process, a shaggy contour, a loss of colonic haustra, with varying degrees of pericolic streakiness; (2) Type II CT (subacute IC), the CT appearance showed concentric and symmetric mild mural thickening and homogeneous attenuation of the wall of the colon with a sharply defined contour and with or without minimal pericolic streakiness; and (3) Type III CT (gangrenous IC): there was circumferential intramural air consistent with pneumatosis coli.

A similar classification was realised by the analysis of MR images and a correlation between the gross appearance of the affected colonic wall on both CT and MRI and the time from the onset.

RESULTS

Patient demographic information (age and sex), pre-existing disease, number of CT and MR examinations during hospitalisation, diagnostic proof (S = surgery, B = biopsy and E = endoscopy) and status at the time of discharge (D = died; L = living) for the 7 patient are summarised in Table 1. The diagnostic CT examinations were performed within a mean of 45.5 h (range, 2-168 h) after the clinical onset of symptoms, while the first MR examinations were performed in 4 patients within 48 h (mean of 14.8 h; range, 1-40 h) after the date of the CT examination, in 5 patients between 48 h and 15 d (mean of 137.5 h, range 54-240 h), and in 3 patients after 15 d from the CT examinations (mean of 524 h, range 384-720 h). Three patients underwent only one MRI examination, 3 patients were studied twice and 1 patient was studied three times, for a total of 12 MRI examinations. The duration of the MRI follow-up of the 7 patients considered varied from 1 to 30 d from the date of the CT examination.

Among the 7 patients, 6 (85.71%) exhibited a segmental involvement of the colon and 1 patient (14.29%) had the entire colon involved. The length of involvement in the patients with a segmental distribution, obtained using 2D reformat reconstructions on CT images, ranged from 145.5 to 1000 mm, with a mean length of 412 mm. The thickness of the wall of the colon in the affected segments varied from 6 to 17.5 mm, with a mean bowel wall thickness of 10.52 mm on CT images, and from 5 to 15 mm, with a mean bowel wall thickness of 8.8 mm, on MR images. Two out of 6 patients (33.33%) with a segmental involvement of the colon exhibited a left-sided and splenic flexure colitis, 1 (16.66%) a left-sided colitis only, 1 (16.66%) a left-sided colitis and IC of the transverse colon and splenic flexure, 1 (16.66%) an IC of the transverse colon only, and 1 (16.66%) an IC of the sigmoid colon. In the 1 patient with ischemic pancolitis, the diagnosis was confirmed at colonoscopy and total colectomy.

The gross appearance of the affected colonic wall at CT, divided into three morphologic groups, according to Balthazar *et al*[14], showed that Type I CT was present in 5 out of 7 patients (71.4%) and Type II CT in 2 out of 7 patients (28.6%). The diagnostic CT examinations in 5 patients with Type I CT appearance was performed within a mean of 36 h (ranging from 2 and 48 h) after the clinical onset of symptoms, showing an acute form of

Table 1 Patients demographic characteristic

Patient	Sex	Age (yr)	Pre-existing disease	Number of CT/MR	Diagnostic proof	Status of discharge
1	F	54	Multiple myeloma relapsed treated with Lenalinomide	1/2	E	L
2	F	89	Ischemic heart disease, hypertension, rheumatoid arthritis	1/1	E	L
3	F	80	Hypertension, thrombotic disease	1/2	E + B	L
4	F	97	Diverticulitis, pancreatitis, hypertension in treatment	1/1	E	L
5	M	67	Crohn's disease	1/1	B	D
6	F	57	Hypertension in treatment	1/2	E	L
7	M	62	Heart failure	1/3	E	L

CT: Computed tomography; MR: Magnetic resonance; F: Female; M: Male; B: Biopsy; E: Endoscopy; D: Died; L: Living.

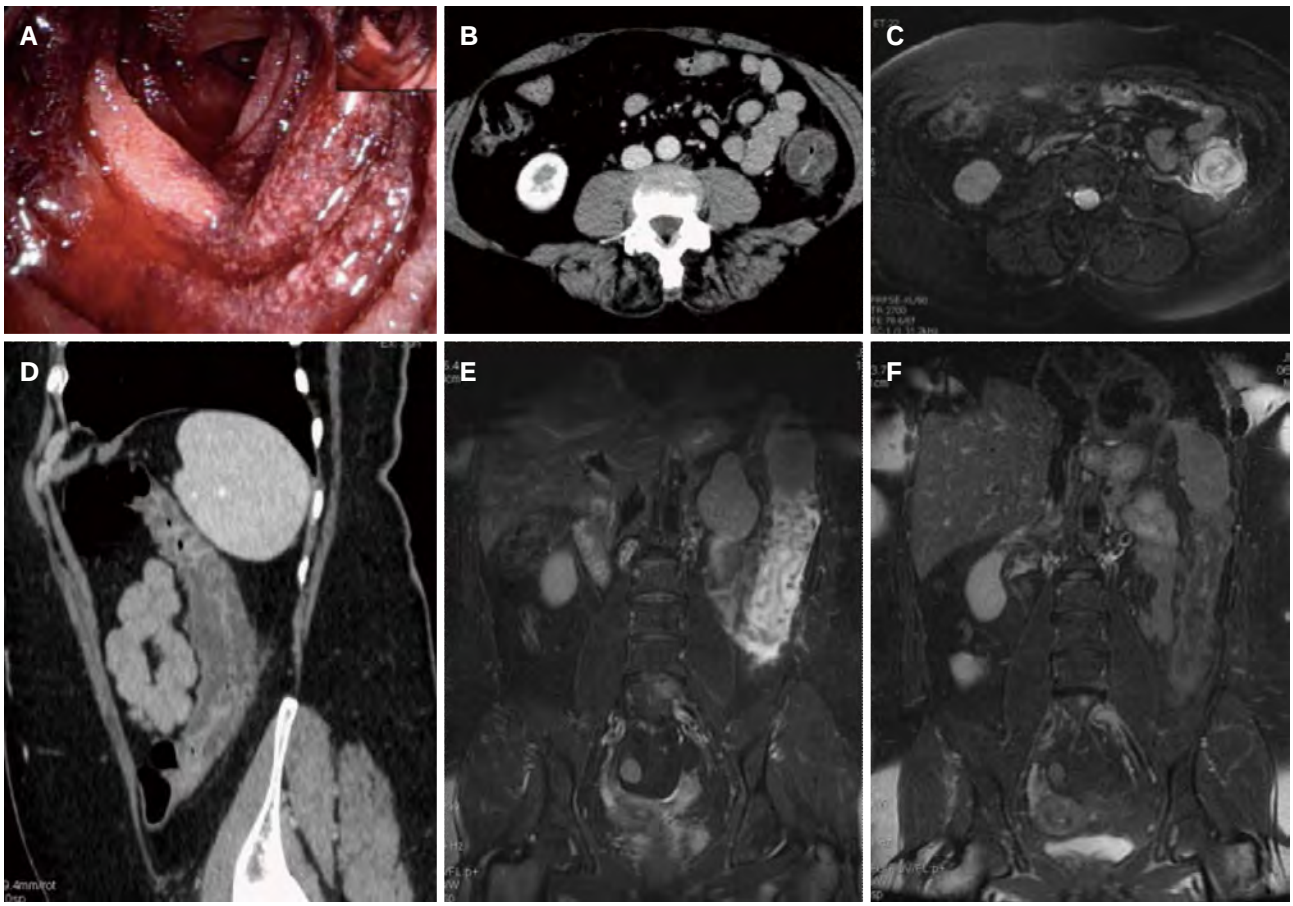


Figure 1 Magnetic resonance imaging follow-up of a patients with ischemic colitis resolved promptly. Ischemic colitis (IC) of left side colon in a 57 year old woman with a recent history of acute hypertensive crisis, who presented with left lower quadrant pain and massive rectal bleeding. A: Endoscopic procedure showed multiple necrotic area; B and C: Contrast-enhanced computed tomography (CT) and axial T2 fast-recovery fast-spin echo sequence (FRFSE) magnetic resonance imaging (MRI), after 32 h from CT, showed acute IC (Type Ⅰ CT and MRI) with wall thickening, three layer sandwich sign and a mild amount of free fluid in the paracolic gutter; D and E: 2D coronal reformat CT and coronal T2 FRFSE MRI, at the same time, showed the entire involved tract; F: Ischemia risolved without complications with conservative therapy as shown in the follow-up MRI.

IC.

As the morphologic CT groups, the gross appearance of the affected colonic wall upon MRI could be divided into 3 groups: (1) Type I MRI, in the first group, wall thickening with a 3 layer sandwich sign showing high signal intensity of the intermediate layer and the low signal intensity of the inner and outer layers, were present in 5 MR examinations (4 patients, 1 patient had 2 MR examinations with an interval of 48 h due to worsening of clinical symptoms). The MR examinations with Type I appearance were performed within a mean of 36 h (ranging from 1 to 54 h) after the CT examination and the same patients with Type I MRI showed the Type I appearance at CT, according to an acute form of IC (Figures 1 and 2); (2) Type Ⅱ MRI, in the second group, which consisted of 2 patients, there was a wall thickening with a 2 layer sign (high signal intensity of the inner layer and low signal intensity of the outer layer. The MR examinations with Type Ⅱ appearance were performed within a mean of 420.5 h (ranging from

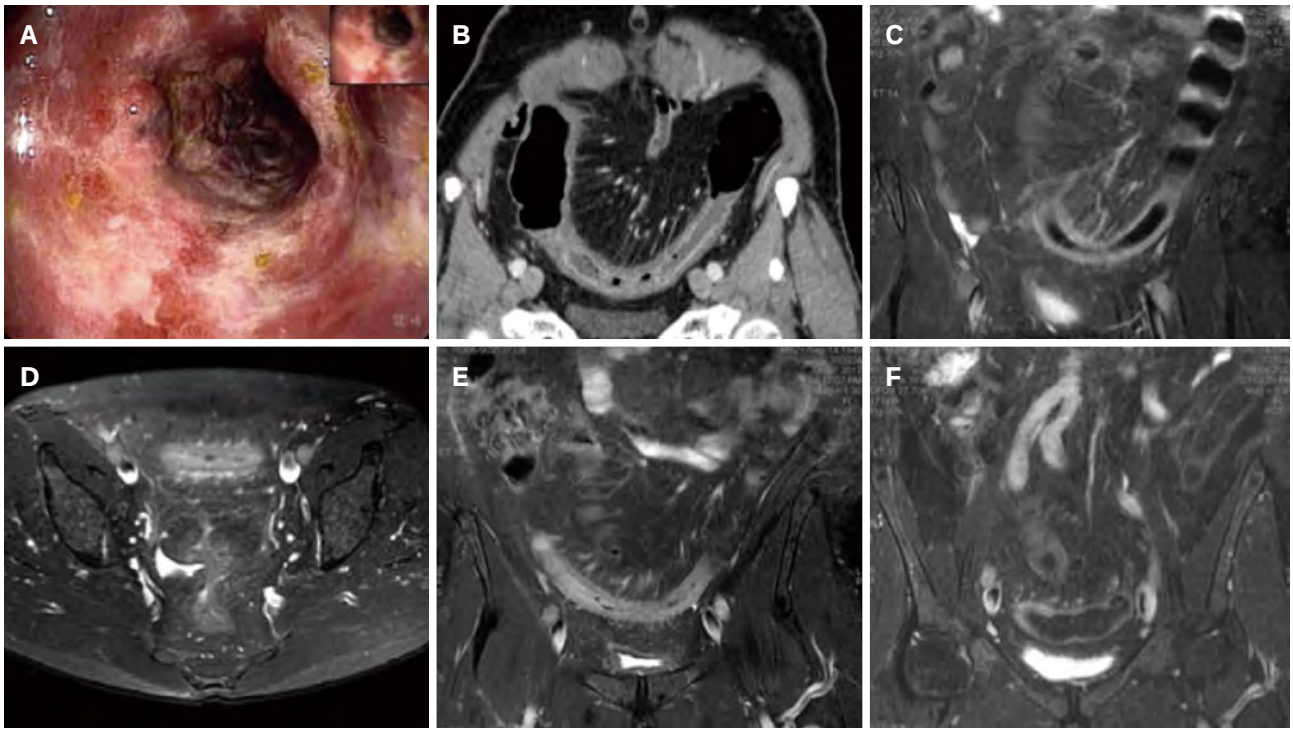


Figure 2 Magnetic resonance imaging follow-up of a patient with ischemic colitis and worsening of clinical symptoms. Ischemic colitis (IC) of sigmoid colon in a 62-year-old man with left lower quadrant pain and elevate lactate dehydrogenase levels, who presented with melena and a recent history of stenting procedures for ischemic cardiopathy. A: Endoscopic procedure showed multiple necrotic area; B: 2D (two dimensional) coronal reformat contrast-enhanced computed tomography (CT) showed acute IC (Type Ⅰ CT); C-E: The patient had 2 magnetic resonance examinations (C and D-E) with an interval of 48 h due to worsening of clinical symptoms, with an increase of the length and thickness of the involved tract (D-E); F: The ischemic process resolved without complication after parenteral nutrition, as showed in the follow-up magnetic resonance imaging, performed after 384 h from the date of CT examination.

121 to 720 h) after the CT examination, according to a subacute form of IC; and (3) Type Ⅲ MRI, in the third group, which consisted of 2 patients, the MR appearance showed a wall thickening with a inversed 2 layer sign (high signal intensity of the outer layer and low signal intensity of the inner layer). The MR examinations with Type Ⅲ appearance were performed within a mean of 420.5 h (ranging from 384 to 457 h) after the CT examination, according to a subacute form of IC.

In the remaining 3 MRI examinations, performed within a mean of 410 h (ranging from 99.5 to 720 h) the colonic wall appeared normal.

No patients had circumferential intramural air consistent with pneumatosis coli.

Mild to moderate amounts of free abdomen fluid were present in all patients (100%) on the CT examination, and in 4 out of 4 of the first MR examinations (performed within 48 h from the date of CT examination) and in 1 out of 5 in the second MRI examinations (performed between 48 h and 15 d from the date of CT examination).

The free fluid was mainly located in the paracolic gutters and in the Douglas. No high-attenuation fluid consistent with blood was present. No reduction in caliber of mesenteric vessels was found.

On the basis of the result of the initial CT examination, endoscopic findings and clinical evaluation, no surgery was performed on any of the patients. Surgery was performed 3 wk after the initial episode because of the development of a sealed-off perforation of the transverse colon in one of the patients.

The site of the affected tract and it's length (mm), gross appearance on CT (type according Balthazar *et al*[14], thickness of the affected wall and presence or absence of peritoneal fluid) and on MRI (type, thickness of the affected colonic wall and presence or absence of peritoneal fluid), in relation to the date of each examination were reported in Table 2.

DISCUSSION

The diagnosis of IC largely depends on clinical suspicion, especially since many other conditions (*e.g.*, infectious colitis, inflammatory bowel disease, diverticulitis, colon cancer) are presented with abdominal pain, diarrhoea and hematochezia[3]. Endoscopy has become the diagnostic test of choice in establishing the diagnosis of IC, although, it can be limited because it could be performed without bowel preparation to prevent hypoperfusion caused by dehydrating cathartics; in addition a minimal air insufflation should be used to prevent perforation[7,8].

From a radiological point of view, a CT examination is actually considered the main technique for the noninvasive diagnosis of mesenteric ischemia and also in cases of acute abdomen from different and various origins, because it can suggest IC when it is unsuspected, can diag-

Table 2 Gross appearance on computed tomography and magnetic resonance imaging

Patient	Involved tract/length (mm)	CT Ⅰ range 2-168 h[1]	MRI Ⅰ within 48 h[2]	MRI Ⅱ > 48 h < 15 d[2]	MRI Ⅲ > 15 d[2]
1	Left side colon and splenic flexure/314	Type Ⅰ/12 mm; free fluid	Type Ⅰ/10 mm; free fluid		Type Ⅲ/6 mm; no free fluid
2	Left side colon, splenic flexure and transverse colon/609	Type Ⅰ/12 mm; free fluid		Type Ⅱ/7 mm; no free fluid	
3	Left side colon and splenic flexure/380	Type Ⅱ/9.5 mm; free fluid		Type Ⅱ/7 mm; no free fluid	normal appearance; no free fluid
4	Transverse colon/219	Type Ⅱ/6 mm; free fluid		normal appearance; no free fluid	
5	Entire colon/1000	Type Ⅰ/8.5 mm; free fluid	Type Ⅰ/9.5 mm; free fluid		
6	Left side colon/218	Type Ⅰ/17.5 mm; free fluid	Type Ⅰ/15 mm; free fluid	Normal appearance; no free fluid	
7	Sigmoid colon/145.5	Type Ⅰ/8.2 mm; free fluid	Type Ⅰ/7 mm; free fluid	Type Ⅰ/8 mm; free fluid	Type Ⅲ/5 mm; no free fluid

[1] After the clinical onset; [2] After the date of the CT examination. MRI: Magnetic resonance imaging; CT: Computed tomography.

nose complications and exclude other illnesses. However it requires the use of ionizing radiation and an iodinates contrast agent, limiting the possibility to use this technique in a short term follow-up[9-14].

Recently Iacobellis *et al*[15] has reported that MRI can play a relevant role in the diagnostic management of acute IC and may be substituted for other invasive endoscopic procedures in the diagnosis and grading of IC when an ischemic injury is suggested. Prior publications have described the feasibility of using MRI to evaluate a full range of colonic disease processes, including only one case of IC but without pathological confirmation and using precontrast and postcontrast imaging[16]. Our study is based on the assumption that a parallel between the experimental colonic ischemic damage in the animal model and humans is reasonable[15]. Then our aim has been to validate the utility of MRI in the clinical diagnosis and follow-up of IC, using combined Fast Imaging Employing Steady State Acquisition on a coronal plane and T2-weighted fast-recovery fast-spin echo sequences, both on an axial and coronal plane. About 71.4% of patients that underwent a CT examination within 48 h showed Type I of gross appearance of an involved colonic wall (wall thickening with heterogeneous enhancement and zones of low attenuation compatible with severe colonic edema and enhancement of the mucosa consistent with an acute process, a shaggy contour, a loss of colonic haustra, with varying degrees of pericolic streakiness). The same patients with Type I CT showed Type I appearance at MRI, according to an acute form of IC. The reason why the gross appearance of the involved colonic wall had a thickened and edematous appearance is related to the fact that usually IC is a form of non-occlusive ischemic disease and in most cases, however, there is no evidence of obstruction of a major artery or vein. Then a decrease in blood flow to 20% of the normal flow, associated with small-vessel disease (hypoxia), and reperfusion injury when the blood flow is reestablished are the responsible factors[17,18]. Consequently, any part of the colon can be involved, with no correlation established between the length and site of the involvement and distribution of the superior mesenteric or inferior mesenteric artery or vein[17,19,20]. Segments commonly affected by IC are the splenic flexure (Griffith point) because the marginal artery of Drummond (a system of arcades connecting the major arteries) is occasionally tenuous here and is absent in upto 5% of patients, and the anastomotic plexus between the inferior mesenteric artery distribution and the hypogastric vascular supply (point of Sudeck) at the rectosigmoid junction, because it is distal to the last collateral connection with proximal arteries[7,21]. In our case population a segmental distribution was apparent in 85.71% (6 patients).

The striking differences in the gross morphology of ischemic segment as detected at MRI is probably related to the timing of the examination and to the pathophysiology of the developing anoxic process. In the initial phases of anoxia, mucosal damage occurs first; with more severe and prolonged forms of anoxia, submucosa hemorrhage, edema, and pericolic congestive and edematous changes developing later due to the reperfusion event. Indeed, in the subacute phase (MRI examination performed between 3 and 30 d) the gross morphology has been changed (MRI Type Ⅱ or Ⅲ) with a reduction of the thickness of the involved wall (mean 7.2 mm, range 5-8 mm) and a double ring appearance (high signal intensity of the inner layer and low signal intensity of the outer layer for Ⅱ type MRI and high signal intensity of the outer layer and low signal intensity of the inner layer for Type Ⅲ MRI), probably for the reduction of edematous phenomena like the CT Type Ⅱ according to Balthazar.

Although up to 85% of cases of IC managed conservatively improve within 1 or 2 d and resolve completely within 1 or 2 wk, close to one-fifth of patients develop peritonitis or deteriorate clinically and require surgery. Surgical resection is required when an irreversible ischemic injury and chronic colitis develop as both can lead to bacteremia and sepsis, colonic stricture, persistent abdominal pain and bloody diarrhoea, and protein-loosing

enteropathy[8]. The advantage of the use of MRI for clinical management of IC, is the possibility to perform a short term follow-up without the employment of ionizing radiation or intravenous contrast material. As demonstrated in our study MRI could be used, instead of CT to suggest the diagnosis of the IC in the proper clinical setting, particular when a segmental distribution is evident, in the depiction of other abnormal conditions that may be seen in patients suspected of having IC, and in a short term follow-up, when a clinical worsening occurs, to adequately manage the patient.

Some limitations of the present study should be outlined. Firstly, our patient selection process does not allow for an evaluation of the sensitivity or specificity of MRI for the diagnosis or detection of IC. The other major limitation of both CT and MR imaging in the diagnosis of colonic ischemia is the lack of specificity. The gross morphologic features overlap with those of inflammatory colitis, although the segmental distribution is more often seen in ischemia[22]. In spite of the limited number of subjects who were examined, we are convinced that MRI can provide a valid imaging for the identification of pathological findings of acute IC. Moreover MRI in combination with clinical suspicion, endoscopic and histological findings, can play a key role in the diagnosis and management of IC. In particular MRI can discriminate patients with urgent operative intervention from patients in which a follow-up can be proposed as an alternative to surgery. All these allow for an earlier detection and effective follow-up of IC with a possible earlier adequate treatment.

ACKNOWLEDGMENTS

We thank Miss Julia Hassall for reviewing the manuscript and Miss Francesca Seri and Mr. Duccio Guerrieri for performing the majority of MR examinations.

COMMENTS

Background

The incidence of ischemic colitis (IC) is underestimated because it often has a mild transient nature, clinical presentation can be nonspecific and highly variable, therefore, the diagnosis largely depends on clinical suspicion. In this context the role of imaging techniques remains controversial. Standard radiology yields non-specific and late findings, while computed tomography (CT), the main technique for the noninvasive diagnosis of mesenteric ischemia, is well suited to confirm the clinical suspicion of IC, to suggest IC when it is unsuspected and to diagnose complications, however it requires the use of radiation and an iodinates contrast agent, limiting the possibility to use this technique in a short term follow-up.

Research frontiers

The role of magnetic resonance imaging (MRI) in the diagnostic management of acute IC is still controversial, and nothing is known about the *in vivo* magnetic resonance findings of IC or about the relationship between MR findings and the onset of clinical symptoms.

Innovations and breakthroughs

To be known, this is the first study using MRI for the evaluation of IC and for the comparison between MRI and CT findings in this pathological condition.

Applications

MRI in combination with clinical suspicion, endoscopic and histological findings, can play a key role in the diagnosis and management of IC. In particular MRI can discriminate patients with urgent operative intervention from patients in which a follow-up can be proposed as an alternative to surgery. In particular the advantage of the use of MRI for clinical management of IC, is the possibility to perform a short term follow-up without the employment of ionizing radiation or intravenous contrast material.

Terminology

Fast imaging employing steady state acquisition sequence (FIESTA): provides images of fluid filled structures with very short acquisition times. The FIESTA sequence uses the T2 steady state contrast mechanism to provide high signal noise ratio images with strong signal from fluid tissues while suppressing background tissue for contrast and anatomic detail of small structures; IC: inflammation of the colon due to colonic ischemia resulting from alterations in systemic circulation or local vasculature.

Peer review

This paper is well written, is original and has good information. It merits to be admitted to publish without susbtantial changes.

REFERENCES

1 **Boley SJ**, Schwartz S, Lash J, Sternhill V. Reversible vascular occlusion of the colon. *Surg Gynecol Obstet* 1963; **116**: 53-60 [PMID: 13968597]
2 **Paterno F**, Longo WE. The etiology and pathogenesis of vascular disorders of the intestine. *Radiol Clin North Am* 2008; **46**: 877-885, v [PMID: 19103137]
3 **Theodoropoulou A**, Koutroubakis IE. Ischemic colitis: clinical practice in diagnosis and treatment. *World J Gastroenterol* 2008; **14**: 7302-7308 [PMID: 19109863 DOI: 10.3748/wjg.14.7302]
4 **Rha SE**, Ha HK, Lee SH, Kim JH, Kim JK, Kim JH, Kim PN, Lee MG, Auh YH. CT and MR imaging findings of bowel ischemia from various primary causes. *Radiographics* 2000; **20**: 29-42 [PMID: 10682769]
5 **Westgeest HM**, Akol H, Schreuder TC. Pure naratriptan-induced ischemic colitis: a case report. *Turk J Gastroenterol* 2010; **21**: 42-44 [PMID: 20533112 DOI: 10.4318/tjg.2010.0047]
6 **Stamatakos M**, Douzinas E, Stefanaki C, Petropoulou C, Arampatzi H, Safioleas C, Giannopoulos G, Chatziconstantinou C, Xiromeritis C, Safioleas M. Ischemic colitis: surging waves of update. *Tohoku J Exp Med* 2009; **218**: 83-92 [PMID: 19478463]
7 **Baixauli J**, Kiran RP, Delaney CP. Investigation and management of ischemic colitis. *Cleve Clin J Med* 2003; **70**: 920-91, 920-91, 920-91, passim [PMID: 14650467]
8 **Elder K**, Lashner BA, Al Solaiman F. Clinical approach to colonic ischemia. *Cleve Clin J Med* 2009; **76**: 401-409 [PMID: 19570972 DOI: 10.3949/ccjm.76a.08089]
9 **Angelelli G**, Scardapane A, Memeo M, Stabile Ianora AA, Rotondo A. Acute bowel ischemia: CT findings. *Eur J Radiol* 2004; **50**: 37-47 [PMID: 15093234 DOI: 10.1016/j.ejrad.2003.11.013]
10 **Romano S**, Romano L, Grassi R. Multidetector row computed tomography findings from ischemia to infarction of the large bowel. *Eur J Radiol* 2007; **61**: 433-441 [PMID: 17157468 DOI: 10.1016/j.ejrad.2006.11.002]
11 **Mazzei MA**, Mazzei FG, Marrelli D, Imbriaco G, Guerrini S, Vindigni C, Civitelli S, Roviello F, Grassi R, Volterrani L. Computed tomographic evaluation of mesentery: diagnostic value in acute mesenteric ischemia. *J Comput Assist Tomogr* 2012; **36**: 1-7 [PMID: 22261763]
12 **Mazzei MA**, Guerrini S, Cioffi Squitieri N, Imbriaco G, Mazzei FG, Volterrani L. Non-obstructive Mesenteric Ischemia after Cardiovascular Surgery: not so uncommon. *Ann Thorac Cardiovas* 2013; In press
13 **Mazzei MA**, Guerrini S, Cioffi Squitieri N, Genovese EA, Mazzei FG, Volterrani L. [Diagnosis of acute mesenteric ischemia/infarction in the era of multislice CT]. *Recenti Prog Med* 2012; **103**: 435-437 [PMID: 23096727 DOI: 10.1701/1166.12884]

14 **Balthazar EJ**, Yen BC, Gordon RB. Ischemic colitis: CT evaluation of 54 cases. *Radiology* 1999; **211**: 381-388 [PMID: 10228517]

15 **Iacobellis F**, Berritto D, Somma F, Cavaliere C, Corona M, Cozzolino S, Fulciniti F, Cappabianca S, Rotondo A, Grassi R. Magnetic resonance imaging: a new tool for diagnosis of acute ischemic colitis? *World J Gastroenterol* 2012; **18**: 1496-1501 [PMID: 22509081 DOI: 10.3748/wjg.v18.i13.1496]

16 **Chung JJ**, Semelka RC, Martin DR, Marcos HB. Colon diseases: MR evaluation using combined T2-weighted single-shot echo train spin-echo and gadolinium-enhanced spoiled gradient-echo sequences. *J Magn Reson Imaging* 2000; **12**: 297-305 [PMID: 10931593]

17 **Brandt LJ**, Boley SJ. Ischemic and vascular lesions of the bowel. In: Sleisenger MH, Fordtran JS, editors. Gastrointestinal disease: pathophysiology, diagnosis, management. 5th ed. Philadelphia: Saunders, 1993: 1940-1945

18 **Zimmerman BJ**, Granger DN. Reperfusion injury. *Surg Clin North Am* 1992; **72**: 65-83 [PMID: 1731390]

19 **Wittenberg J**, Athanasoulis CA, Williams LF, Paredes S, O' Sullivan P, Brown B. Ischemic colitis. Radiology and pathophysiology. *Am J Roentgenol Radium Ther Nucl Med* 1975; **123**: 287-300 [PMID: 1115306]

20 **Bharucha AE**, Tremaine WJ, Johnson CD, Batts KP. Ischemic proctosigmoiditis. *Am J Gastroenterol* 1996; **91**: 2305-2309 [PMID: 8931407]

21 **Roger AI**, David S. Intestinal blood flow and disease of vascular impairment. In: Haubrich WS, Schaffner F, Berk JE, editors. Gastroenterology. 5th ed. Philadelphia: Saunders, 1995: 1212-1234

22 **Philpotts LE**, Heiken JP, Westcott MA, Gore RM. Colitis: use of CT findings in differential diagnosis. *Radiology* 1994; **190**: 445-449 [PMID: 8284397]

P- Reviewer Rodrigo L **S- Editor** Wen LL **L- Editor** A **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i8.1264

World J Gastroenterol 2013 February 28; 19(8): 1264-1270
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Ensure preparation and capsule endoscopy: A two-center prospective study

Eva Niv, Baruch Ovadia, Yulia Ron, Ervin Santo, Elisabeth Mahajna, Zamir Halpern, Zvi Fireman

Eva Niv, Yulia Ron, Ervin Santo, Zamir Halpern, Department of Gastroenterology, Tel Aviv Sourasky Medical Center, Tel Aviv 64239, Israel
Baruch Ovadia, Elisabeth Mahajna, Zvi Fireman, Department of Gastroenterology, Hillel Yaffe Medical Center, Hadera 38100, Israel
Author contributions: Niv E, Fireman Z, Santo E and Halpern Z designed the study; Niv E, Ovadia B, Ron Y and Mahajna E performed the research; Niv E and Fireman Z wrote the paper.
Correspondence to: Eva Niv, MD, Department of Gastroenterology, Tel Aviv Sourasky Medical Center, Weizmann Street 6, Tel Aviv 64239, Israel. niv_em@netvision.net.il
Telephone: +972-3-6974280 Fax: +972-3-6974622
Received: October 18, 2011 Revised: February 10, 2012
Accepted: March 10, 2012
Published online: February 28, 2013

Abstract

AIM: To compare small bowel (SB) cleanliness and capsule endoscopy (CE) image quality following Ensure®, polyethylene glycol (PEG) and standard preparations.

METHODS: A preparation protocol for CE that is both efficacious and acceptable to patients remains elusive. Considering the physiological function of the SB as a site for the digestion and absorption of food and not as a stool reservoir, preparation consisting of a liquid, fiber-free formula ingested one day before a CE study might have an advantage over other kinds of preparations. We conducted a prospective, blind-to-preparation, two-center study that compared four types of preparations. The participants' demographic and clinical data were collected. Gastric and SB transit times were calculated. The presence of bile in the duodenum was scored by a single, blinded-to-preparation gastroenterologist expert in CE, as was cleanliness within the proximal, middle and distal part of the SB. A four-point scale was used (grade 1 = no bile or residue, grade 4 $\geqslant$ 90% of lumen full of bile or residual material).

RESULTS: The 198 consecutive patients who were referred to CE studies due to routine medical reasons were divided into four groups. They all observed a 12-h overnight fast before undergoing CE. Throughout the 24 h preceding the fast, control group 1 (n = 45 patients) ate light unrestricted meals, control group 2 (n = 81) also ate light meals but free of fruits and vegetables, the PEG group (n = 50) ate unrestricted light meals and ingested the PEG preparation, and the Ensure group (n = 22) ingested only the Ensure formula. Preparation with Ensure improved the visualization of duodenal mucosa (a score of 1.76) by decreasing the bile content compared to preparation with PEG (a score of 2.9) (P = 0.053). Overall, as expected, there was less residue and stool in the proximal part of the SB than in the middle and distal parts in all groups. The total score of cleanliness throughout the length of the SB showed some benefit for Ensure (a score of 1.8) over control group 2 (a score of 2) (P = 0.06). The cleanliness grading of the proximal and distal parts of the SB was similar in all four groups (P = 0.6 for both). The cleanliness in the middle part of the SB in the PEG (a score of 1.8) and Ensure groups (a score of 1.7) was equally better than that of control group 2 (a score of 2.1) (P = 0.057 and P = 0.07, respectively). All 50 PEG patients had diarrhea as an anticipated side effect, compared with only one patient in the Ensure group.

CONCLUSION: Preparation with Ensure, a liquid, fiber-free formula has advantages over standard and PEG preparations, with significantly fewer side effects than PEG.

© 2013 Baishideng. All rights reserved.

Key words: Capsule endoscopy; Preparation; Polyethylene glycol; Ensure; Fiber-free diet; Image quality

Niv E, Ovadia B, Ron Y, Santo E, Mahajna E, Halpern Z, Fireman Z. Ensure preparation and capsule endoscopy: A two-center

prospective study. *World J Gastroenterol* 2013; 19(8): 1264-1270 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i8/1264.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i8.1264

INTRODUCTION

Capsule endoscopy (CE) has become a commonly applied technology for examining the small bowel (SB) mucosa[1-8]. A review of the literature revealed that there is no universally accepted protocol for bowel preparation before a CE study[9-23]. A proper preparation is one that decreases the presence of debris, food remnants, bile and stool content in the intestinal lumen and significantly increases diagnostic accuracy. Preparation by a 12-h fast and light meals one day before the procedure, as suggested by the manufacturer of the capsule, is inadequate. Investigators have prepared the bowels of their patients with different doses of purgatives [polyethylene glycol (PEG) or sodium phosphate solution] or prokinetics (erythromycin, metoclopramide, domperidone and tegaserod) and/or anti-bubble agents (simethicone)[9-23]. The main disadvantage of purgatives is that they are not well tolerated by patients. Moreover, the efficacy of both purgatives and prokinetics is controversial.

The physiologic function of the small intestine is very different from that of the colon. While the colon functions as a stool reservoir and is full of stool even during a fast, the small intestine is free of stool or any content after a 24- to 48-h fast. Since instructing patients to observe such prolonged fasting before undergoing CE is unreasonable, ingestion of a liquid, fiber-free formula instead of food during the 24 h preceding a CE examination would appear to be a more practical option for ensuring sufficient cleanliness of the small intestine as well as good patient compliance. This idea has not been investigated before.

The aim of this two-center prospective study was to compare the SB cleanliness and the quality of CE images of patients who were prepared by ingestion of Ensure (a fiber-free liquid formula) with those of patients who were assigned the PEG and two control groups.

MATERIALS AND METHODS

This prospective, blind-to-preparation investigation was conducted in two Israeli medical centers, the Tel-Aviv Sourasky Medical Center (TASMC) and the Hillel-Yaffe Medical Center (HYMC). The protocol of the study was approved by the local Helsinki committees of both centers. Consecutive patients who were referred to CE studies were asked to sign informed consent forms to participate in the study. The usual medical indications for CE referral were: (1) suspected Crohn's disease (CD); (2) gastrointestinal (GI) bleeding of unknown origin (normal gastroscopy and colonoscopy); (3) iron deficiency anemia of unknown origin (normal gastroscopy and colonoscopy, negative celiac serology); (4) suspected malabsorption disease; (5) unexplained abdominal pain; (6) unexplained chronic diarrhea; and (7) abnormal SB findings on abdominal computerized tomogram (CT).

The exclusion criteria were: age < 18 and > 70 year, pregnancy, previous surgery involving the GI tract, previous intestinal obstruction, and refusal to participate in the study. Because of its sucrose content, diabetes mellitus was an exclusion criterion for the Ensure group only. The relevant demographic and medical data were collected, including: age, gender, the indication for a CE study, chronic diseases, medications, previous surgeries, and previous GI endoscopic examinations.

Study group assignment

The patients were divided into four groups and they all observed a 12-h overnight fast before undergoing CE, whatever preparation protocol they followed.

The patients who were evaluated at HYMC were randomly divided into two groups. Those assigned to the PEG group were instructed to eat light meals and to ingest two liters of PEG one day before undergoing CE. Those assigned to the Ensure group were instructed to drink up to four cans of the Ensure Plus® formula ad libitum throughout 24 h, and to stop all other food/beverage intake except for water. Ensure Plus® (Abbott Laboratories) is a popular, fiber-free, polymeric-balanced formula with a caloric content of 355 kcal/can, 13 g protein, 50.1 g carbohydrates, 11.4 g fat, minerals, trace elements and vitamins.

The patients who underwent the CE study due to medical indications in TASMC during the same period of time comprised the two control groups: the control group 1 patients were instructed to eat light meals during the day before the CE, and the control group 2 patients followed a low-fiber diet (without fruits, vegetables and fiber supplements) for 2 d before CE and ate light meals one day before undergoing CE.

The CE procedure was standard in both centers. All patients ingested a PillCam™ SB 2 wireless video capsule (Given® Diagnostic Imaging System, Yokneam, Israel) with a small amount of water. An array of 8 sensors was attached to the abdominal wall, and a recorder with a battery was fastened around their waists with a belt. The patients were allowed to drink liquids two hours after ingesting the capsule and to eat a light meal four hours later. Eight hours after capsule ingestion, the recorder was disconnected and the sensors were removed. The recorded digital information was downloaded onto the computer and the images were analyzed using RAPID® software. The CE films were examined by gastroenterologists with CE expertise in both medical centers. Afterwards, a single independent expert who was unaware of the provenance of the films reviewed all films for the presence of bile and degree of cleanliness.

The following data were collected and analyzed from the CE studies: (1) Indications for the CE study grouped into 3 categories (suspected CD, obscure GI bleeding, others); (2) Gastric transit time as calculated from the

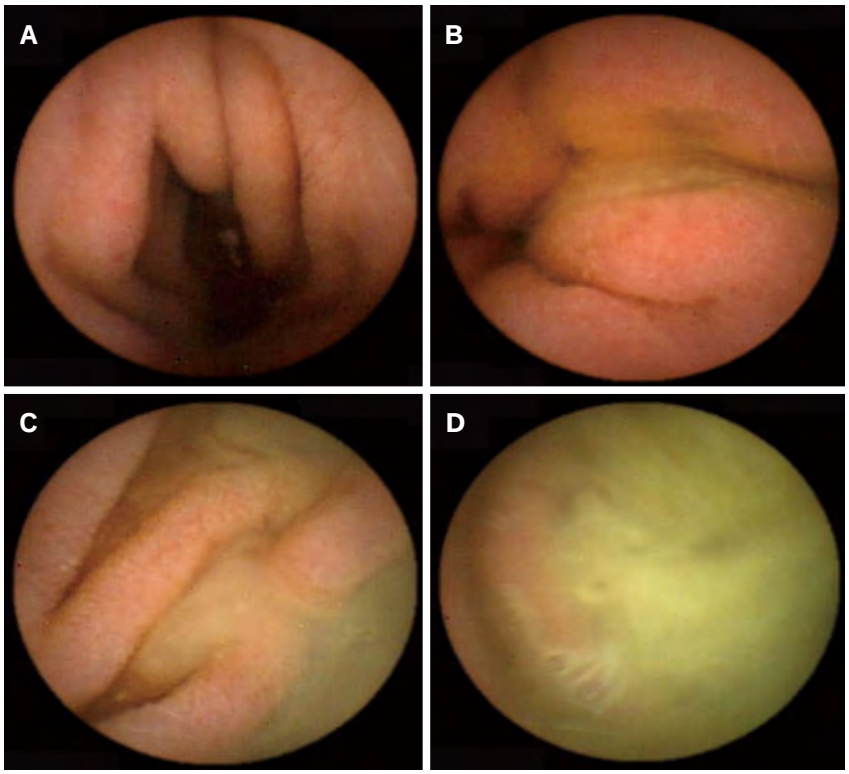


Figure 1 Grading of the presence of bile in the duodenum. A: Grade 1; B: Grade 2; C: Grade 3; D: Grade 4.

first view of the gastric mucosa to the first image of the duodenum, expressed in minutes; (3) SB transit time as calculated from the first view of the duodenum up to the first cecal image, expressed in minutes; (4) Final diagnosis grouped into six categories (established CD, angiodysplasia, polyps, nonspecific findings, normal examination, others); (5) Cecum arrival: a CE study was defined as having been completed if the capsule reached the cecum. An abdominal X ray was performed to exclude capsule retention in cases of non-arrival; (6) The bile presence score: the presence of bile in the duodenum lumen was evaluated using a scale of 1 to 4, with 1 representing none and 4 indicating more than 90% of the lumen being full of bile (Figure 1); (7) The cleanliness score: SB cleanliness was evaluated by a 4-point scale, with 1 indicating no residual material in the lumen and 4 indicating more than 90% of the lumen having residual material (Figure 2). The SB section of the CE study was divided into three parts: proximal, middle and distal. Each part received a separate cleanliness score; (8) Extra-SB findings, *i.e.*, the number of recorded findings in the esophagus, stomach and cecum; and (9) Follow-up, including the number of Ensure cans that were consumed, possible side effects of Ensure and PEG, level of satisfaction from preparation in PEG and Ensure groups, patient compliance with the preparation's protocol.

Statistical analysis

Descriptive statistics are given as mean and standard deviation for continuous variables and frequency distribution for categorical variables. Comparisons between patients with different protocols of preparation with regard to demographics (age, gender), clinical parameters (indications for CE study) and CE data (final diagnosis, gastric and SB transit times, cecal arrival rate, duodenal bile score and cleanliness score) were performed using the Chi-square, Fisher's Exact and unpaired *t*-tests. The non-parametric Mann-Whitney test was also applied if the continuous parameters did not follow a normal distribution. Pairwise comparisons were performed when the results of the overall tests were significant. The False Discovery Rate method for adjustment of significance level was used. Comparison of continuous variables between groups was performed by one-way analysis of variance (ANOVA) and the non-parametric ANOVA Kruskal-Wallis test.

Ryan-Einot-Gabriel-Welsch pair-wise comparisons between groups were employed when the ANOVA indicated significant results. Additionally, post-hoc tests using Mann-Whitney tests with Bonferroni adjustment were performed when Kruskal-Wallis test revealed significant relations between groups and other clinical parameters. The relationships between transit time and other continuous parameters were examined by the Spearman's correlation coefficients. All statistical analyses were performed using SAS for Windows 9.2. The statistical tests were defined as having a confidence interval of 95%. $P < 0.05$ was considered statistically significant.

RESULTS

A total of 139 patients were screened to participate in the PEG and Ensure groups. Sixty-seven of them were

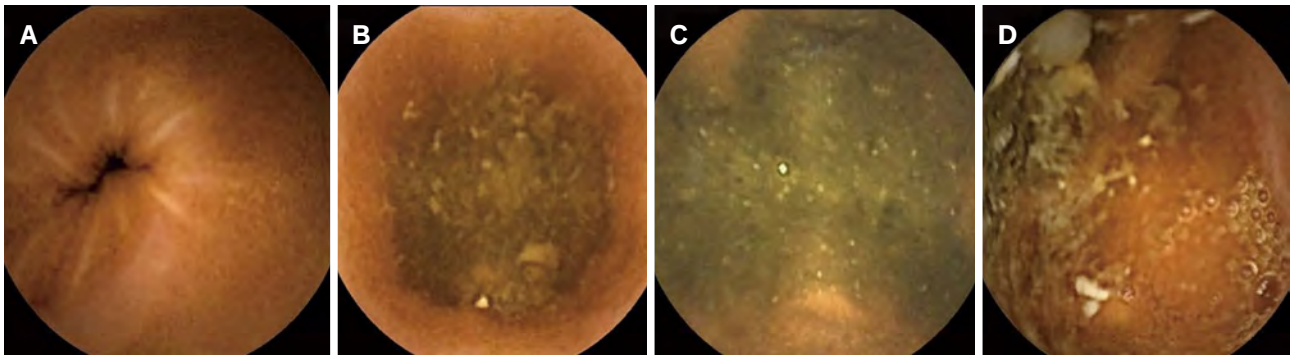


Figure 2 Grading of small bowel cleanliness. A: Grade 1; B: Grade 2; C: Grade 3; D: Grade 4.

Table 1 Demographics of the entire study population *n* (%)

	Group 1 Overnight fast	Group 2 Low fiber diet and overnight fast	Group 3 PEG and overnight fast	Group 4 Ensure and overnight fast	*P* value
Number of patients	45	81	50	22	
Male/female	21/24	42/39	27/23	8/14	
Average age (yr)	55.3	53	48.5	41.1	0.0138 (groups 1 and 2 *vs* 4)
Indication for CE					
Suspected CD	11 (24.4)	20 (24.7)	18 (36)	13 (59)	0.0151 (groups 1 and 2 *vs* 4)
Obscure GI bleeding	29 (64.4)	50 (61.7)	24 (48)	6 (27.3)	0.0124 (groups 1 and 2 *vs* 4)
Others	5 (11.2)	11 (13.6)	8 (16)	3 (13.7)	0.9265
Number of incomplete cases[1]	3 (6.7)	5 (6.2) +1 gastroparesis	7 (14)	3 (13.6) +2 gastroparesis	0.14

[1]Incomplete cases = non-arrival to the cecum. CE: Capsule endoscopy; CD: Crohn's disease; PEG: Polyethylene glycol; GI: Gastrointestinal.

excluded from the study due to the following reasons: About 13 patients had diabetes mellitus, 18 patients were younger than 18 years, 11 patients had previous intestinal surgeries, 8 patients had an urgent CE study, and 17 patients refused to participate in the study. The remaining 72 patients were divided into either the PEG (n = 50) or Ensure (n = 22) groups. Control group 1 consisted of 45 patients and control group 2 consisted of 81 patients.

Table 1 presents the demographic data of these four groups of patients. The PEG and Ensure groups were younger than both control groups. About 24%-25% of all patients in the control groups were referred to the CE study in order to rule out CD, about 60%-65% to identify a reason for obscure GI bleeding and about 11%-14% for other indications. The percentage of cases with suspected CD was higher in the PEG and Ensure groups than in the control groups. The reason for these age and referral differences between the study and control groups was related to the refusal of more of the older patients to drink PEG or Ensure. The rate of cases with non-arrival of the capsule to the cecum was not significantly different between the groups (P = 0.14). Capsule retention was excluded in all cases of incomplete studies by abdominal X-ray. One control patient and two Ensure patients had an extremely prolonged gastric transit time (> 4 h) without any anatomic reason on gastroscopy. These three patients were considered as having gastroparesis and were not included in the evaluation of the rate of non-arrival to the cecum. These patients with a gastroparesis were 25, 33 and 56 years old with no previous intestinal surgery and no diabetes mellitus. They were referred to CE study to exclude CD and celiac disease and to identify a reason for iron deficiency anemia.

In order to provide a reliable analysis of image quality of CE studies, all cases of incomplete studies (non-arrival to cecum and gastroparesis) and those with incomplete data were excluded from the analyses in Table 2. All four resultant groups were very similar to the original groups with respect to age and indications for CE study. Overall, 107 of those 157 cases had a normal study or non-specific findings (68%). The rate of positive findings by CE was 32%. Twelve of 53 patients with suspected CD were diagnosed as having definite CD, with a yield of 23% (7.6% of all CE studies). There was an 18% rate of various extra-SB findings. Both gastric transit time and SB transit time for the four groups showed no statistical difference (P = 0.29 and P = 0.7, respectively), ranging between 29.1-40.1 min and 220.4-228 min for gastric and SB transit times, respectively.

The presence of duodenal bile was similar in both control groups (Table 3). Much more bile interfered with the visualization of duodenal mucosa in the PEG group (a score of 2.9) than in the Ensure group (a score of 1.76), and the difference almost reached a level of significance (P = 0.053). Overall, as expected, there was less residue and stool in the proximal part of the SB than in the middle and distal parts in all groups. The cleanliness grading of the proximal and distal parts of the SB was similar in

Table 2 Parameters of study population with complete date *n* (%) (mean ± SD)

	Group 1 Overnight fast	Group 2 Low fiber diet and overnight fast	Group 3 PEG and overnight fast	Group 4 Ensure and overnight fast	*P* value
Number of patients	36	61	43	17	
Male/female	15/21	34/27	24/19	5/12	
Average age (yr)	55.9	53	46.9	41.9	
Indication for CE					
Suspected CD	8 (22.2)	17 (27.8)	17 (39.5)	10 (58.9)	
Obscure GI bleeding	23 (63.9)	37 (60.7)	18 (41.9)	4 (23.5)	
Others	5 (13.9)	7 (22.5)	8 (18.6)	3 (17.6)	
Final SB diagnosis					
Established CD	2 (5.56)	6 (9.8)	2 (4.6)	2 (11.8)	
Angioectasia	4 (11.1)	11 (18)	4 (2.3)	1 (5.9)	
SB Polyp	1 (2.8)	0	0	2 (11.8)	
Celiac disease	0	3 (4.9)	0	0	
Nonspecific findings	2 (5.56)	7 (11.5)	5 (11.6)	3 (17.6)	
Normal study	25 (69.4)	29 (47.5)	29 (67.4)	7 (42.2)	
Others	2 (5.56)	5 (8.2)	3 (7)	2 (11.8)	
Extra-SB findings	5 (13.9)	16 (26.2)	6 (14)	4 (23.5)	
Mean gastric transit time (min)	29.1 ± 35.5	34.9 ± 31.3	35.8 ± 29.8	40.1 ± 41	General = 0.29; 0.075 (group 1 *vs* group 2); 0.08 (group 1 *vs* group 3)
Mean SB transit time(min)	228 ± 95.4	220.4 ± 81.4	239.6 ± 84	222.4 ± 100.4	General = 0.7

CE: Capsule endoscopy; SB: Small bowel; CD: Crohn's disease; GI: Gastrointestinal.

Table 3 Scoring of bile presence and cleanliness of small bowel (mean ± SD)

	Group 1 Overnight fast	Group 2 Low fiber diet and overnight fast	Group 3 PEG and overnight fast	Group 4 Ensure and overnight fast	*P* value
Number of patients	36	61	43	17	
Duodenal bile score	1.96 ± 0.7	1.9 ± 0.6	2.9 ± 0.6	1.76 ± 0.6	General = 0.15; 0.053 (group 3 *vs* group 4)
Cleanliness of proximal SB	1.8 ± 0.6	1.8 ± 0.4	1.7 ± 0.4	1.6 ± 0.34	General = 0.6
Cleanliness of middle SB	1.9 ± 0.7	2.1 ± 0.6	1.8 ± 0.6	1.7 ± 0.5	General = 0.16; 0.057 (group 2 *vs* group 3); 0.07 (group 2 *vs* group 4)
Cleanliness of distal SB	2.2 ± 0.7	2.2 ± 0.6	2.3 ± 0.8	2.1 ± 0.6	General = 0.6
Total score of clearness	1.97 ± 0.7	2 ± 0.6	1.9 ± 0.6	1.8 ± 0.5	General = 0.4; 0.06 (group 2 *vs* group 4)

SB: Small bowel; PEG: Polyethylene glycol.

all four groups ($P = 0.6$ and $P = 0.6$, respectively). The scores of the middle part of the SB in the PEG and Ensure groups were lower than that of the control group 2 ($P = 0.057$ and $P = 0.07$, respectively). The total cleanliness score for the entire length of the SB showed some difference between that same control group and the Ensure group ($P = 0.06$), with a benefit for the latter.

Patient compliance among the PEG and Ensure groups was similar (1.25 and 1.2, respectively, $P = 0.7$), as was subjective feeling during the preparation for CE ($P = 0.163$). The patients succeeded in drinking an average of two cans of Ensure, ranging from 0.25 to 4 cans per day. All 50 PEG patients had diarrhea during the day they drank it, compared to only one of the 22 patients in the Ensure group.

DISCUSSION

Our prospective, blind-to-preparation, two-center study demonstrated that a preparation by a liquid, fiber-free formula (Ensure) before a CE study has several advantages over other types of preparation. First, it improved the visualization in the duodenum by slightly decreasing the amount of bile compared with the control groups and by decreasing the amount of bile to a level that almost reached statistical significance compared with the PEG group ($P = 0.053$). Secondly, the total score of cleanliness throughout the length of the SB showed some benefit for the Ensure preparation over one of the control groups ($P = 0.06$). Finally, the cleanliness in the middle part of the SB of the patients in both the PEG and Ensure groups was better than that of one of control groups ($P = 0.057$ and $P = 0.07$, respectively). However, all patients in the PEG group had diarrhea as a side effect, compared to only one patient in the Ensure group.

Many studies have dealt with the issue of preparation before a CE study by means of different kinds of

prokinetics and purgatives[9-23]. In contrast, our current study approached this problem by considering the physiological function of the SB as a place for the digestion and absorption of food and not as a reservoir for stools. As such, we expected that preparation with a liquid, fiber-free formula for one day before the CE study (followed by a 12-h overnight fast) should ensure SB cleanliness by keeping the amount of residue in the SB to a minimum. The results of our study bore out these expectations by demonstrating a positive impact of Ensure preparation on the bile score in the duodenum compared with the score for the PEG group and a total cleanliness score compared with one of the control groups. The Ensure preparation proved itself as good as PEG in terms of cleanliness in the middle part of the SB, with much less reported diarrhea. The statistical differences that showed strong trends but that rarely decreased below 0.05 to indicate significance could probably be related to the relatively small number of patients in the Ensure preparation group.

Our choice of Ensure Plus to serve as a very low-residue diet was arbitrary. We could have used any other commercially available free-fiber liquid formula and even a milkshake or soy-milkshake.

The efficacy of the PEG preparation is controversial. Some studies showed its benefit over a 12-h fast[17,18,20], while others reported no advantage[16,23]. It is obvious that preparation with 2 liters of PEG is as good as 4 liters[20]. More bile in the images of the PEG group led to poorer image quality in the duodenum than that of the Ensure group. The cleanliness in the middle part of the SB was similar to that of the Ensure group and better than that of the control groups, without there being any effect on the cleanliness of the proximal and distal parts. However, diarrhea as a common side effect was a significant disadvantage of PEG compared with Ensure and the control groups.

This study has a number of limitations. Although we succeeded in recruiting enough patients in the PEG and control groups for conducting capsule studies, the Ensure group was small. Therefore, some of our results could not reach a level of statistical significance. In addition, there were some basic differences between the groups: the PEG and ENSURE groups included younger populations with a higher percentage of CD patients. One important advantage of this study was the fact that all the scoring was done by a single blind-to-preparation expert in CE studies.

In summary, this prospective, two-center study raised, for the first time, the option of preparation for CE study with a liquid, fiber-free formula, and showed that it has some advantage over standard preparation or/and PEG, with significantly fewer side effects than PEG.

ACKNOWLEDGMENTS

Esther Eshkol is thanked for editorial assistance.

COMMENTS

Background

Capsule endoscopy (CE) has become a commonly used technology for examining the small bowel (SB) mucosa. A proper preparation decreases the amount of debris, food remnants, bile and stool present in the intestinal lumen and significantly increases diagnostic accuracy. However, there is no universally accepted protocol for SB preparation before a CE study. A standard preparation by 12-h fast and light meals one day before the procedure is inadequate. Preparations with purgatives are not well tolerated by patients, and their efficacy is controversial.

Research frontiers

Considering the physiological function of the SB as a site for the digestion and absorption of food and not as a stool reservoir, a preparation consisting of a liquid, fiber-free formula (like Ensure®) ingested one day before a CE study might have an advantage over other kinds of preparations. The aim of this two-center prospective, blind-to-preparation study was to compare the SB cleanliness and the quality of CE images of patients who were prepared by ingestion of Ensure® with those of patients who were assigned to the polyethylene glycol (PEG) group and two control groups that received standard preparation.

Innovations and breakthroughs

Preparation with Ensure® improved the visualization of duodenal mucosa by decreasing the bile content compared to preparation with PEG. The total score of cleanliness throughout the length of the SB showed some benefit for Ensure® over one of control groups. The cleanliness in the middle part of the SB in the PEG and Ensure® groups was equally better over of one of the control groups. As expected, all 50 PEG patients had diarrhea as a side effect, compared with only one patient in the Ensure group. This study presents for the first time a novel idea that preparation for undergoing CE of the SB should be completely different from the preparation for endoscopic examination of a colon and should be based on fasting and a liquid, fiber-free formula and not on purgatives.

Applications

This study results suggest that the preparation with Ensure®, a liquid, fiber-free formula has advantages over standard and PEG preparations, with significantly fewer side effects than PEG.

Terminology

Capsule endoscopy: CE is a technology that uses a swallowed wireless video capsule for photographing the inside of the esophagus, stomach, and small intestine. The images are transmitted it to a belt-holded recorder. It is a well-accepted tool which provides a direct and noninvasive approach to view the entire small bowel mucosa. This technology has enriched the knowledge on small bowel pathology and revolutionized the overall management of SB diseases.

Peer review

The manuscript is well-written and refers to an important issue in light of the increasing utility of the CE modality. The authors present a novel approach to a common "real life" issue and provide a new opportunity for an easy and convenient preparation.

REFERENCES

1 **Iddan G**, Meron G, Glukhovsky A, Swain P. Wireless capsule endoscopy. *Nature* 2000; **405**: 417 [PMID: 10839527 DOI: 10.1038/35013140]

2 **Scapa E**, Jacob H, Lewkowicz S, Migdal M, Gat D, Gluckhovski A, Gutmann N, Fireman Z. Initial experience of wireless-capsule endoscopy for evaluating occult gastrointestinal bleeding and suspected small bowel pathology. *Am J Gastroenterol* 2002; **97**: 2776-2779 [PMID: 12425547 DOI: 10.1111/j.1572-0241.2002.07021.x]

3 **Costamagna G**, Shah SK, Riccioni ME, Foschia F, Mutignani M, Perri V, Vecchioli A, Brizi MG, Picciocchi A, Marano P. A prospective trial comparing small bowel radiographs and video capsule endoscopy for suspected small bowel disease. *Gastroenterology* 2002; **123**: 999-1005 [PMID: 12360460]

4 **Ell C**, Remke S, May A, Helou L, Henrich R, Mayer G. The

first prospective controlled trial comparing wireless capsule endoscopy with push enteroscopy in chronic gastrointestinal bleeding. *Endoscopy* 2002; **34**: 685-689 [PMID: 12195324 DOI: 10.1055/s-2002-33446]
5 **Fireman Z**, Glukhovsky A, Jacob H, Lavy A, Lewkowicz S, Scapa E. Wireless capsule endoscopy. *Isr Med Assoc J* 2002; **4**: 717-719 [PMID: 12440238 DOI: 10.1136/gut.52.3.390]
6 **Mylonaki M**, Fritscher-Ravens A, Swain P. Wireless capsule endoscopy: a comparison with push enteroscopy in patients with gastroscopy and colonoscopy negative gastrointestinal bleeding. *Gut* 2003; **52**: 1122-1126 [PMID: 12865269 DOI: 10.1136/gut.52.8.1122]
7 **Rey JF**, Ladas S, Alhassani A, Kuznetsov K. European Society of Gastrointestinal Endoscopy (ESGE). Video capsule endoscopy: update to guidelines (May 2006). *Endoscopy* 2006; **38**: 1047-1053 [PMID: 17058174 DOI: 10.1055/s-2006-944874]
8 **Mishkin DS**, Chuttani R, Croffie J, Disario J, Liu J, Shah R, Somogyi L, Tierney W, Song LM, Petersen BT. ASGE Technology Status Evaluation Report: wireless capsule endoscopy. *Gastrointest Endosc* 2006; **63**: 539-545 [PMID: 16564850 DOI: 10.1016/j.gie.2006.01.014]
9 **Villa F**, Signorelli C, Rondonotti E, de Franchis R. Preparations and prokinetics. *Gastrointest Endosc Clin N Am* 2006; **16**: 211-220 [PMID: 16644451 DOI: 10.1016/j.giec.2006.01.020]
10 **de Franchis R**, Avgerinos A, Barkin J, Cave D, Filoche B. ICCE consensus for bowel preparation and prokinetics. *Endoscopy* 2005; **37**: 1040-1045 [PMID: 16189787]
11 **Niv Y**. Efficiency of bowel preparation for capsule endoscopy examination: a meta-analysis. *World J Gastroenterol* 2008; **14**: 1313-1317 [PMID: 18322940 DOI: 10.3748/wjg.14.1313]
12 **Caddy GR**, Moran L, Chong AK, Miller AM, Taylor AC, Desmond PV. The effect of erythromycin on video capsule endoscopy intestinal-transit time. *Gastrointest Endosc* 2006; **63**: 262-266 [PMID: 16427932 DOI: 10.1016/j.gie.2005.07.043]
13 **Leung WK**, Chan FK, Fung SS, Wong MY, Sung JJ. Effect of oral erythromycin on gastric and small bowel transit time of capsule endoscopy. *World J Gastroenterol* 2005; **11**: 4865-4868 [PMID: 16097060]
14 **Niv E**, Bonger I, Barkay O, Halpern Z, Mahajna E, Depsames R, Kopelman Y, Fireman Z. Effect of erythromycin on image quality and transit time of capsule endoscopy: a two-center study. *World J Gastroenterol* 2008; **14**: 2561-2565 [PMID: 18442206 DOI: 10.3748/wjg.14.2561]
15 **Selby W**. Complete small-bowel transit in patients undergoing capsule endoscopy: determining factors and improvement with metoclopramide. *Gastrointest Endosc* 2005; **61**: 80-85 [PMID: 15672061 DOI: 10.1016/S0016-5107(04)02462-9]
16 **Fireman Z**, Paz D, Kopelman Y. Capsule endoscopy: improving transit time and image view. *World J Gastroenterol* 2005; **11**: 5863-5866 [PMID: 16270399]
17 **Viazis N**, Sgouros S, Papaxoinis K, Vlachogiannakos J, Bergele C, Sklavos P, Panani A, Avgerinos A. Bowel preparation increases the diagnostic yield of capsule endoscopy: a prospective, randomized, controlled study. *Gastrointest Endosc* 2004; **60**: 534-538 [PMID: 15472674]
18 **van Tuyl SA**, den Ouden H, Stolk MF, Kuipers EJ. Optimal preparation for video capsule endoscopy: a prospective, randomized, single-blind study. *Endoscopy* 2007; **39**: 1037-1040 [PMID: 18072052 DOI: 10.1055/s-2007-966988]
19 **Wei W**, Ge ZZ, Lu H, Gao YJ, Hu YB, Xiao SD. Purgative bowel cleansing combined with simethicone improves capsule endoscopy imaging. *Am J Gastroenterol* 2008; **103**: 77-82 [PMID: 18005366 DOI: 10.1111/j.1572-0241.2007.01633.x]
20 **Park SC**, Keum B, Seo YS, Kim YS, Jeen YT, Chun HJ, Um SH, Kim CD, Ryu HS. Effect of bowel preparation with polyethylene glycol on quality of capsule endoscopy. *Dig Dis Sci* 2011; **56**: 1769-1775 [PMID: 21161380 DOI: 10.1007/s10620-010-1500-2]
21 **Postgate A**, Tekkis P, Patterson N, Fitzpatrick A, Bassett P, Fraser C. Are bowel purgatives and prokinetics useful for small-bowel capsule endoscopy? A prospective randomized controlled study. *Gastrointest Endosc* 2009; **69**: 1120-1128 [PMID: 19152909 DOI: 10.1016/j.gie.2008.06.044]
22 **Wi JH**, Moon JS, Choi MG, Kim JO, Do JH, Ryu JK, Shim KN, Lee KJ, Jang BI, Chun HJ. Bowel preparation for capsule endoscopy: a prospective randomized multicenter study. *Gut Liver* 2009; **3**: 180-185 [PMID: 20431743 DOI: 10.5009/gnl.2009.3.3.180]
23 **Kalantzis C**, Triantafyllou K, Papadopoulos AA, Alexandrakis G, Rokkas T, Kalantzis N, Ladas SD. Effect of three bowel preparations on video-capsule endoscopy gastric and small-bowel transit time and completeness of the examination. *Scand J Gastroenterol* 2007; **42**: 1120-1126 [PMID: 17710680 DOI: 10.1080/00365520701251601]

P- Reviewer Tóth E **S- Editor** Lv S **L- Editor** A
E- Editor Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i8.1271

World J Gastroenterol 2013 February 28; 19(8): 1271-1277
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Effect of alternative antibiotics in treatment of cefotaxime resistant spontaneous bacterial peritonitis

Ahmed Abouelkhair Badawy, Tarik Ibrahim Zaher, Samar Mahmoud Sharaf, Mohamed Hassan Emara, Noha Elsaid Shaheen, Talaat Fathy Aly

Ahmed Abouelkhair Badawy, Tarik Ibrahim Zaher, Mohamed Hassan Emara, Noha Elsaid Shaheen, Talaat Fathy Aly, Department of Tropical Medicine, Zagazig University Faculty of Medicine, Zagazig 44519, Egypt
Samar Mahmoud Sharaf, Department of Clinical Pathology, Zagazig University Faculty of Medicine, Zagazig 44519, Egypt
Author contributions: Badawy AA, Zaher TI, Emara MH, Shaheen NE and Aly TF shared in study design and recuited the patients; Sharaf SM collected the samples and performed all laboratory work up; all authors shared in literature review analysis, writing the manuscript, manuscript revision and approval.
Correspondence to: Dr. Mohamed Hassan Emara, MD, Department of Tropical Medicine, Zagazig University Faculty of Medicine, Zagazig 44519, Egypt. emara_20007@yahoo.com
Telephone: +20-55-2301523 Fax: +20-55-2301523
Received: September 14, 2012 Revised: October 16, 2012
Accepted: November 11, 2012
Published online: February 28, 2013

Abstract

AIM: To evaluate effective alternative antibiotics in treatment of cefotaxime-resistant spontaneous bacterial peritonitis.

METHODS: One hundred cirrhotic patients with spontaneous bacterial peritonitis [ascitic fluid polymorphonuclear cell count (PMNLs) ⩾ 250 cells/mm^3 at admission] were empirically treated with cefotaxime sodium 2 g/12 h and volume expansion by intravenous human albumin. All patients were subjected to history taking, complete examination, laboratory tests (including a complete blood cell count, prothrombin time, biochemical tests of liver and kidney function, and fresh urine sediment), chest X-ray, a diagnostic abdominal paracentesis, and the sample subjected to total and differential cell count, chemical examination, aerobic and anaerobic cultures. Patients were divided after 2 d by a second ascitic PMNL count into group Ⅰ; patients sensitive to cefotaxime (n = 81), group Ⅱ (n = 19); cases resistant to cefotaxime (less than 25% decrease in ascitic PMNL count). Patients of group Ⅱ were randomly assigned into meropenem (n = 11) or levofloxacin (n = 8) subgroups. All patients performed an end of treatment ascitic PMNL count. Patients were considered improved when: PMNLs decreased to < 250 cells/mm^3, no growth in previously positive culture cases, and improved clinical manifestations with at least 5 d of antibiotic therapy.

RESULTS: Age, sex, and Child classes showed no significant difference between group Ⅰ and group Ⅱ. Fever and abdominal pain were the most frequent manifestations and were reported in 82.7% and 80.2% of patients in group Ⅰ and in 94.7% and 84.2% of patients in group Ⅱ, respectively. Patients in group Ⅱ had a more severe ascitic inflammatory response than group Ⅰ and this was demonstrated by more ascitic lactate dehydrogenase (LDH) [median: 540 IU/L (range: 150-1200 IU/L) *vs* median: 240 IU/L (range: 180-500 IU/L), P = 0.000] and PMNL [median: 15 000 cell/mm^3 (range: 957-23 822 cell/mm^3) *vs* 3400 cell/mm^3 (range: 695-26 400 cell/mm^3), P = 0.000] counts. Ascitic fluid culture was positive in 32% of cases. Cefotaxime failed in 19% of patients; of these patients, 11 (100%) responded to meropenem and 6 (75%) responded to levofloxacin. Two patients with failed levofloxacin therapy were treated according to the *in vitro* culture and sensitivity (one case was treated with vancomycin and one case was treated with ampicillin/sulbactam). In group Ⅱ the meropenem subgroup had higher LDH (range: 108-860 IU/L *vs* 120-491 IU/L, P = 0.042) and PMNL counts (range: 957-23 822 cell/mm^3 *vs* 957-15 222 cell/mm^3, P = 0.000) at initiation of the alternative antibiotic therapy; there was no significant difference in the studied parameters between patients responsive to meropenem and patients responsive to levofloxacin at the end of therapy (mean ± SD: 316.01 ± 104.03

PMNLs/mm^3 *vs* 265.63 ± 69.61 PMNLs/mm^3, *P* = 0.307). The isolated organisms found in group Ⅱ were; *enterococci*, *acinetobacter*, expanded-spectrum β-lactamase producing *Escherichia coli*, β-lactamase producing *Enterobacter* and *Staphylococcus aureus*.

CONCLUSION: Empirical treatment with cefotaxime is effective in 81% of cases; meropenem is effective in cefotaxime-resistant cases.

© 2013 Baishideng. All rights reserved.

Key words: Spontaneous bacterial peritonitis; Cefotaxime; Ascitic polymorphonuclear count; Cirrhosis; Meropenem; Levofloxacin

Badawy AA, Zaher TI, Sharaf SM, Emara MH, Shaheen NE, Aly TF. Effect of alternative antibiotics in treatment of cefotaxime resistant spontaneous bacterial peritonitis. *World J Gastroenterol* 2013; 19(8): 1271-1277 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i8/1271.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i8.1271

INTRODUCTION

Spontaneous bacterial peritonitis (SBP) is infection of ascitic fluid in cirrhotic patients. In 1985, results of a randomized trial demonstrated that cefotaxime, as a third-generation cephalosporin, achieved cure in SBP episodes for 85% of patients, compared to 56% of patients who received ampicillin plus tobramycin[1]. Since then, cefotaxime has become the empiric antibiotic of choice for the treatment of SBP. Mortality rates reduced from 90% when it was first described to around 20%-40%[2]. The widespread use of diagnostic paracentesis, as well as the prompt initiation of empiric antibiotic therapy, based on the results of ascitic polymorphonuclear (PMNL) cell count, has contributed in the improvement of survival in these patients[3]. Recent reports showed that resistance to third generation cephalosporins (including cefotaxime) is increasing[4,5] and this may increase the need to develop alternative antibiotics. The primary aim of this study was to evaluate effective alternative antibiotics in treatment of cefotaxime-resistant spontaneous bacterial peritonitis.

MATERIALS AND METHODS

Study design

This prospective study was conducted at the Tropical Medicine Department, Zagazig University Hospitals, Zagazig, Sharkia Governorate, Egypt during the period from October 2010 to October 2011. This study was approved by the Ethical committee of our institution. Written informed consents were obtained from all patients. For patients with any grade of hepatic encephalopathy, the written informed consents were obtained from the first degree relatives.

Patient management

All patients were inpatients and underwent diagnostic abdominal paracentesis with a differential leucocyte count, microbiological culture, and biochemical analysis at admission. Cefotaxime sodium 2 g/12 h (*iv*) was used as the initial empirical therapy because it is supported by the current guidelines as the first line of therapy for SBP[2]. It was subsequently maintained or replaced depending on the clinical course, a second abdominal paracentesis with PMNL count, and the *in vitro* susceptibility of the isolated microorganisms, up to a planned period of 5-7 d in a hospitalized basis.

After 48 h, a second abdominal paracentesis was done; cases with < 25% reduction in PMNL count were considered resistant to cefotaxime and were then randomly assigned to receive either meropenem 1 g/8 h (*iv*) or levofloxacin 1 g/12 h (*iv* drip). They were randomized by matching the sample (resistant group) and were classified into matched pairs; one from each pair was randomly enrolled into either the meropenem subgroup or the levofloxacin subgroup. One patient who was initially assigned to the levofloxacin subgroup was moved to the meropenem subgroup due to drug availability at the time of therapy. The final number was 11 patients in the meropenem subgroup and 8 in the levofloxacin subgroup.

The choice of these antibiotics was not only a matter of availability but also mainly due to previous reports of activity against bacterial species causing SBP[6,7] both *in vitro*[8] and *in vivo*[9] at national[10] and international[11] levels.

Five days after initiation of alternative antibiotic therapy an end of treatment diagnostic abdominal paracentesis was performed; cases with < 25% reduction in PMNL count from the second puncture counts were treated according to the results of *in vitro* culture and sensitivity that were available. All cases with initially positive ascitic fluid culture were re-cultured. Intravenous expansion was performed with human albumin 20 g *iv* infusion at the time of diagnosis and after 48 h.

All patients underwent a final diagnostic paracentesis with a differential leucocyte count at the end of therapy. The antibiotic dosage was adjusted to renal function throughout the treatment period. Diuretics were routinely discontinued at the time of diagnosis of SBP and therapeutic paracentesis was not allowed during the study.

Clinical and laboratory assessment

All patients were subjected to history taking, complete clinical examination, laboratory tests (including a complete blood cell count, prothrombin time, biochemical tests of liver and kidney function, and fresh urine sediment), chest x-ray film, a diagnostic abdominal paracentesis, and the sample was subjected to total and differential cell count, chemical examination, aerobic and anaerobic culture. These were performed in all the cirrhotic patients with ascites on the day of admission and

Table 1 Demographic and clinical features of all patients *n* (%)

Variable	Group Ⅰ (*n* = 81)	Group Ⅱ (*n* = 19)	χ^2	*P* value
Gender				
Male	48 (59.3)	11 (57.9)	0.01	0.91
Female	33 (40.7)	8 (42.1)		
Age (yr) mean ± SD	49.4 ± 7.74	51.5 ± 8.08	1.04	0.29
Abdominal pain				
Yes	65 (80.2)	16 (84.2)	0.01	0.9
No	16 (19.8)	3 (15.8)		
Fever				
Yes	67 (82.7)	18	0.93	0.33
No	14 (17.3)	1 (5.3)		
Child Class				
B	17 (21)	1 (5.3)	1.62	0.2
C	64 (79)	18 (94.7)		
Abdominal tenderness				
Yes	43 (53.1)	12 (63.2)	0.11	0.427
No	38 (46.9)	7 (36.8)		
Upper GI bleeding				
Yes	8 (9.9)	4 (21.1)	1.820	0.177
No	73 (90.1)	15 (78.9)		
Encephalopathy				
Yes	25 (30.9)	12 (63.2)	6.885	0.009
No	56 (69.1)	7 (36.8)		
Asymptomatic				
Yes	15 (18.5)	0 (0.00)	4.08	0.04
No	66 (81.5)	19 (100)		

GI: Gastrointestinal.

whenever they developed symptoms and signs suspicious for SBP (*i.e.*, fever, change in mental status, abdominal pain, development of renal failure, hypotension, *etc.*) during the hospitalization period.

The ascitic fluid samples were collected under aseptic conditions in tubes containing ethylenediamine tetraacetic acid anticoagulant and then tested to determine white blood cell and PMNL counts by automated cell blood counter; chemical analysis for glucose, lactate dehydrogenase (LDH) and total protein were also done. All the specimens were analyzed within one hour. Moreover, 10 mL of ascitic fluid was inoculated directly at the patient's bedside into aerobic and anaerobic blood culture bottles for bacteriological examination.

Microbiological analysis

Ascitic fluid cultures were performed at the time of the initial paracentesis in the blood culture bottles (Remaux, France) at the bedside. All were incubated aerobically and anaerobically at 37 ℃ for 2 d before a subculture was done. Cases were considered negative after 7 d. All initially positive cases were re-cultured and cases were considered resolved from infection when PMNLs count had dropped to < 250/mm^3 and all culture cases were negative.

Definitions

Cirrhosis: Cirrhosis diagnosed based on clinical, biochemical, histological and/or radiological findings and Child class was assessed for each patient.

SBP: SBP was defined as the presence of ⩾ 250 PMNLs/mm^3 in the ascitic fluid[2].

Resolution of SBP: Resolution of SBP was defined as reduction of the elevated ascitic fluid PMNL count to < 250/mm^3, negative culture in previously positive cases with resolution of the clinical manifestations and normalization of the impaired renal function.

Resistance: Resistance to empiric therapy was considered when < 25% reduction in PMNL count from the base line was achieved after 48 h[2].

Exclusion criteria: (1) Non-cirrhotic ascites (including malignant ascites); (2) Cases with secondary bacterial peritonitis; and (3) Bacterascites (*i.e.*, positive ascitic fluid culture with < 250 neutrophils/mm^3).

Statistical analysis

Data were checked, entered and analyzed using SPSS Version 15. Data were expressed as mean ± SD for quantitative variables, number and percentage for qualitative ones. χ^2 or Fisher exact, *t* test and paired *t* test were used when appropriate. $P < 0.05$ was considered significant.

RESULTS

General characteristics and clinical presentation

Baseline characteristics of patients are presented in Table 1. Age and gender were comparable in group Ⅰ and group Ⅱ. In group Ⅰ, the numbers in Child class B and C were 21% and 79% while in group Ⅱ they were 5.3% and 94.7%, respectively. This means that most cefotaxime resistance cases were associated with advanced liver disease.

Fever and abdominal pain were the most frequent manifestations and were reported in 82.7% and 94.7% of patients in group Ⅰ and in 80.2% and 84.2% of patients in group Ⅱ, respectively. Asymptomatic patients were found in 18.5% of the cefotaxime sensitive group, but all patients of the resistant group presented with symptoms and signs. Regarding the laboratory parameters (Table 2), patients in group Ⅱ had a greater peripheral blood leucocyte response than group Ⅰ.

Microorganisms

Ascitic fluid culture was positive in only 32% of cases. There were 26 ascitic fluid culture positive cases within group Ⅰ (out of 81); the isolated organisms were *Escherichia coli* (*E. coli*) in 13 (50%), *Klebsiella* in 7 (27%), *Enterobacter*, and *Strep. viridans* each in 2 (8%), *Pneumococci* and *S. aureus* each in 1 (4%) (Figure 1A). There were 6 ascitic fluid culture positive cases within group Ⅱ (out of 19); the isolated organisms were *E. coli* in 2 (33%), *Enterobac-*

Table 2 Laboratory parameters of all patients at admission

Variable/mean ± SD	Group Ⅰ (n = 81)	Group Ⅱ (n = 19)	t	P value
Laboratory parameters				
INR	1.87 ± 0.48	1.85 ± 0.57	0.091	0.927
Albumin (g/dL)	2.16 ± 0.32	2.23 ± 0.22	0.852	0.396
Total bilirubin (mg/dL)	5.45 ± 4.50	4.23 ± 2.60	1.133	0.260
Direct bilirubin (mg/dL)	3.44 ± 3.25	2.50 ± 1.95	1.202	0.232
Creatinine (mg/dL)	1.62 ± 0.85	1.80 ± 0.59	0.882	0.38
Urea (mg/dL)	83.87 ± 37.16	88.33 ± 42.99	0.457	0.649
Potassium (mmol/L)	3.76 ± 0.65	3.65 ± 0.79	0.586	0.559
Sodium (mmol/L)	130.36 ± 9.26	129.93 ± 10.53	0.178	0.859
Platelets (× 10/mL)	53.2 ± 20.1	54.6 ± 31.5	0.235	0.815
Ascitic fluid parameters				
Glucose (mg/dL)	121.05 ± 41.62	101.52 ± 53.032	1.744	0.084
Protein (mg/dL)	1389.95 ± 840.65	1553.63 ± 566.38	0.805	0.423
LDH (IU/L)	240 (180-500)	540 (108-1200)	4.406	0.000
PMNLs (/mm^3)	3400 (695-264 00)	15 000 (957-23 822)	3.852	0.000

Data are presented by mean ± SD or median (range). INR: International normalized ratio; WBC: White blood cell; LDH: Lactate dehydrogenase; PMNLs: Polymorph nuclear leucocytes.

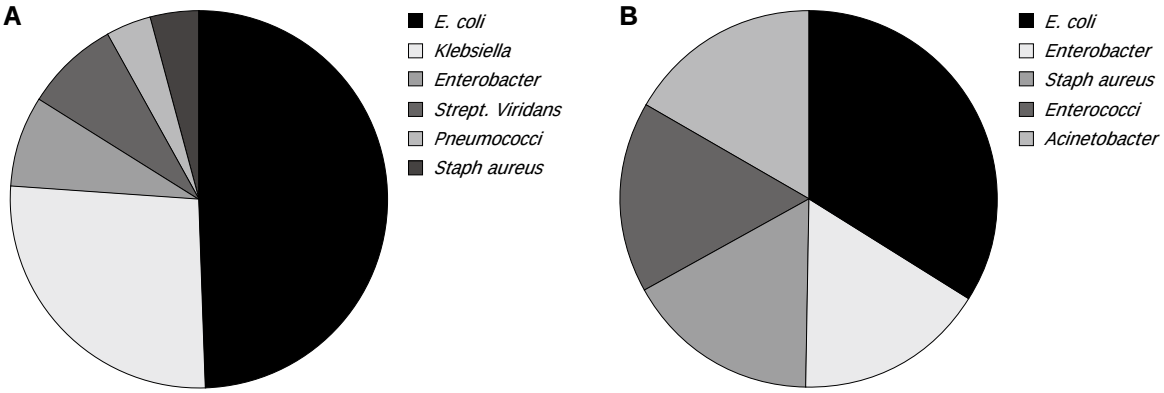


Figure 1 Isolated organisms in groups. A: Group Ⅰ; B: Group Ⅱ. *E. coli*: *Escherichia coli*.

ter, *S. aureus*, *Enterococci* and *Acinetobacter* each in one (16%) (Figure 1B).

Ascitic fluid analysis

Data for all patients are presented in Tables 2 and 3. Patients in group Ⅱ had a more severe ascitic inflammatory response than group Ⅰ and this was demonstrated by the more ascitic LDH and PMNL counts. In group Ⅰ, apart from the significant reduction in PMNL count, none of the biochemical parameters showed significant changes at the end of therapy. In group Ⅱ the meropenem subgroup had higher LDH and PMNL count at initiation of the alternative antibiotic therapy; there was no significant difference in the studied parameters between patients responsive to meropenem and levofloxacin at the end of therapy.

Treatment outcomes

Cases were treated for a median of 5-12 d. Cases in group Ⅰ (n = 81) were treated by cefotaxime for 5-7 d, while patients in group Ⅱ were treated as follows: the meropenem subgroup was treated for 5 d, the levofloxacin subgroup was treated for 5 d, 2 cases who were resistant to levofloxacin were treated with vancomycin in one case and ampicillin/sulbactam in one case, according to the *in vitro* culture and sensitivity results, for further 5 d and were recultured and examined by an end of treatment ascitic PMNL count before being considered cured.

The clinical and laboratory parameters of group Ⅱ patients showed no major improvement with the initial empirical therapy; whereas noticeable improvements in many of the parameters were achieved when the alternative antibiotic therapy was given. This was obvious in hepatic encephalopathy (HE) which was encountered in 30.9% of patients in group Ⅰ and 63.2% of patients in group Ⅱ (grade Ⅱ-Ⅲ HE), where an improvement of consciousness level was achieved after antibiotic treatment of SBP. All patients of group Ⅱ, in spite of starting treatment of HE, showed no major improvement with cefotaxime, but improvement of consciousness level occurred after the resolution of SBP by the alternative antibiotic therapy.

Table 3 Ascitic fluid parameters in group Ⅱ at the beginning and end of alternative therapy

Variable	Meropenem subgroup (n = 11)	Levofloxacin subgroup (n = 8)	t	P value
At initiation of alternative therapy				
Glucose (mg/dL)	100.45 ± 80.33	113.50 ± 9.58	0.390	0.702
Protein (mg/dL)	1850.00 ± 529.26	1360.83 ± 572.08	1.771	0.097
LDH (IU/L)	450 (108-1200)	237 (120-831)	2.223	0.042
PMNLs/mm^3	18 061 (957-23822)	3540 (957-15222)	3.622	0.000
At end of therapy				
Glucose (mg/dL)	102.81 ± 39.35	124.00 ± 29.17	1.150	0.268
Protein (mg/dL)	1660.00 ± 418.85	1163.33 ± 516.78	2.156	0.052
PMNLs/mm^3	316.01 ± 104.03	265.63 ± 69.61	1.056	0.307

Data are presented by mean ± SD or median (range). LDH: Lactate dehydrogenase; PMNLs: Polymorph nuclear leucocytes.

DISCUSSION

Cefotaxime as a standard treatment of SBP has been tried for many years with many reports of evolving resistance to cefotaxime and other third-generation cephalosporins in SBP[2,5]. Resistance to cefotaxime in this work is 19%, close to 21.5% recently reported by Ariza *et al*[5].

In this study cefotaxime resistance is not related to age or sex of patients, and the male predominance in our study is directly related to the higher rates of cirrhosis and risk of exposure and is in agreement with previous studies from other parts of the world[12,13].

Clinical manifestations of SBP are nonspecific; the most frequently encountered symptoms and signs are fever (69%) and abdominal pain (59%),[14]. This was the position in our study where fever and abdominal pain were the most frequent clinical manifestations.

However approximately 10% of patients with SBP are asymptomatic[14]; in our study asymptomatic patients made up 18.5% of the cefotaxime sensitive group, but all patients in the resistant group had clinical manifestations. This notion, together with the low yield of ascitic fluid culture, raised the value of PMNL count ⩾ 250 cell/mm^3 as the most important parameter for the diagnosis of SBP.

It is well known that infection in cirrhotic patients, especially SBP, precipitates HE and has been related to variceal bleeding both in terms of pathogenesis of portal pressure increase and severity of bleeding episodes[15] hence the related mortality was reduced by prompt antibiotic therapy[16] and this is confirmed by our study.

SBP occurs due to bacterial translocation, mainly in patients with advanced cirrhosis and severe liver functional damage[13,14,17]; this is confirmed in this study as SBP developed only in patients with Child classes B and C.

In this study, resistance to cefotaxime is associated with more advanced liver disease, because 94.7% and 5.3% of cases were Child C and B respectively. This notion needs further evaluation.

The low proportion of positive ascitic fluid cultures is probably due to the relatively low concentration of bacteria in the ascitic fluid as compared with the infections in other organic fluids (*e.g.*, urine)[18]. For the same reason, a therapy based on the isolation of the responsible bacteria is seldom achievable and antibiotic treatment cannot be delayed to the moment when microbiological results are available[19]. That is why cefotaxime as an empirical therapy should be begun without delay.

In the cefotaxime sensitive group gram negative bacteria were the most frequent (22/26; 85%) and *E. coli* was the most predominant; however Gram positive bacteria were isolated in 4 patients. In the resistant group, bacteria were isolated in 6 patients; these results are closely similar to Taşkiran *et al*[20] study which revealed positive ascitic fluid culture in 29.4% of cases with predominantly Gram negative bacteria. Angeloni *et al*[12] revealed positive cultures in 30% of episodes with predominantly Gram negative bacteria; in these episodes the percentage of treatment failure of the initial therapy with cefotaxime was 32% and this result is similar to our findings.

In our patients, cefotaxime failed because the isolated organisms were intrinsically resistant to cefotaxime as *Enterococci* (one patient) and *Acinetobacter* spp (one patient) or were capable of degrading the expanded-spectrum cephalosporins as expanded-spectrum β-lactamase producing (ESBL) *E. coli* (two patients) or β-lactamase producing *Enterobacter* species (one patient) or bacteria with inherent insufficient susceptibility to cefotaxime, such as *S. aureus* (one patient). Our study supports previous reports[21] about the introduction of new organisms in the development of SBP.

The efficacy of empiric antibiotic treatment can rarely be based on the amelioration of symptoms or on microbiological results. Therefore, a reduction of PMNL count below 250 cell/mm^3 or a 25% reduction of the initial value has been suggested as the main criterion for establishing the efficacy of antibiotics and the need for switching therapy. Our study confirms the validity of such an approach. Based on PMNL count, we identified the failure of the initial therapy on time.

Meropenem use was successful in 100% of resistant cases. Levofloxacin was successful in 75% of resistant cases, a result lower than reported for *in vivo*[9,22] and *in vitro* isolates where all aerobic isolates of SBP were sensitive to levofloxacin[23]. Other studies recommended meropenem use, not only in severe cases but also when ESBL producing *E. coli* is seen in the cultures and points to a relation between quinolone resistance and ESBL production[7,24]. Types of the isolated bacteria in group Ⅱ of our study explain the efficacy of meropenem over levofloxacin.

Albumin use was suggested in SBP management[25], although it was not included in the treatment protocols. However the guidelines for the prevention and treatment of hepato-renal syndrome[26] suggested that albumin administration may reduce the incidence of renal failure and mortality in patients with SBP; consequently, all

our patients received albumin. Some patients recorded a temporary increase in serum creatinine. All these patients showed improvement of kidney function with the use of proper antibiotics and resolution of SBP.

In conclusion, our study suggests that use of cefotaxime as the first line of treatment is considered valid, since a switch to another antibiotic was necessary only in 19% of our cases and we suggest meropenem as an effective alternative in resistant cases. This study also confirmed the value of a second abdominal paracentesis 48 h after initiation of empiric therapy to detect resistant cases early.

COMMENTS

Background

Spontaneous bacterial peritonitis is infection of ascitic fluid in cirrhotic patients with high mortality rates. Cefotaxime has become the empiric antibiotic of choice for the treatment since 1985. Mortality rates reduced from 90% when cefotaxime was first described to around 20%-40%. Recent reports showed that resistance to third generation cephalosporins (including cefotaxime) is increasing and this may increase the need to develop alternative antibiotics.

Research frontiers

Eradication of ascitic fluid infection is the goal of antibiotic therapy. This study confirmed the validity of cefotaxime as an empiric first line therapy for spontaneous bacterial peritonitis and identified meropenem as an effective alternative in cases of cefotaxime resistance.

Innovations and breakthroughs

Cefotaxime as the empiric first line therapy for spontaneous bacterial peritonitis is effective in 81% of cases. Meropenem is effective in 100% of cefotaxime-resistant cases, while levofloxacin is effective in 75% of cefotaxime-resistant cases.

Applications

This article emphasises the value of meropenem and levofloxacin as effective alternatives to cefotaxime in resistant cases.

Terminology

Spontaneous bacterial peritonitis is infection of ascitic fluid in patients with cirrhosis; Cefotaxime (a third generation cephalosporin) is utilized primarily for its activity against Gram-negative aerobic organisms. The drug exhibits moderate activity against Gram-positive bacteria. Meropenem is an ultra-broad spectrum injectable antibiotic used to treat a variety of infections. It is a beta lactam antibiotic and belongs to the subgroup of carbapenems; Levofloxacin is a synthetic chemotheraputic antibiotic which belongs to fluoroquinolone drug class and is used to treat severe or life-threatening bacterial infections or bacterial infections that have failed to respond to other antibiotic classes.

Peer review

The clinical study focused on the effect of cefotaxime as the empiric first line therapy for spontaneous bacterial peritonitis and effectiveness of meropenem and levofloxacin as alternatives in case of cefotaxime resistance. This will be useful for future planning of therapy and clinical application.

REFERENCES

1 **Felisart J**, Rimola A, Arroyo V, Perez-Ayuso RM, Quintero E, Gines P, Rodes J. Cefotaxime is more effective than is ampicillin-tobramycin in cirrhotics with severe infections. *Hepatology* 1985; **5**: 457-462 [PMID: 3888810]

2 **European Association for the Study of the Liver**. EASL clinical practice guidelines on the management of ascites, spontaneous bacterial peritonitis, and hepatorenal syndrome in cirrhosis. *J Hepatol* 2010; **53**: 397-417 [PMID: 20633946]

3 **Riggio O**, Angeloni S. Ascitic fluid analysis for diagnosis and monitoring of spontaneous bacterial peritonitis. *World J Gastroenterol* 2009; **15**: 3845-3850 [PMID: 19701963]

4 **Umgelter A**, Reindl W, Miedaner M, Schmid RM, Huber W. Failure of current antibiotic first-line regimens and mortality in hospitalized patients with spontaneous bacterial peritonitis. *Infection* 2009; **37**: 2-8 [PMID: 19169633]

5 **Ariza X**, Castellote J, Lora-Tamayo J, Girbau A, Salord S, Rota R, Ariza J, Xiol X. Risk factors for resistance to ceftriaxone and its impact on mortality in community, healthcare and nosocomial spontaneous bacterial peritonitis. *J Hepatol* 2012; **56**: 825-832 [PMID: 22173153]

6 **Horinek E**, Fish D. Spontaneous bacterial peritonitis. *AACN Adv Crit Care* 2009; **20**: 121-125; quiz 127 [PMID: 19411866]

7 **Heo J**, Seo YS, Yim HJ, Hahn T, Park SH, Ahn SH, Park JY, Park JY, Kim MY, Park SK, Cho M, Um SH, Han KH, Kim HS, Baik SK, Kim BI, Cho SH. Clinical features and prognosis of spontaneous bacterial peritonitis in korean patients with liver cirrhosis: a multicenter retrospective study. *Gut Liver* 2009; **3**: 197-204 [PMID: 20431746]

8 **Pfaller MA**, Jones RN. A review of the in vitro activity of meropenem and comparative antimicrobial agents tested against 30,254 aerobic and anaerobic pathogens isolated world wide. *Diagn Microbiol Infect Dis* 1997; **28**: 157-163 [PMID: 9327242]

9 **Ahmad M**, Ali AA, Mumtaz M, Iqbal J, Mughal AA. Spontaneous bacterial peritonitis; Microbiological analysis of ascitic fluid in patients with complicated liver cirrhosis. *Professional Med J Jan* 2011; **18**: 557-561

10 **El-Bendary MM**, Abdel-Aziz M, El-Sherbiny WA, Farag RE, El-Gilany A, Zaghloul MH. Spontaneous bacterial peritonitis: clinico-epidemiological and microbiological study. *Benha Med J* 2009; **26**: 287-306

11 **Yakar T**, Güçlü M, Serin E, Alişkan H, Husamettin E. A recent evaluation of empirical cephalosporin treatment and antibiotic resistance of changing bacterial profiles in spontaneous bacterial peritonitis. *Dig Dis Sci* 2010; **55**: 1149-1154 [PMID: 19424797]

12 **Angeloni S**, Leboffe C, Parente A, Venditti M, Giordano A, Merli M, Riggio O. Efficacy of current guidelines for the treatment of spontaneous bacterial peritonitis in the clinical practice. *World J Gastroenterol* 2008; **14**: 2757-2762 [PMID: 18461661]

13 **Sun L**, Zhang JV, Zhao J, Bai WT, Huang CX, Jia ZS, Lian JQ, Sun YT. Changes in the profiles of bacteria causing spontaneous bacterial peritonitis: A recent twelve-year study. *Afr J Microbiol Res* 2010; **4**: 527

14 **Levison ME**, Bush LM. Peritonitis and intraperitoneal abcesses. In: Mandell GL, Bennett JE, Dolin R. Principles and Practice of Infectious Diseases. 6th ed. Philadelphia: Elsevier, 2005: 927-951

15 **Goulis J**, Patch D, Burroughs AK. Bacterial infection in the pathogenesis of variceal bleeding. *Lancet* 1999; **353**: 139-142 [PMID: 10023916]

16 **Bernard B**, Grangé JD, Khac EN, Amiot X, Opolon P, Poynard T. Antibiotic prophylaxis for the prevention of bacterial infections in cirrhotic patients with gastrointestinal bleeding: a meta-analysis. *Hepatology* 1999; **29**: 1655-1661 [PMID: 10347104]

17 **Tandon P**, Garcia-Tsao G. Bacterial infections, sepsis, and multiorgan failure in cirrhosis. *Semin Liver Dis* 2008; **28**: 26-42 [PMID: 18293275]

18 **Rimola A**, García-Tsao G, Navasa M, Piddock LJ, Planas R, Bernard B, Inadomi JM. Diagnosis, treatment and prophylaxis of spontaneous bacterial peritonitis: a consensus document. International Ascites Club. *J Hepatol* 2000; **32**: 142-153 [PMID: 10673079]

19 **Hoefs JC**. Spontaneous bacterial peritonitis: prevention and therapy. *Hepatology* 1990; **12**: 776-781 [PMID: 2210680]

20 **Taşkiran B**, Colakoğlu O, Sözmen B, Unsal B, Aslan SL, Buyraç Z. Comparison of cefotaxime and ofloxacin in treatment of

spontaneous bacterial peritonitis. *Turk J Gastroenterol* 2004; **15**: 34-38 [PMID: 15264119]
21 **Fernández J**, Navasa M, Gómez J, Colmenero J, Vila J, Arroyo V, Rodés J. Bacterial infections in cirrhosis: epidemiological changes with invasive procedures and norfloxacin prophylaxis. *Hepatology* 2002; **35**: 140-148 [PMID: 11786970]
22 **Bert F**, Noussair L, Lambert-Zechovsky N, Valla D. Viridans group streptococci: an underestimated cause of spontaneous bacterial peritonitis in cirrhotic patients with ascites. *Eur J Gastroenterol Hepatol* 2005; **17**: 929-933 [PMID: 16093870]
23 **Cormican MG**, Runyon BA, Jones RN. In vitro activity of levofloxacin and FK-037 against aerobic isolates from spontaneous bacterial peritonitis. *J Chemother* 1995; **7**: 197-200 [PMID: 7562013]
24 **Paterson DL**. Recommendation for treatment of severe infections caused by Enterobacteriaceae producing extended-spectrum beta-lactamases (ESBLs). *Clin Microbiol Infect* 2000; **6**: 460-463 [PMID: 11168179]
25 **Sort P**, Navasa M, Arroyo V, Aldeguer X, Planas R, Ruiz-del-Arbol L, Castells L, Vargas V, Soriano G, Guevara M, Ginès P, Rodés J. Effect of intravenous albumin on renal impairment and mortality in patients with cirrhosis and spontaneous bacterial peritonitis. *N Engl J Med* 1999; **341**: 403-409 [PMID: 10432325]
26 **Salerno F**, Gerbes A, Ginès P, Wong F, Arroyo V. Diagnosis, prevention and treatment of hepatorenal syndrome in cirrhosis. *Gut* 2007; **56**: 1310-1318 [PMID: 17389705 DOI: 10.1136/gut.2006.107789]

P- Reviewer Butterworth J **S- Editor** Gou SX
L- Editor O'Neill M **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i8.1278

World J Gastroenterol 2013 February 28; 19(8): 1278-1282
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Radiofrequency ablation during continuous saline infusion can extend ablation margins

Toru Ishikawa, Tomoyuki Kubota, Ryoko Horigome, Naruhiro Kimura, Hiroki Honda, Akito Iwanaga, Keiichi Seki, Terasu Honma, Toshiaki Yoshida

Toru Ishikawa, Tomoyuki Kubota, Ryoko Horigome, Naruhiro Kimura, Hiroki Honda, Akito Iwanaga, Keiichi Seki, Terasu Honma, Toshiaki Yoshida, Department of Gastroenterology and Hepatology, Saiseikai Niigata Daini Hospital, Niigata 950-1104, Japan

Author contributions: Ishikawa T designed the research; Ishikawa T, Kubota T, Horigome R, Kimura N and Honda H performed the research; Ishikawa T conceived the study, wrote and drafted the manuscript; the other authors participated in the preparation of the manuscript.

Correspondence to: Toru Ishikawa, MD, Department of Gastroenterology and Hepatology, Saiseikai Niigata Daini Hospital, 280-7 Teraji, Niigata 950-1104, Japan. toruishi@ngt.saiseikai.or.jp
Telephone: +81-25-2336161 Fax: +81-25-2338880
Received: September 14, 2012 Revised: November 28, 2012
Accepted: December 22, 2012
Published online: February 28, 2013

Abstract

AIM: To determine whether fluid injection during radiofrequency ablation (RFA) can increase the coagulation area.

METHODS: Bovine liver (1-2 kg) was placed on an aluminum tray with a return electrode affixed to the base, and the liver was punctured by an expandable electrode. During RFA, 5% glucose; 50% glucose; or saline fluid was infused continuously at a rate of 1.0 mL/min through the infusion line connected to the infusion port. The area and volume of the thermocoagulated region of bovine liver were determined after RFA. The Joule heat generated was determined from the temporal change in output during the RFA experiment.

RESULTS: No liquid infusion was 17.3 ± 1.6 mL, similar to the volume of a 3-cm diameter sphere (14.1 mL). Mean thermocoagulated volume was significantly larger with continuous infusion of saline (29.3 ± 3.3 mL) than with 5% glucose (21.4 ± 2.2 mL), 50% glucose (16.5 ± 0.9 mL) or no liquid infusion (17.3 ± 1.6 mL). The ablated volume for RFA with saline was approximately 1.7-times greater than for RFA with no liquid infusion, representing a significant difference between these two conditions. Total Joule heat generated during RFA was highest with saline, and lowest with 50% glucose.

CONCLUSION: RFA with continuous saline infusion achieves a large ablation zone, and may help inhibit local recurrence by obtaining sufficient ablation margins. RFA during continuous saline infusion can extend ablation margins, and may be prevent local recurrence.

© 2013 Baishideng. All rights reserved.

Key words: Radiofrequency ablation; *Ex vivo* bovine liver; Large ablation zone; Saline; Glucose; Joule heat

Ishikawa T, Kubota T, Horigome R, Kimura N, Honda H, Iwanaga A, Seki K, Honma T, Yoshida T. Radiofrequency ablation during continuous saline infusion can extend ablation margins. *World J Gastroenterol* 2013; 19(8): 1278-1282 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i8/1278.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i8.1278

INTRODUCTION

Radiofrequency ablation (RFA) is a technique by which deposition of electromagnetic energy is used to thermally ablate hepatic tumor tissue[1] and is now the standard method for local treatment of hepatic malignancy[2-9].

Several types of device are currently used for such therapy, such as the Cool-tip system (single electrode) made by Covidien, and the RFA systems made by Boston Scientific and AngioDynamics (the Starburst XL electrode)[10]. The former uses a single-electrode puncture needle, whereas the latter both employ a needle elec-

trode with an expandable array of fine needles extending from the tip. The expandable array of the StarBurst XL electrode is also fitted with an infusion port. To date, various techniques have been devised to secure adequate ablation margins[11]. We investigated whether ablation margins could be extended by fluid infusion from the infusion port of an expandable electrode.

We conducted an *ex vivo* experiment on bovine liver to investigate whether ablation margins could be extended by fluid infusion from a StarBurst XL electrode in order to avoid the influence of the cooling effect *in vivo* experiment.

MATERIALS AND METHODS

RFA setting and ablation protocol

A StarBurst XL electrode (AngioDynamics; Queensbury, NY) and RITA1500X generator (AngioDynamics) were used to provide RFA. One to two kilograms of bovine liver form an abattoir was used which had been exposed to room temperature for several hours until the baseline mean liver temperature ranged between 15 ℃ and 21 ℃, placed on an aluminum tray with a return electrode affixed to the base and the liver was punctured by the StarBurst XL electrode, with a maximum array expansion of 5 cm in diameter, under ultrasonographic guidance by convex probe using a Logiq 7 ultrasound device (GE Healthcare, Tokyo, Japan). Stepped ablation was performed, with the electrode first expanded to 2 cm after reaching the set temperature of 105 ℃, then expanded by 3 cm after reaching the set temperature of 105 ℃, with single ablation maintained at each diameter for 7 min. An infusion line was connected to the infusion port on the electrode, and the other end of the infusion line was connected to a syringe pump. Continuous liquid infusion was initiated at the same time as radiofrequency output. Three infusion liquids were used with the continuous infusion rate was set at 1.0 mL/min: 5% glucose; 50% glucose; and saline[12].

After stopping radiofrequency output, specimens were sectioned along the longitudinal and transverse axes of each lesion. Measurement of the coagulation diameter perpendicular to the electrode axis was based on consensus of two observers (Ishikawa T, Kubota T). Macroscopic changes in specimens have been demonstrated to correlate well with coagulation necrosis at histological examination[13]. Furthermore, the central white area of the RF-induced ablation zone was found to correspond to the necrotic zone[14].

The liver blocks containing lesions were dissected along the axis along which the electrode was inserted, and the central white area of the RF-induced ablation zone was found to correspond to the necrotic zone[15]. For macroscopic examination, two observers used calipers to measure, in the central white area of coagulation necrosis in each pathologic specimen, the overlapping width, the longest diameter of each ablation sphere along the electrode, and the shortest diameter at the midpoint between the two electrodes[16].

The region of discoloration indicating ablation of the bovine liver was measured, and the volume of ablation was calculated using the formula for calculating the volume of an ellipsoid ($V = 4/3abc\pi$).

An intergroup comparison of calculated thermocoagulated volumes was performed using Student's *t*-test with Bonferroni correction.

In addition, the generated Joule heat was calculated from the data for output changes over time during the RFA experiment.

Statistical analysis

In the intergroup comparison of calculated thermocoagulated volumes, an unpaired Student's *t*-test was used and *P* values were calculated with a correction of the α significance level by Bonferroni's method based on the number of test repetitions. For all statistical analyses, values of $P < 0.05$ were considered statistically significant.

RESULTS

Figure 1 shows cross-sectional photographs of ablated regions of liver and graphs of changes over time in temperature, output and impedance during RFA for each continuous infusion condition. Changes in impedance over time after array expansion to 3 cm tended to gradually increase in RFA without infusion and with glucose, but the steady state of impedance was seen when RFA was used with saline (Figure 1). The ablation zone also widened and presented a circular shape when saline was used.

Experimental result shows mean ± SD values for dimensions of ablation, calculated volume, mean output during RFA, and calculated Joule heat. Mean thermocoagulated volume was significantly larger with continuous infusion of saline (29.3 ± 3.3 mL) than with 5% glucose (21.4 ± 2.2 mL), 50% glucose (16.5 ± 0.9 mL) or no liquid infusion (17.3 ± 1.6 mL) ($P < 0.01$). The ablated volume for RFA with saline was approximately 1.7-times greater than for RFA with no liquid infusion, representing a significant difference between these two conditions.

An increasing trend in ablation volume was also seen in RFA with 5% glucose, although not to the same extent as with saline. In RFA with 50% glucose, the ablated volume was very similar to RFA with no liquid infusion, and differed significantly from RFA with saline.

Mean radiofrequency output tended to be higher in RFA with saline than with no liquid infusion, but tended to be low in RFA with glucose. The mean total Joule heat generated during RFA was highest with saline, and low with glucose.

DISCUSSION

RFA is a therapeutic technique that utilizes the principle of conductive heating, in which heat is generated by friction from moving electrons when current, is passed through a conductor[17]. Specifically, by passing electricity

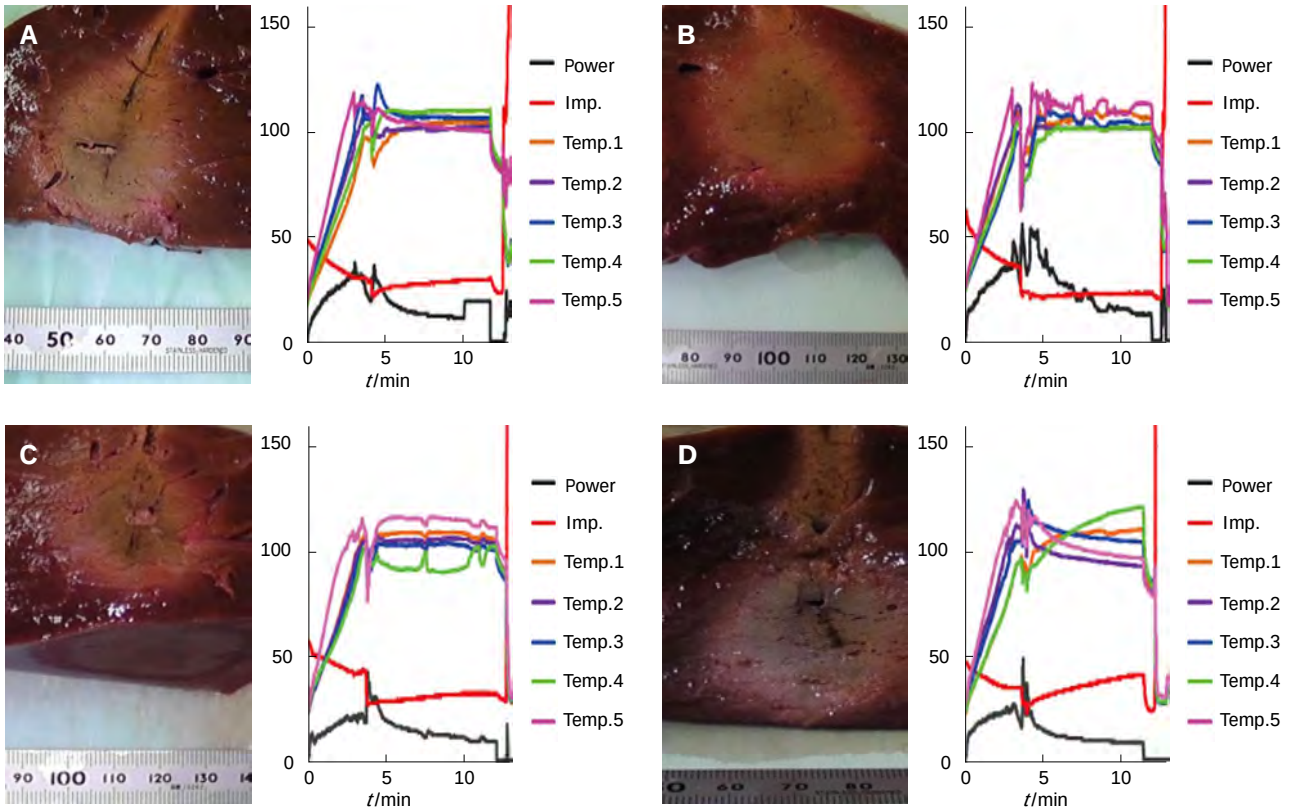


Figure 1 Transection of liver specimen with radiofrequency ablation lesion (on the left side) and graphic depiction of changes occurring in temperature (middle) with tissue impedance (red line) and power (black line) during radiofrequency ablation (on the right side) in the four groups. A: No continuous liquid infusion; B: Saline; C: 5% glucose; D: 50% glucose.

through a needle electrode inserted into the human body, coagulation and necrosis of a malignant neoplasm or other lesion tissue is induced[18,19]. The basic principle of RFA as treatment for hepatic malignancy is the generation of Joule heat by high-frequency current in living tissue between the needle electrode and a return electrode. Although the heat generated by this high-frequency current arises in all the conducting tissue pathways, high temperatures only develop in tissues near the electrode, where the current density is high. Barely any heat arises in tissues further from the electrode because of the fall in current density and the cooling (or radiator) effect of blood flow.

Although this principle brings about ablation in tissue near the electrode, the vapor microbubbles generated in the ablation region are removed by blood flow, lowering the electrical conductivity of tissue and consequently sometimes preventing adequate ablation of the target zone[2]. Efficient generation of conductive heating is therefore necessary to achieve adequate ablation margins[20]. Temperature-controlled systems for facilitating adequate ablation of the target zone by preventing excessive heating of tissue have been commercialized, but our experiments aimed to test the effect of RFA used in combination with continuous infusion of a liquid to the target tissue. Specifically, we conducted *ex vivo* experiments on bovine liver to investigate whether infusion of a liquid from the infusion port of a StarBurst XL electrode could enhance the efficiency of conductive heating.

The mean ablated volume when RFA was performed by StarBurst XL electrode with no liquid infusion was 17.3 ± 1.6 mL, similar to the volume of a 3-cm diameter sphere (14.1 mL). On the other hand, when RFA was used with a continuous infusion of liquid, the mean ablation volume was 29.3 ± 3.3 mL with saline, 21.4 ± 2.2 mL with 5% glucose, and 16.5 ± 0.9 mL with 50% glucose ($P < 0.01$). This shows that the ablated region expanded significantly when saline was used. In RFA with continuous liquid infusion, the ablated volume was significantly higher than in RFA without liquid infusion, and when saline in particular was used, the mean point estimate volume increased approximately 1.7-fold. In order to maintain the steady state of impedence for RFA during saline continuous injection, current density between electrodes should therefore be decreased to within the range within which current causes an increase in tissue temperatures, without boiling. We speculate that changing the electrical conductance of the tissue by infusing saline could be a good solution. Furthermore, we propose the following three reasons for these results.

First, in RFA without liquid infusion, water in tissues near the electrode forms microbubbles, some of which move to the area around the tissue. The moisture percentage near the electrode thus decreases and electrical conductivity of the tissue falls. This is supported by the finding that impedance gradually increased during RF

output. In contrast, when RFA is used with continuous liquid infusion, the moisture percentage in tissue near the electrode remains stable or is increased, maintaining or increasing electrical conductivity and leading to expansion of the ablated region. Furthermore, saline was likely more effective in extending the ablated region than glucose when used with RFA because saline is a conductive solution.

Second, ablation of tissue near the electrode results from the thermal conversion effect of Joule heat due to a high current density. The thermal conversion effect of Joule heat alone is insufficient to induce ablation of the surrounding tissue, and we speculate that such ablation is the result of synergistic effects involving, for example, heat conduction from tissue near the electrode to surrounding tissue, or transfer of heated water or high-temperature microbubbles generated near the electrode to the surrounding tissue. Infusion of liquid would promote this transfer of heated water or high-temperature microbubbles to the surrounding tissue, with the ultimate effect of extending the ablated region.

Third, the type of infusion liquid was relevant, with a significant difference in ablated volume seen between the saline and 50% glucose solutions. This is probably because although a highly concentrated glucose solution would be expected to extend the ablation region through the effects described in the two points above, the non-ionic nature of the solution may lower the electrical conductivity of the target tissue. Considering that ablated volumes were similar in RFA with 50% glucose infusion and without any liquid infusion, the negative effects of this highly concentrated but non-ionic solution cancelled out the effects described in the first two points above, resulting in no marked extension of the ablated region.

Shorter treatment time (RFA time) may be another effect of RFA with continuous liquid infusion. This could be due to an increase in thermocoagulation efficiency at the target tissue arising from the transfer of high-temperature microbubbles or heated water to the surrounding tissue, as suggested in the second point above.

Furthermore, RFA with continuous liquid infusion tended to produce a larger thermocoagulated region with a lower total Joule heat compared to RFA without liquid infusion. Saline infusion in particular demonstrated high ablation efficiency at the target tissue, and could therefore prove highly useful[21].

This high ratio reveals the strong ablative action on the target tissue, and suggests that RFA with continuous saline infusion can achieve efficient ablation of tissue near the electrode. While this *ex-vivo* procedure cannot be directly adopted for use in clinical settings, RFA during continuous saline infusion can achieve a large thermocoagulation region in a short time and may be able to inhibit local recurrence by securing ablation margins.

There are several limitations to this study. The most important limitation was the absence of blood flow. *In vivo*, the volume of coagulation will be smaller and the amount of applied energy will be higher due to the removal of heat by the presence of blood flow[22]. This *ex-vivo* procedure cannot be directly adopted for use in a clinical setting. Using the same RF device, the size of the ablation zone *in vivo* was smaller than when *ex vivo*[23]. However, RFA during saline injection has potentially broad clinical merit and can assist in the planning of a methodological strategy. The relationship between parameters was calculated for a particular RF system, therefore, a different multipolar RF system would change the mathematical modeling. Moreover, the physiologic variability of thermal and electrical tissue properties is supposed to lead to a variable efficiency of applied energy under *in vivo* conditions. Consequently, the accuracy of mathematical modeling derived from *ex vivo* data will be restricted in the clinical application of multipolar RF ablation. Therefore, mathematical modeling has to be adjusted for *in vivo* conditions and due to the physiologic variability[24] of parameters the accuracy of predicted volumes is supposed to be reduced.

Finally, the findings of this study reflect certain model-specific characteristics, such as its hypovascularity, outcomes in other more clinically relevant tumor model may differ. Therefore, extrapolation to other types of tumors should be performed with caution.

In conclusion, we confirmed the effect of continuous liquid infusion in expanding the RFA thermocoagulated region in *ex vivo* experiments using a Starburst XL electrode system. Our results indicate that RFA with continuous saline infusion can significantly expand the volume of thermocoagulation in temperature-controlled RFA systems. RFA with continuous saline infusion can achieve a large thermocoagulation region in a short time and may be able to inhibit local recurrence by securing ablation margins[25,26].

COMMENTS

Background

Radiofrequency ablation (RFA) is now the standard method for local treatment of hepatic malignancy. To date, various techniques have been devised to secure adequate ablation margins. This study was investigated whether ablation margins could be extended by fluid infusion from the infusion port of an expandable electrode.

Research frontiers

In this study, authors confirmed that ablation margins by RFA could be extended by saline infusion from the infusion port of an expandable electrode.

Innovations and breakthroughs

While this *ex-vivo* procedure cannot be directly adopted for use in clinical settings, RFA during continuous saline infusion can achieve a large thermocoagulation region in a short time and may be able to inhibit local recurrence by securing ablation margins.

Applications

In this study, RFA with continuous saline infusion can achieve a large thermocoagulation region in a short time and may be able to inhibit local recurrence by securing ablation margins.

Peer review

This is a very novel study that Starburst RFA device and infusion saline and dextrose to study differences in burn volume with RFA. The results are interesting and suggest that RFA during continuous saline infusion can extend ablation margins.

REFERENCES

1 **Callstrom MR**, Charboneau JW. Technologies for ablation of hepatocellular carcinoma. *Gastroenterology* 2008; **134**: 1831-1835 [PMID: 18486619 DOI: 10.1053/j.gastro.2008.05.022]
2 **Rossi S**, Di Stasi M, Buscarini E, Cavanna L, Quaretti P, Squassante E, Garbagnati F, Buscarini L. Percutaneous radiofrequency interstitial thermal ablation in the treatment of small hepatocellular carcinoma. *Cancer J Sci Am* 1995; **1**: 73-81 [PMID: 9166457]
3 **Livraghi T**. Treatment of hepatocellular carcinoma by interventional methods. *Eur Radiol* 2001; **11**: 2207-2219 [PMID: 11702161 DOI: 10.1007/s003300100889]
4 **Rhim H**, Lim HK, Choi D. Current status of radiofrequency ablation of hepatocellular carcinoma. *World J Gastrointest Surg* 2010; **2**: 128-136 [PMID: 21160861 DOI: 10.4240/wjgs.v2.i4.128]
5 **Rhim H**, Lim HK. Radiofrequency ablation of hepatocellular carcinoma: pros and cons. *Gut Liver* 2010; **4** Suppl 1: S113-S118 [PMID: 21103289 DOI: 10.5009/gnl.2010.4.S1.S113]
6 **Livraghi T**, Mäkisalo H, Line PD. Treatment options in hepatocellular carcinoma today. *Scand J Surg* 2011; **100**: 22-29 [PMID: 21482502]
7 **Künzli BM**, Abitabile P, Maurer CA. Radiofrequency ablation of liver tumors: Actual limitations and potential solutions in the future. *World J Hepatol* 2011; **3**: 8-14 [PMID: 21307982]
8 **Izumi N**. Recent advances of radiofrequency ablation for early hepatocellular carcinoma. *J Gastroenterol Hepatol* 2011; **26** Suppl 1: 115-122 [PMID: 21199522 DOI: 10.1111/j.1440-1746.2010.06543.x]
9 **Himoto T**, Kurokohchi K, Watanabe S, Masaki T. Recent advances in radiofrequency ablation for the management of hepatocellular carcinoma. *Hepat Mon* 2012; **12**: e5945 [PMID: 23162600 DOI: 10.5812/hepatmon.5945]
10 **Denys AL**, De Baere T, Kuoch V, Dupas B, Chevallier P, Madoff DC, Schnyder P, Doenz F. Radio-frequency tissue ablation of the liver: in vivo and ex vivo experiments with four different systems. *Eur Radiol* 2003; **13**: 2346-2352 [PMID: 12942277 DOI: 10.1007/s00330-003-1970-0]
11 **Goldberg SN**, Stein MC, Gazelle GS, Sheiman RG, Kruskal JB, Clouse ME. Percutaneous radiofrequency tissue ablation: optimization of pulsed-radiofrequency technique to increase coagulation necrosis. *J Vasc Interv Radiol* 1999; **10**: 907-916 [PMID: 10435709 DOI: 10.1016/S1051-0443(99)70136-3]
12 **Livraghi T**, Goldberg SN, Monti F, Bizzini A, Lazzaroni S, Meloni F, Pellicanò S, Solbiati L, Gazelle GS. Saline-enhanced radio-frequency tissue ablation in the treatment of liver metastases. *Radiology* 1997; **202**: 205-210 [PMID: 8988212]
13 **McGahan JP**, Griffey SM, Budenz RW, Brock JM. Percutaneous ultrasound-guided radiofrequency electrocautery ablation of prostate tissue in dogs. *Acad Radiol* 1995; **2**: 61-65 [PMID: 9419526 DOI: 10.1016/S1076-6332(05)80248-X]
14 **Lee JD**, Lee JM, Kim SW, Kim CS, Mun WS. MR imaging-histopathologic correlation of radiofrequency thermal ablation lesion in a rabbit liver model: observation during acute and chronic stages. *Korean J Radiol* 2001; **2**: 151-158 [PMID: 11752986 DOI: 10.3348/kjr.2001.2.3.151]
15 **Lee JD,** Lee JM, Kim SW, Kim CS, Mun WS. MR imaging-histopathologic correlation of radiofrequency thermal ablation lesion in a rabbit liver model: observation during acute and chronic stages. *Korean J Radiol* 2001; **2**: 151-158 [PMID: 11752986]
16 **Lee JM**, Han JK, Kim SH, Sohn KL, Lee KH, Ah SK, Choi BI. A comparative experimental study of the in-vitro efficiency of hypertonic saline-enhanced hepatic bipolar and monopolar radiofrequency ablation. *Korean J Radiol* 2003; **4**: 163-169 [PMID: 14530645 DOI: 10.3348/kjr.2003.4.3.163]
17 **Solbiati L**, Ierace T, Goldberg SN, Sironi S, Livraghi T, Fiocca R, Servadio G, Rizzatto G, Mueller PR, Del Maschio A, Gazelle GS. Percutaneous US-guided radio-frequency tissue ablation of liver metastases: treatment and follow-up in 16 patients. *Radiology* 1997; **202**: 195-203 [PMID: 8988211]
18 **Goldberg SN**, Gazelle GS, Mueller PR. Thermal ablation therapy for focal malignancy: a unified approach to underlying principles, techniques, and diagnostic imaging guidance. *AJR Am J Roentgenol* 2000; **174**: 323-331 [PMID: 10658699 DOI: 10.1117/12.348726]
19 **Gazelle GS**, Goldberg SN, Solbiati L, Livraghi T. Tumor ablation with radio-frequency energy. *Radiology* 2000; **217**: 633-646 [PMID: 11110923]
20 **Nakazawa T**, Kokubu S, Shibuya A, Ono K, Watanabe M, Hidaka H, Tsuchihashi T, Saigenji K. Radiofrequency ablation of hepatocellular carcinoma: correlation between local tumor progression after ablation and ablative margin. *AJR Am J Roentgenol* 2007; **188**: 480-488 [PMID: 17242258 DOI: 10.2214/AJR.05.2079]
21 **Miao Y**, Ni Y, Yu J, Zhang H, Baert A, Marchal G. An ex vivo study on radiofrequency tissue ablation: increased lesion size by using an "expandable-wet" electrode. *Eur Radiol* 2001; **11**: 1841-1847 [PMID: 11511912 DOI: 10.1007/s003300100891]
22 **Lu DS**, Raman SS, Vodopich DJ, Wang M, Sayre J, Lassman C. Effect of vessel size on creation of hepatic radiofrequency lesions in pigs: assessment of the "heat sink" effect. *AJR Am J Roentgenol* 2002; **178**: 47-51 [PMID: 11756085]
23 **de Baere T**, Denys A, Wood BJ, Lassau N, Kardache M, Vilgrain V, Menu Y, Roche A. Radiofrequency liver ablation: experimental comparative study of water-cooled versus expandable systems. *AJR Am J Roentgenol* 2001; **176**: 187-192 [PMID: 11133564]
24 **Montgomery RS**, Rahal A, Dodd GD, Leyendecker JR, Hubbard LG. Radiofrequency ablation of hepatic tumors: variability of lesion size using a single ablation device. *AJR Am J Roentgenol* 2004; **182**: 657-661 [PMID: 14975966]
25 **Gananadha S**, Morris DL. Saline infusion markedly reduces impedance and improves efficacy of pulmonary radiofrequency ablation. *Cardiovasc Intervent Radiol* 2004; **27**: 361-365 [PMID: 15366219 DOI: 10.1007/PL00021046]
26 **Hänsler J**, Frieser M, Schaber S, Kutschall C, Bernatik T, Müller W, Becker D, Hahn EG, Strobel D. Radiofrequency ablation of hepatocellular carcinoma with a saline solution perfusion device: a pilot study. *J Vasc Interv Radiol* 2003; **14**: 575-580 [PMID: 12761310 DOI: 10.1097/01.RVI.0000071096.76348.3B]

P- Reviewers Ooi LL, Kambadakone A
S- Editor Gou SX **L- Editor** A **E- Editor** Lu YJ

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i8.1283

World J Gastroenterol 2013 February 28; 19(8): 1283-1291
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Ethnicity association of *Helicobacter pylori* virulence genotype and metronidazole susceptibility

Hanafiah Alfizah, Awang Hamat Rukman, Ahmad Norazah, Razlan Hamizah, Mohamed Ramelah

Hanafiah Alfizah, Department of Medical Medical Microbiology and Immunology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, 56000 Cheras, Kuala Lumpur, Malaysia
Awang Hamat Rukman, Department of Medical Microbiology and Parasitology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia
Ahmad Norazah, Bacteriology Unit, Institute for Medical Research, Jalan Pahang, 50588 Kuala Lumpur, Malaysia
Razlan Hamizah, Department of Medicine, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, 56000 Cheras, Kuala Lumpur, Malaysia
Mohamed Ramelah, Centre for Innovative Collaboration, Universiti Kebangsaan Malaysia, 43600 Bangi, Selangor, Malaysia
Author contributions: All authors contributed equally in this study.
Supported by Grant from the Ministry of Science, Technology and Innovation, Malaysia, No. 06-02-0055-PR0073/05
Correspondence to: Hanafiah Alfizah, PhD, Department of Medical Microbiology and Immunology, Faculty of Medicine, Universiti Kebangsaan Malaysia Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia. alfizah@ppukm.ukm.edu.my
Telephone: +60-3-91455442 Fax: +60-3-91456671
Received: October 17, 2012 Revised: December 29, 2012
Accepted: January 11, 2013
Published online: February 28, 2013

Abstract

AIM: To characterise the *cag* pathogenicity island in *Helicobacter pylori* (*H. pylori*) isolates by analysing the strains' *vacA* alleles and metronidazole susceptibilities in light of patient ethnicity and clinical outcome.

METHODS: Ninety-five *H. pylori* clinical isolates obtained from patients with dyspepsia living in Malaysia were analysed in this study. Six genes in the *cag*PAI region (*cagE*, *cagM*, *cagT*, *cag13*, *cag10* and *cag67*) and *vacA* alleles of the *H. pylori* isolates were identified by polymerase chain reaction. The isolates' metronidazole susceptibility was also determined using the *E*-test method, and the resistant gene was characterised by sequencing.

RESULTS: More than 90% of the tested isolates had at least one gene in the *cag*PAI region, and *cag67* was predominantly detected in the strains isolated from the Chinese patients, compared with the Malay and Indian patients ($P < 0.0001$). The majority of the isolates (88%) exhibited partial deletion (rearrangement) in the *cag*PAI region, with nineteen different patterns observed. Strains with intact or deleted *cag*PAI regions were detected in 3.2% and 8.4% of isolates, respectively. The prevalence of *vacA* s1m1 was significantly higher in the Malay and Indian isolates, whereas the isolates from the Chinese patients were predominantly genotyped as *vacA* s1m2 ($P = 0.018$). Additionally, the isolates from the Chinese patients were more sensitive to metronidazole than the isolates from the Malay and Indian patients ($P = 0.047$). Although we attempted to relate the *cag*PAI genotypes, *vacA* alleles and metronidazole susceptibilities to disease outcome, no association was observed. The *vacA* alleles were distributed evenly among the strains with intact, partially deleted or deleted *cag*PAI regions. Interestingly, the strains exhibiting an intact *cag*PAI region were sensitive to metronidazole, whereas the strains with a deleted *cag*PAI were more resistant.

CONCLUSION: Successful colonisation by different *H. pylori* genotypes is dependent on the host's genetic makeup and may play an important role in the clinical outcome.

© 2013 Baishideng. All rights reserved.

Key words: *Helicobacter pylori*; *cag* pathogenicity island; *vacA* alleles; Metronidazole susceptibility

Alfizah H, Rukman AH, Norazah A, Hamizah R, Ramelah M. Ethnicity association of *Helicobacter pylori* virulence genotype and metronidazole susceptibility. *World J Gastroenterol*

2013; 19(8): 1283-1291 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i8/1283.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i8.1283

INTRODUCTION

Helicobacter pylori (*H. pylori*) demonstrates great genetic diversity, as evidenced by the apparently unlimited number of unique strains differing in genome size, gene order, gene content and allelic profile[1]. The high levels of genetic diversity in clinical isolates and the presence of certain virulence genotypes may confer a selective advantage to certain strains in different hosts. Therefore, the virulence genotype of *H. pylori* is a potentially useful predictor of the clinical outcome of gastric mucosal colonisation.

H. pylori possess a number of virulence determinants that modulate the organism's interaction with a host[2,3]. These virulence factors include the secreted, vacuolating cytotoxin VacA and the gene products of the pathogenicity island (*cag*PAI). The presence of these genetic loci correlates with the more severe *H. pylori*-associated pathologies, peptic ulcers and gastric cancer. Screening for the *cag*PAI genes is frequently performed due to the genes' involvement in virulence characteristics. An infection with *cag*PAI-positive *H. pylori* strains significantly increases the risk of developing severe gastric mucosal inflammation, duodenal ulceration and gastric cancer[4-6]. It has been observed that only one-half to two-thirds of isolates from Western patients express the *cag*PAI region, whereas almost all of the East Asian strains express this region. As there is limited information on the structure of the *cag*PAI in Malaysian patients, the role of *cag*PAI rearrangement in *H. pylori* isolates from various ethnic populations within this country should be investigated.

The *vacA* gene is another *H. pylori* virulence factor that exhibits great polymorphism and diverse allelic combination. The variable structure, resulting in different allelic arrangements in the gene, is related to differences in cytotoxin production and to the distinct clinical outcomes of *H. pylori* infection[7-9]. For example, a recent study performed at our institute has demonstrated a correlation between the presence of genetic polymorphisms in the *vacA* gene and the severity of gastritis[10].

H. pylori infection can be effectively cured by antibiotics. More specifically, metronidazole is commonly used in conjunction with either amoxicillin or clarithromycin and a proton-pump inhibitor to eradicate *H. pylori* infection[11,12]. Metronidazole is also used in the treatment of other diseases, such as gynaecological infections, and has contributed to the increased antibiotic resistance of *H. pylori*[13]. In Malaysia, the prevalence of metronidazole-resistant strains is 37%, which may be one of the factors contributing to the failed eradication of *H. pylori* infection in the country[14].

The population of Malaysia consists of three major ethnic groups (Malay, Chinese and Indian) that are historically associated with differences in *H. pylori* infection[15-17]. *H. pylori*-associated gastroduodenal diseases typically do not reflect the prevalence of *H. pylori* infection[15]. Furthermore, geographical and population differences in *H. pylori* virulence factors and disease severity underscore the importance of investigating the relationship between the genotypes of the causative strains and the clinical outcome. Therefore, to further understand the dynamics of the genetic traits of the *H. pylori cag*PAI regions, *vacA* alleles and metronidazole susceptibilities in Malaysia's multi-ethnic population, we investigated the possible association between these *H. pylori* virulence factors and infection in patients of different ethnicities and clinical outcomes.

MATERIALS AND METHODS

Patients

Patients with dyspepsia who had undergone an upper gastrointestinal scope (OGDS) at Universiti Kebangsaan Malaysia Medical Centre (Kuala Lumpur, Malaysia) between May 2004 and December 2007 were recruited. Any patients who had received antibiotics 4 wk prior to OGDS were excluded. Written informed consent was obtained from each patient, and the protocol was approved by the Research Ethics Committee (protocol number FF-075-2003) of the University.

H. pylori culture

Biopsies were subcultured for *H. pylori* on Columbia agar base (Oxoid, Basingstoke, England) containing Dent's supplement (Oxoid, Basingstoke, England) and 7% defibrinated sheep blood. The plates were incubated at 37 ℃ for 5 d under microaerophilic conditions.

DNA extraction

H. pylori genomic DNA was prepared using a High Pure polymerase chain reaction (PCR) Template Preparation Kit (Roche, Mannheim, Germany) according to the manufacturer's instructions.

Detection of metronidazole susceptibility and resistance genotype

Metronidazole susceptibility was determined using the E-test method (AB Biodisk, Solna, Sweden). First, an *H. pylori* culture suspension with a McFarland turbidity of 3.0 was used to inoculate Columbia agar supplemented with 10% defibrinated sheep blood. The plates were then incubated at 35 ± 2 ℃ for 3-5 d under microaerophilic conditions. The isolates were classified as resistant to metronidazole when the MIC value was > 8 μg/mL[18]. The *rdxA* genes were amplified by PCR using specific primers, as previously described[19], and then subjected to sequencing.

Detection of cagPAI genes

Two loci were selected in the *cag* I region (*cagE* and

cagM), and four loci were chosen from the *cag* Ⅱ region (*cagT*, *cag13*, *cag10* and *cag67*). Additionally, the insertion sequence IS605 was selected. The presence of these selected genes was determined by PCR using specific primers, as previously described[20]. PCR was conducted in 25 μL volumes containing 1 × PCR buffer, 0.2 mmol/L dNTP mix, 10 pmol of each primer, 1 U Taq polymerase (BioTherm, GeneCraft, Germany) and 1 μL of the DNA sample. The PCR amplification conditions consisted of 35 cycles at 94 ℃ for 1 min, 50-55 ℃ for 30 s, 72 ℃ for 1 min, and one cycle for the final extension at 72 ℃ for 10 min. The amplified product was electrophoresed on a 1% agarose gel.

Detection of vacA alleles

The *vacA* genotyping was performed by multiplex PCR using specific primers, as previously described[10,21]. PCR was conducted in 25-μL volumes containing 1 × PCR buffer, 0.2 mmol/L dNTP mix, 6 pmol of each primer, 0.75 U Taq polymerase (BioTherm, GeneCraft, Germany) and 1 μL of the DNA sample. The PCR amplification conditions consisted of an initial denaturation step at 94 ℃ for 4 min, followed by 30 cycles of denaturation at 94 ℃ for 30 s, primer annealing at 55 ℃ for 30 s, extension at 72 ℃ for 30 s, and one cycle for the final extension at 72 ℃ for 7 min. The amplified products were electrophoresed on a 1.5% agarose gel.

Statistical analysis

A statistical analysis was performed using the χ^2 test. A value of $P < 0.05$ was considered statistically significant.

RESULTS

H. pylori clinical isolates

A total of 95 clinical *H. pylori* isolates were obtained from 40 male and 55 female patients with a mean age of 55.47 ± 16.87 years (age range of 17-89 years) and from three different ethnic groups (12 Malays, 59 Chinese and 24 Indians). The endoscopic findings were as follows: nonulcer dyspepsia (n = 76), gastric ulcer (n = 9), duodenal ulcer (n = 6) and both gastric and duodenal ulcers (n = 2). The OGDS findings were not available for two of the patients. Nonulcer dyspepsia was further classified as endoscopically normal stomach (n = 12), antrum-predominant gastritis (n = 43), corpus-predominant gastritis (n = 2) and pangastritis (n = 19). No significant association was found between patient ethnicity (Chinese versus non-Chinese) and the endoscopic findings (nonulcer dyspepsia versus peptic ulcer).

cagPAI genotype

Of the 95 *H. pylori* isolates, 87 (91.6%) were *cag*PAI-positive, and the remaining 8 (8.4%) lacked all selected *cag*PAI genes. For the *cag*PAI-positive strains, intact *cag*PAI genes were detected only in 3 isolates, whereas other isolates exhibited partially deleted (rearranged) *cag*PAI genes. Additionally, the *IS605* gene was detected in 4 (4.2%) isolates. Further analyses indicated that the positivity of the *H. pylori* isolates was 61.1% (58/95), 53.7% (51/95), 4.2% (4/95), 82.1% (78/95), 76.8% (73/95) and 65.3% (62/95) for *cag67*, *cag10*, *cag13*, *cagT*, *cagM* and *cagE*, respectively.

Table 1 Distribution of *Helicobacter pylori cag*PAI genes isolated from patients of different ethnicities *n* (%)

*cag*PAI genes	Ethnic groups			*P* value
	Malay	Chinese	Indian	
cag Ⅰ region				
cagE	7 (58.3)	41 (69.5)	14 (58.3)	0.541
cagM	9 (75.0)	45 (76.3)	19 (79.2)	0.948
cag Ⅱ region				
cagT	11 (91.7)	49 (83.1)	18 (75.0)	
cag13	0	4 (6.8)	0	
cag10	6 (50.0)	29 (49.2)	16 (66.7)	0.336
cag67	6 (50.0)	47 (79.7)	5 (20.8)	< 0.0001

As shown in Table 1, the presence of *cagE* and *cagM* in the *cag*I region was distributed evenly among the isolates from patients of different ethnicities, with no significant differences between the ethnicities. The *cagT* gene was frequently detected in isolates from the Malay patients, whereas *cag13* was only detected in the isolates from the Chinese patients. The presence of *cag67* was significantly higher in the isolates from the Chinese patients than in the isolates from the Malay patients and was lowest in the isolates from the Indian patients. The *cag*PAI genes were detected in greater than 50% of the isolates from the nonulcer and ulcer patients, except for *cag13*, which was only detected in the patients with a normal stomach or gastritis. Thus, different *cag*PAI genes were present in the majority of the isolates examined, irrespective of disease state.

Of the 95 clinical isolates, only 3 (3.2%) contained an intact *cag*PAI, whereas 84 (88.4%) exhibited partial deletions within the *cag*PAI region, and 8 (8.4%) lacked the entire selected gene in the *cag*PAI. As shown in Table 2, thirteen isolates appeared to have a diverged *cag*PAI, in which the two selected genes for the *cag* Ⅰ region had been deleted, although the genes in the *cag* Ⅱ region were conserved, with at least one gene present. In contrast, ten strains had only one gene in either the *cag* Ⅰ or the *cag* Ⅱ region. The single gene detected was *cagM*, *cagT* and *cag67* in 3, 6 and 1 isolate, respectively. The most commonly partially deleted *cag*PAI genotypes were detected in 26 (27.4%), 16 (16.8%) and 13 (13.7%) isolates, with *cag*PAI regions of types C, I and N, respectively. These isolates exhibited at least four genes in the *cag*PAI region, and the genes in the *cag*I region were conserved in these isolates. Additionally, the *IS605* gene was detected in the isolates from the Chinese (n = 2) and Indian (n = 2) patients. Three of these isolates contained all of the selected genes for *cag*PAI, except *cag13*, and one isolate lacked the *cag13* and *cag10* genes.

As shown in Table 3, three isolates with an intact *cag*-

Table 2 *cag*PAI genotypes of *Helicobacter pylori* clinical isolates *n* (%)

*cag*PAI type	*cag* Ⅱ region				*cag* Ⅰ region		Total
	cag67	*cag10*	*cag13*	*cagT*	*cagM*	*cagE*	
A	+	+	+	+	+	+	3 (3.2)
B	+	-	+	-	-	-	1 (1.1)
C	+	+	-	+	+	+	26 (27.4)
D	+	+	-	+	+	-	2 (2.1)
E	+	+	-	-	+	+	1 (1.1)
F	+	+	-	-	+	-	1 (1.1)
G	+	+	-	-	-	-	1 (1.1)
H	+	-	-	-	-	-	1 (1.1)
I	+	-	-	+	+	+	16 (16.8)
J	+	-	-	+	+	-	2 (2.1)
K	+	-	-	+	-	+	1 (1.1)
L	+	-	-	-	+	+	1 (1.1)
M	+	-	-	+	-	-	2 (2.1)
N	-	+	-	+	+	+	13 (13.7)
O	-	+	-	+	+	-	2 (2.1)
P	-	+	-	+	-	-	2 (2.1)
Q	-	-	-	+	+	+	1 (1.1)
R	-	-	-	+	+	-	2 (2.1)
S	-	-	-	+	-	-	6 (6.3)
T	-	-	-	-	+	-	3 (3.2)
U	-	-	-	-	-	-	8 (8.4)

PAI were obtained from the Chinese patients. Nineteen of the 59 (32.2%) isolates from the Chinese patients possessed partially deleted *cag*PAI regions of type C, compared with 25% and 16.7% of the isolates from the Malay and Indian patients, respectively. Interestingly, the isolates from the Indian patients showed a high proportion of partially deleted *cag*PAI regions of type N (37.5%) and deleted *cag*PAI regions (16.7%). An analysis of the disease state, with reference to strain genotype, revealed a relationship between the *cag*PAI genotype and disease severity. The isolates from those patients with peptic-ulcer disease (PUD) primarily exhibited *cag*PAI regions of type C, which contained more genes in the rearranged *cag*PAI genotypes. In contrast, a larger number of isolates from the patients with pangastritis or antrum-predominant gastritis possessed *cag*PAI regions of type I or N, respectively, compared with the isolates from the patients with peptic ulcers.

vacA genotype

The *vacA* s1m1, s1m2 and s2m2 genotypes were identified in 51.6% (49/95), 42.1% (40/95) and 4.2% (4/94) of the isolates, respectively. Additionally, two (2.1%) isolates possessed the *vacA* s1m1m2 genotype. The *vacA* s1m1 genotype was further classified as *vacA* s1am1 (63.3%, 31/49), s1cm1 (22.4%, 11/49) or s1as1cm1 (14.3%, 7/49), and the *vacA* s1m2 genotype was further classified as *vacA* s1am2 (65%, 26/40), s1cm2 (22.5%, 9/40) or s1as1cm2 (12.5%, 5/40). An analysis of the distribution of the *vacA* alleles with respect to patient ethnicity and disease state is shown in Table 4. We found a significant distribution of different *vacA* alleles according to patient ethnicity. The *vacA* s1m1 genotype was significantly more prevalent in the isolates from the Malay and Indian patients, whereas the *vacA* s1m2 genotype was predominantly detected in the isolates from the Chinese patients ($P = 0.018$). The *vacA* s2m2 genotype was only isolated from the Malay and Indian patients, and a mixed genotype of the m region was identified in the isolates from the Chinese patients. Further analyses of the *vacA* subgenotypes with respect to patient ethnicity revealed that the highest proportion of isolates harbouring the *vacA* s1am1 subgenotype were from Indian patients (93.8%, 15/16). The prevalence of the *vacA* s1cm1 genotype was similar in proportion in the isolates from the Malay (37.5%, 3/8) and Chinese (32%, 8/25) patients, although this subgenotype was not detected in the isolates from the Indian patients. In contrast, the *vacA* s1am2 subgenotype was detected in all of the ethnic groups, whereas the *vacA* s1cm2 subgenotype was only identified in the isolates from the Chinese patients. No significant difference was observed in the distribution of the *vacA* s1m1 and s1m2 strains with respect to the disease state ($P = 0.686$).

Metronidazole susceptibility

In this study, metronidazole resistance was observed in 45.3% (43/95) of the isolates. The resistant strains exhibited MIC values ranging from 8 to > 256 mg/L, with an MIC value > 256 mg/L for the majority of the strains (62.8%, 27/43). As shown in Table 4, a strong association was observed between metronidazole susceptibility and patient ethnicity. The Chinese patients were more prone to infection with metronidazole-sensitive *H. pylori* strains, whereas the Malay and Indian patients were more likely to be infected with the antibiotic-resistant strains. The strains isolated from the patients with PUD were more sensitive to metronidazole than the strains from the patients with pangastritis, although this difference was not statistically significant. DNA sequence analyses of the *rdxA* gene revealed the presence of missense, frameshift and nonsense mutations in 30, 3 and 4 isolates, respectively. All of the tested metronidazole-sensitive strains harboured only missense mutations, whereas all three types of mutations were identified in the antibiotic-resistant strains.

Distribution of H. pylori cagPAI genotypes, vacA alleles and metronidazole susceptibilities

As shown in Table 5, the isolates with intact *cag*PAI regions carrying the *vacA* s1m2 allele were sensitive to metronidazole. Of the isolates with a rearranged *cag*PAI region, the type C strains predominantly harboured the *vacA* s1m2 allele, whereas a high proportion of the types I and N *cag*PAI strains harboured the *vacA* s1m1 allele. However, these strains were found to be more sensitive to metronidazole. In contrast, the isolates exhibiting a deleted *cag*PAI region predominantly carried the *vacA* s1m2 allele and were more resistant to metronidazole.

Table 3 Distribution of strains with intact, partially deleted (rearranged) or deleted *cag*PAI regions among patients of different ethnicities and disease states *n* (%)

*cag*PAI status	Ethnic group			Disease state			
	Malay (*n* = 12)	Chinese (*n* = 59)	Indian (*n* = 24)	Normal (*n* = 12)	Gastritis (antral) (*n* = 43)	Pangastritis (*n* = 19)	PUD (*n* = 17)
Intact							
Type A	0	3 (5.1)	0	1 (8.3)	2 (4.7)	0	0
Partially deleted							
Type C	3 (25.0)	19 (32.2)	4 (16.7)	3 (25.0)	9 (20.9)	7 (36.8)	7 (41.2)
Type I	2 (16.7)	14 (23.7)	0	2 (16.7)	5 (11.6)	5 (26.3)	3 (17.6)
Type N	2 (16.7)	2 (3.4)	9 (37.5)	1 (8.3)	7 (16.3)	2 (10.5)	2 (11.8)
Deleted							
Type U	1 (8.3)	3 (5.1)	4 (16.7)	1 (8.3)	3 (7.0)	1 (5.3)	2 (11.8)

PUD: Peptic-ulcer disease.

Table 4 Distribution of *vacA* alleles and metronidazole susceptibilities among *Helicobacter pylori* clinical isolates from patients of different ethnicities and disease states *n* (%)

*cag*PAI status	Ethnic group			Disease state			
	Malay (*n* = 12)	Chinese (*n* = 59)	Indian (*n* = 24)	Normal (*n* = 12)	Gastritis (antral) (*n* = 43)	Pangastritis (*n* = 19)	PUD (*n* = 17)
Intact							
Type A	0	3 (5.1)	0	1 (8.3)	2 (4.7)	0	0
Partially deleted							
Type C	3 (25.0)	19 (32.2)	4 (16.7)	3 (25.0)	9 (20.9)	7 (36.8)	7 (41.2)
Type I	2 (16.7)	14 (23.7)	0	2 (16.7)	5 (11.6)	5 (26.3)	3 (17.6)
Type N	2 (16.7)	2 (3.4)	9 (37.5)	1 (8.3)	7 (16.3)	2 (10.5)	2 (11.8)
Deleted							
Type U	1 (8.3)	3 (5.1)	4 (16.7)	1 (8.3)	3 (7.0)	1 (5.3)	2 (11.8)

PUD: Peptic-ulcer disease.

DISCUSSION

The *cag*PAI and *vacA* genotypes are widely used to characterise the virulence of *H. pylori* and the relationship of such virulence to disease severity, although direct associations with peptic ulcers and gastric cancer have not been established[22,23]. Rather, the development of severe histological changes in the gastric mucosa may depend on the synergistic effect of bacterial and host factors[24].

The genotypic characteristics of the *H. pylori cag*PAI genes show great variability worldwide. The rearrangement of the *cag*PAI varies between different populations and geographical regions, with variations of 50%-65% in areas of the Indian subcontinent[25,26], 32% in France[27], 9% in Sweden[28] and the United States[29] and 1% in Japan[30]. In the present study, more than 90% of the clinical isolates were positive for at least one of the selected *cag*PAI genes. The *cag*PAI genes tested in this study did not include the *cagA* gene, as we previously demonstrated[31] that the majority of the *H. pylori* strains circulating in our study population were *cagA*-positive. In the six genetic loci studied, the most frequently deleted gene was *cag13*, and the least frequently deleted gene was *cagT*. The IS605 insertion sequence was present in less than 5% of the *H. pylori* isolates examined, and the majority of the isolates harboured a rearranged *cag*PAI gene. Analyses of the individual *cag*PAI genes showed that *cag67* was more conserved in the isolates from the Chinese patients, whereas the rearrangement of *cagE* occurred at a higher frequency in the isolates from the Malay and Indian patients, although this difference was not significant.

We also observed that the majority of the *H. pylori* clinical isolates expressed partially deleted *cag*PAI regions and that only few isolates exhibited either intact or deleted *cag*PAI regions. Previous studies have reported the occurrence of different proportions of intact *cag*PAI in their isolates. Ali *et al*[32] found that 37.4% of isolates exhibited intact *cag*PAI regions, whereas other researchers[30,33] reported that more than 96% of isolates contained intact *cag*PAI. In the present study, the isolates expressing intermediate genotypes (deletions within the *cag*PAI region) were more commonly encountered than those isolates with intact or deleted *cag*PAI regions. Moreover, a large proportion of the isolates exhibiting *cag*PAI rearrangement lacked an IS605 sequence, suggesting that this insertion sequence did not play a role in *cag*PAI disruption in the clinical isolates.

Additionally, we found that no single gene in the *cag*PAI region can be used as a genetic marker for an intact *cag*PAI because a large proportion of the clinical isolates exhibited gene rearrangement in the *cag*PAI region. Due

Table 5 Distribution of *Helicobacter pylori cag*PAI genotypes, *vacA* alleles and metronidazole susceptibilities *n* (%)

*cag*PAI status	*vacA*		Metronidazole susceptibility		*vacA*/metronidazole susceptibility			
	s1m1	s1m2	Sensitive	Resistant	s1m1/sensitive	s1m1/resistant	s1m2/sensitive	s1m2/resistant
Intact (n = 2)[1]	0	2 (100)	2 (100)	0	0	0	2 (100)	0
Partially deleted								
Type C (n = 26)	10 (38.5)	16 (61.5)	14 (53.8)	12 (46.2)	5 (19.2)	5 (19.2)	9 (34.6)	7 (26.9)
Type I (n = 16)	12 (75.0)	4 (25.0)	10 (62.5)	6 (37.5)	7 (43.8)	5 (31.2)	3 (18.8)	1 (6.2)
Type N (n = 13)	11 (84.6)	2 (15.4)	8 (61.5)	5 (38.5)	7 (53.8)	4 (50.0)	1 (12.5)	1 (12.5)
Deleted (n = 6)[2]	1 (16.7)	5 (83.3)	3 (37.5)	5 (62.5)	0	1 (16.7)	2 (33.3)	3 (50.0)

[1]One strain with the *vacA* s1m1m2 genotype was not included; [2]Two strains with the *vacA* s2m2 genotype were not included.

to the region's large size (approximately 40 kbp), the absence of these genes in certain clinical isolates did not necessarily indicate the complete absence of the *cag*PAI, as indicated in 9 isolates positive for a single gene. Regarding *cag*PAI rearrangement, 19 different genotypes (types B to T) have been identified. The three most common *cag*PAI genotypes identified in the clinical strains revealed that the genes at the left end of the *cag*PAI region were more unstable and prone to rearrangement than the genes in the middle and at the right end of the *cag*PAI region. This finding was supported by analyses of the individual genes, which demonstrated more gene rearrangement at the left end of the *cag*PAI region (*cag13*, *cag10* and *cag67*). Therefore, the genes in the middle and at the right end of the *cag*PAI region may play an important role in the pathogenesis of *H. pylori* infection in the study population.

In the current study, specific *cag*PAI genotypes were isolated from patients of different ethnicities. All of the isolates with intact *cag*PAI regions and less *cag*PAI rearrangement were from the Chinese patients, whereas the Indian patients were infected with strains exhibiting more rearranged or deleted *cag*PAI regions, and the Malay patients tended to be infected with various *cag*PAI genotypes. Thus, the presence of different *cag*PAI genotypes in different ethnicities may be related to the genetic characteristics of both the colonising *H. pylori* strain and the host. Although the relationship between the *cag*PAI genotype and disease state was unclear, we observed that certain types of infecting strains exhibited different *cag*PAI rearrangements in nonulcer and ulcer patients.

A significant difference was observed in the prevalence of *vacA* genotypes among patients of distinct ethnicities. As the Chinese patients were more likely to be infected with the *vacA* s1m2 strains, these strains may be regarded as more pathogenic, consistent with previous reports showing a higher frequency of peptic ulcers and gastric cancer in the Chinese patients in a similar study population[15,34]. In our study population, the smaller proportion of patients with PUD than NUD may have contributed to the lack of a significant association between the endoscopic findings and patient ethnicity, as analysis was conducted on the samples with positive cultures. Additionally, we demonstrated that the *vacA* s1m2 genotype was significantly associated with enhanced gastric inflammation[10]. In contrast, the *vacA* s2m2 strains were isolated only from the Malay and Indian patients, suggesting that these strains are less virulent. Consistent with this finding, Chinese ethnicity has been associated with infection by strains lacking the *vacA* s2m2 allele[35,36]. As Miernyk *et al*[37] recently reported a high proportion of *vacA* s2m2 strains isolated from Alaskan natives, it would be interesting to examine whether the disease outcome in Alaskan natives is similar to the outcome in the Malay and Indian patients in our study population. The current study also revealed the proportions of different *vacA* subgenotypes in each ethnic population. More specifically, the *vacA* s1cm1 genotype was not detected in the isolates from the Indian patients, whereas the *vacA* s1cm2 genotype was only detected in the isolates from the Chinese patients.

A previous report demonstrated a higher prevalence of resistance to metronidazole than to other antibiotics in *H. pylori* isolates from Malaysia[14]. In the current study, we attempted to further relate the metronidazole susceptibility of *H. pylori* to patient ethnicity and to correlate this susceptibility with the *cag*PAI and *vacA* genotypes. We noted a significant association between the metronidazole-sensitive *H. pylori* strains and Chinese ethnicity, whereas the Malay and Indian patients were more likely to be infected with the metronidazole-resistant strains. This finding may reflect differences in metronidazole use between different ethnic populations. The pattern of metronidazole susceptibility in different ethnicities paralleled the specific genotypes of the infecting strains. For example, the strains of the specific *cag*PAI genotype and *vacA* allele isolated from the Chinese patients were more sensitive to metronidazole.

The presence of frameshift and nonsense mutations in the antibiotic-resistant *H. pylori* strains suggested that these mutations confer resistance to metronidazole. However, more than half of the resistant strains exhibited missense mutations that were also detected in the antibiotic-sensitive strains. This finding implied that *rdxA* is not the only gene involved in the metronidazole-resistant phenotype and thus is not a reliable epidemiological marker. Rather, other genes or mechanisms may be implicated in the generation of resistance[38-40].

We observed no association between the specific *H. pylori* genotypes and the strains' antibiotic susceptibilities

in severe disease. These results may be complicated by the fact that most of the patients in our study population had gastritis. Although the *cag*PAI and *vacA* alleles are important virulence factors in infection, the development of disease is likely to involve a highly complex interplay of many bacterial and/or host factors.

The distribution of the *vacA* s1m2 genotype was broad, as this genotype was detected in the majority of the strains with intact *cag*PAI regions or *cag*PAI rearrangement of type C (positive for more *cag*PAI genes than other types) and the metronidazole-resistant strains. This finding may indicate that the *vacA* s1m2 strains have variable pathogenic properties when combined with other genotypic characteristics. The isolates with the deleted *cag*PAI regions were also primarily linked to metronidazole resistance. As these isolates may induce less inflammation in the host gastric epithelia, their genotypic characteristics may reduce antibiotic delivery and thus hinder the eradication of *H. pylori*. In contrast, the *vacA* s1m2 genotype could not contribute to metronidazole resistance, as this genotype was distributed evenly between the antibiotic-sensitive and -resistant strains[41,42].

In conclusion, we report a large proportion of *H. pylori* isolates harbouring *cag*PAI rearrangement and demonstrate that metronidazole susceptibility varies with patient ethnicity. The distinct distribution of the *H. pylori* *cag*PAI genotypes, *vacA* alleles and metronidazole susceptibilities in the different ethnicities of Malaysia may contribute to varying risk of gastroduodenal diseases. These distinct, patient ethnicity-associated *H. pylori* genotypes may have important clinical and epidemiological implications. Finally, the present study of *H. pylori*-specific genotypes from different host genetic backgrounds enhances our understanding of bacterium-host interactions and bacterial ecology in various niches. Further information on the characteristics of *H. pylori* will allow a more precise identification of virulent strains and a better definition of risk factors in susceptible hosts.

ACKNOWLEDGMENTS

We thank Universiti Kebangsaan Malaysia for providing both the permission and the facilities to conduct and publish this research. We are also grateful to all of the staff of the Endoscopy and Histopathology Units at UKMMC for their technical help.

COMMENTS

Background

The prevalence of *Helicobacter pylori* (*H. pylori*) infection in Malaysia consistently revealed ethnic differences, with Indians exhibiting higher infection rates than the Chinese and Malays. In contrast, peptic-ulcer disease and gastric carcinoma are known to be more prevalent in the Chinese and lowest in the Indians. The great genetic diversity of *H. pylori* may play an important role in the consequences of infection in different hosts. The *cag*PAI and *vacA* genes are well-established *H. pylori* virulence factors that interact with the host cells and disrupt downstream signalling pathways. The *cag*PAI structures, *vacA* alleles and metronidazole susceptibilities of different strains in hosts of varying genetic makeup may enhance the knowledge of bacterium-host interactions in *H. pylori* infections in multiethnic populations.

Research frontiers

The specific genotypes stemming from the *cag*PAI and *vacA* alleles were identified in *H. pylori* strains isolated from different ethnic groups. The strains isolated from the Chinese and peptic-ulcer disease (PUD) patients were more sensitive to antibiotics, indicating a selective advantage that occurred during early infection and persisted in chronic infection. In the present study, however, no association between the specific *H. pylori* genotypes and PUD could be determined due to the small number of PUD cases. Consistent with this observation, previous epidemiological studies have reported that less than 20% of patients infected with *H. pylori* are diagnosed as PUD.

Innovations and breakthroughs

Past reports have highlighted the importance of *H. pylori* genetic diversity in chronic infection. The current study emphasises and adds to findings from the same institution, demonstrating that the genetic background of the *H. pylori* strains may play an important role in the risk of gastroduodenal disease in different ethnic groups.

Applications

The results of this study provide insight into the effects of the genomic diversity of *H. pylori* on hosts of different genetic backgrounds. Therefore, these findings can be used in the development of advanced screening tools for diagnosing and determining the prognosis of *H. pylori* infection.

Terminology

The term *cag*PAI is defined as the *cag* pathogenicity island, a common gene sequence believed to be responsible for pathogenesis. This sequence contains approximately 40 kbp of nucleotides encoding over 40 genes. The pathogenicity island is typically absent from the *H. pylori* strains isolated from human carriers of *H. pylori* who remain asymptomatic.

Peer review

The authors investigated the diversity of the *H. pylori* virulence factors (the *cag*PAI and *vacA* alleles) and metronidazole susceptibilities of strains isolated from patients of different ethnicities. The association between specific *H. pylori* genotypes and patient ethnicity provides insight into the pathogenesis of *H. pylori* infection in different hosts and possibly the different risk factors in *H. pylori* infection.

REFERENCES

1 **Cooke CL**, Huff JL, Solnick JV. The role of genome diversity and immune evasion in persistent infection with Helicobacter pylori. *FEMS Immunol Med Microbiol* 2005; **45**: 11-23 [PMID: 15949928 DOI: 10.1016/j.femsim.2005.04.002]

2 **Leanza AG**, Matteo MJ, Crespo O, Antelo P, Olmos J, Catalano M. Genetic characterisation of Helicobacter pylori isolates from an Argentinean adult population based on cag pathogenicity island right-end motifs, lspA-glmM polymorphism and iceA and vacA genotypes. *Clin Microbiol Infect* 2004; **10**: 811-819 [PMID: 15355412]

3 **Salama NR**, Gonzalez-Valencia G, Deatherage B, Aviles-Jimenez F, Atherton JC, Graham DY, Torres J. Genetic analysis of Helicobacter pylori strain populations colonizing the stomach at different times postinfection. *J Bacteriol* 2007; **189**: 3834-3845 [PMID: 17337568 DOI: 10.1128/JB.01696-06]

4 **Hatakeyama M**. Helicobacter pylori and gastric carcinogenesis. *J Gastroenterol* 2009; **44**: 239-248 [PMID: 19271114]

5 **Kumar S**, Kumar A, Dixit VK. Evidences showing association of interleukin-1B polymorphisms with increased risk of gastric cancer in an Indian population. *Biochem Biophys Res Commun* 2009; **387**: 456-460 [PMID: 19607807]

6 **Segal ED**, Cha J, Lo J, Falkow S, Tompkins LS. Altered states: involvement of phosphorylated CagA in the induction of host cellular growth changes by Helicobacter pylori. *Proc Natl Acad Sci USA* 1999; **96**: 14559-14564 [PMID: 10588744]

7 **Nagiyev T**, Yula E, Abayli B, Koksal F. Prevalence and gen-

otypes of Helicobacter pylori in gastric biopsy specimens from patients with gastroduodenal pathologies in the Cukurova Region of Turkey. *J Clin Microbiol* 2009; **47**: 4150-4153 [PMID: 19846654 DOI: 10.1128/JCM.00605-09]

8 **Basso D**, Zambon CF, Letley DP, Stranges A, Marchet A, Rhead JL, Schiavon S, Guariso G, Ceroti M, Nitti D, Rugge M, Plebani M, Atherton JC. Clinical relevance of Helicobacter pylori cagA and vacA gene polymorphisms. *Gastroenterology* 2008; **135**: 91-99 [PMID: 18474244 DOI: 10.1053/j.gastro.2008.03.041]

9 **Nogueira C**, Figueiredo C, Carneiro F, Gomes AT, Barreira R, Figueira P, Salgado C, Belo L, Peixoto A, Bravo JC, Bravo LE, Realpe JL, Plaisier AP, Quint WG, Ruiz B, Correa P, van Doorn LJ. Helicobacter pylori genotypes may determine gastric histopathology. *Am J Pathol* 2001; **158**: 647-654 [PMID: 11159201 DOI: 10.1016/S002-9440(10)64006-0]

10 **Alfizah H**, Ramelah M, Rizal AM, Anwar AS, Isa MR. Association of Malaysian Helicobacter pylori virulence polymorphisms with severity of gastritis and patients' ethnicity. *Helicobacter* 2012; **17**: 340-349 [PMID: 22967117 DOI: 10.1111/j.1523-5378.2012.00956.x]

11 **Suzuki H**, Nishizawa T, Hibi T. Helicobacter pylori eradication therapy. *Future Microbiol* 2010; **5**: 639-648 [PMID: 20353303 DOI: 10.2217/fmb.10.25]

12 **Malfertheiner P**, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D, Hunt R, Rokkas T, Vakil N, Kuipers EJ. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. *Gut* 2007; **56**: 772-781 [PMID: 17170018 DOI: 10.1136/gut.2006.101634]

13 **Boyanova L**, Mitov I. Geographic map and evolution of primary Helicobacter pylori resistance to antibacterial agents. *Expert Rev Anti Infect Ther* 2010; **8**: 59-70 [PMID: 20014902]

14 **Ahmad N**, Zakaria WR, Mohamed R. Analysis of antibiotic susceptibility patterns of Helicobacter pylori isolates from Malaysia. *Helicobacter* 2011; **16**: 47-51 [PMID: 21241412 DOI: 10.1111/j.1523-5378.2010.00816.x]

15 **Alfizah H**, Rizal AM, Isa MR, Aminuddin A, Jasmi AY, Ramelah M. Four year analysis of Helicobacter pylori infection among patients with dyspepsia in Universiti Kebangsaan Malaysia Medical Centre. *Med Health* 2010; **5**: 13-21

16 **Goh KL**. Epidemiology of Helicobacter pylori infection in Malaysia--observations in a multiracial Asian population. *Med J Malaysia* 2009; **64**: 187-192 [PMID: 20527265]

17 **Kaur G**, Naing NN. Prevalence and ethnic distribution of Helicobacter pylori infection among endoscoped patients in North Eastern Peninsular Malaysia. *Mal J Med Sci* 2003; **10**: 66-70

18 **Kim JJ**, Reddy R, Lee M, Kim JG, El-Zaatari FA, Osato MS, Graham DY, Kwon DH. Analysis of metronidazole, clarithromycin and tetracycline resistance of Helicobacter pylori isolates from Korea. *J Antimicrob Chemother* 2001; **47**: 459-461 [PMID: 11266421 DOI: 10.1093/jac/47.4.459]

19 **Solcà NM**, Bernasconi MV, Piffaretti JC. Mechanism of metronidazole resistance in Helicobacter pylori: comparison of the rdxA gene sequences in 30 strains. *Antimicrob Agents Chemother* 2000; **44**: 2207-2210 [PMID: 10898705 DOI: 10.1128/AAC.44.8.2207-2210.2000]

20 **Maeda S**, Yoshida H, Ikenoue T, Ogura K, Kanai F, Kato N, Shiratori Y, Omata M. Structure of cag pathogenicity island in Japanese Helicobacter pylori isolates. *Gut* 1999; **44**: 336-341 [PMID: 10026317 DOI: 10.1136/gut.44.3.336]

21 **Atherton JC**, Cao P, Peek RM, Tummuru MK, Blaser MJ, Cover TL. Mosaicism in vacuolating cytotoxin alleles of Helicobacter pylori. Association of specific vacA types with cytotoxin production and peptic ulceration. *J Biol Chem* 1995; **270**: 17771-17777 [PMID: 7629077 DOI: 10.1074/jbc.270.30.17771]

22 **Patra R**, Chattopadhyay S, De R, Datta S, Chowdhury A, Ramamurthy T, Nair GB, Berg DE, Mukhopadhyay AK. Intact cag pathogenicity island of Helicobacter pylori without disease association in Kolkata, India. *Int J Med Microbiol* 2011; **301**: 293-302 [PMID: 21195664 DOI: 10.1016/j.ijmm.2010.10.003]

23 **Suerbaum S**, Michetti P. Helicobacter pylori infection. *N Engl J Med* 2002; **347**: 1175-1186 [PMID: 12374879 DOI: 10.1056/NEJMra020542]

24 **Höcker M**, Hohenberger P. Helicobacter pylori virulence factors--one part of a big picture. *Lancet* 2003; **362**: 1231-1233 [PMID: 14568748 DOI: 10.1016/S0140-6736(03)14547-3]

25 **Kauser F**, Khan AA, Hussain MA, Carroll IM, Ahmad N, Tiwari S, Shouche Y, Das B, Alam M, Ali SM, Habibullah CM, Sierra R, Megraud F, Sechi LA, Ahmed N. The cag pathogenicity island of Helicobacter pylori is disrupted in the majority of patient isolates from different human populations. *J Clin Microbiol* 2004; **42**: 5302-5308 [PMID: 15528729 DOI: 10.1128/JCM.42.11.5302-5308.2004]

26 **Kumar S**, Kumar A, Dixit VK. Diversity in the cag pathogenicity island of Helicobacter pylori isolates in populations from North and South India. *J Med Microbiol* 2010; **59**: 32-40 [PMID: 19815664]

27 **Audibert C**, Burucoa C, Janvier B, Fauchère JL. implication of the structure of the Helicobacter pylori cag pathogenicity island in induction of interleukin-8 secretion. *Infect Immun* 2001; **69**: 1625-1629 [PMID: 11179336 DOI: 10.1128/IAI.69.3.1625-1629.2001]

28 **Nilsson C**, Sillén A, Eriksson L, Strand ML, Enroth H, Normark S, Falk P, Engstrand L. Correlation between cag pathogenicity island composition and Helicobacter pylori-associated gastroduodenal disease. *Infect Immun* 2003; **71**: 6573-6581 [PMID: 14573679]

29 **Hsu PI**, Hwang IR, Cittelly D, Lai KH, El-Zimaity HM, Gutierrez O, Kim JG, Osato MS, Graham DY, Yamaoka Y. Clinical presentation in relation to diversity within the Helicobacter pylori cag pathogenicity island. *Am J Gastroenterol* 2002; **97**: 2231-2238 [PMID: 12358238 DOI: 10.1111/j.1572-0241.2002.05977.x]

30 **Ikenoue T**, Maeda S, Ogura K, Akanuma M, Mitsuno Y, Imai Y, Yoshida H, Shiratori Y, Omata M. Determination of Helicobacter pylori virulence by simple gene analysis of the cag pathogenicity island. *Clin Diagn Lab Immunol* 2001; **8**: 181-186 [PMID: 11139216 DOI: 10.1128/CDLI.8.1.181-186.2001]

31 **Ramelah M**, Aminuddin A, Alfizah H, Isa MR, Jasmi AY, Tan HJ, Rahman AJ, Rizal AM, Mazlam MZ. cagA gene variants in Malaysian Helicobacter pylori strains isolated from patients of different ethnic groups. *FEMS Immunol Med Microbiol* 2005; **44**: 239-242 [PMID: 15866222 DOI: 10.1016/j.femsim.2005.02.001]

32 **Ali M**, Khan AA, Tiwari SK, Ahmed N, Rao LV, Habibullah CM. Association between cag-pathogenicity island in Helicobacter pylori isolates from peptic ulcer, gastric carcinoma, and non-ulcer dyspepsia subjects with histological changes. *World J Gastroenterol* 2005; **11**: 6815-6822 [PMID: 16425389]

33 **Mukhopadhyay AK**, Kersulyte D, Jeong JY, Datta S, Ito Y, Chowdhury A, Chowdhury S, Santra A, Bhattacharya SK, Azuma T, Nair GB, Berg DE. Distinctiveness of genotypes of Helicobacter pylori in Calcutta, India. *J Bacteriol* 2000; **182**: 3219-3227 [PMID: 10809703 DOI: 10.1128/JB.182.11.3219-3227.2000]

34 **Lim CC**, Halimah Y. Cancer incidence in Malaysia, 2nd Report. National Cancer Registry Report. Malaysia: Ministry of Health Malaysia, 2003: 78–79

35 **Jang S**, Jones KR, Olsen CH, Joo YM, Yoo YJ, Chung IS, Cha JH, Merrell DS. Epidemiological link between gastric disease and polymorphisms in VacA and CagA. *J Clin Microbiol* 2010; **48**: 559-567 [PMID: 19955279 DOI: 10.1128/JCM.01501-09]

36 **Wang J**, van Doorn LJ, Robinson PA, Ji X, Wang D, Wang

Y, Ge L, Telford JL, Crabtree JE. Regional variation among vacA alleles of Helicobacter pylori in China. *J Clin Microbiol* 2003; **41**: 1942-1945 [PMID: 12734231 DOI: 10.1128/JCM.41.5.1942-1945.2003]

37 **Miernyk K**, Morris J, Bruden D, McMahon B, Hurlburt D, Sacco F, Parkinson A, Hennessy T, Bruce M. Characterization of Helicobacter pylori cagA and vacA genotypes among Alaskans and their correlation with clinical disease. *J Clin Microbiol* 2011; **49**: 3114-3121 [PMID: 21752979 DOI: 10.1128/JCM.00469-11]

38 **Francesco VD**, Zullo A, Hassan C, Giorgio F, Rosania R, Ierardi E. Mechanisms of Helicobacter pylori antibiotic resistance: An updated appraisal. *World J Gastrointest Pathophysiol* 2011; **2**: 35-41 [PMID: 21860834 DOI: 10.4291/wjgp.v2.i3.35]

39 **Jiang K**, Zhang JZ, Pan GZ. Mechanism of metronidazole resistance of Helicobacter pylori. *Zhongguo Xiaohua Jibing Zazhi* 2001; **2**: 95-99

40 **Hoffman PS**, Goodwin A, Johnsen J, Magee K, Veldhuyzen van Zanten SJ. Metabolic activities of metronidazole-sensitive and -resistant strains of Helicobacter pylori: repression of pyruvate oxidoreductase and expression of isocitrate lyase activity correlate with resistance. *J Bacteriol* 1996; **178**: 4822-4829 [PMID: 8759844]

41 **Elviss NC**, Owen RJ, Breathnach A, Palmer C, Shetty N. Helicobacter pylori antibiotic-resistance patterns and risk factors in adult dyspeptic patients from ethnically diverse populations in central and south London during 2000. *J Med Microbiol* 2005; **54**: 567-574 [PMID: 15888466]

42 **Godoy AP**, Ribeiro ML, Benvengo YH, Vitiello L, Miranda Mde C, Mendonça S, Pedrazzoli J. Analysis of antimicrobial susceptibility and virulence factors in Helicobacter pylori clinical isolates. *BMC Gastroenterol* 2003; **3**: 20 [PMID: 12911839 DOI: 10.1186/1471-230X-3-20]

P- Reviewer Mori N **S- Editor** Gou SX **L- Editor** A
E- Editor Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i8.1292

World J Gastroenterol 2013 February 28; 19(8): 1292-1298
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Abnormal splenic artery diameter/hepatic artery diameter ratio in cirrhosis-induced portal hypertension

Dao-Bing Zeng, Chuan-Zhou Dai, Shi-Chun Lu, Ning He, Wei Wang, Hong-Jun Li

Dao-Bing Zeng, Chuan-Zhou Dai, Shi-Chun Lu, Department of Hepatobiliary Surgery and You-An Liver Transplantation Center, Beijing You-An Hospital, Capital Medical University, Beijing 100069, China
Ning He, Wei Wang, Hong-Jun Li, Department of Radiology, Beijing You-An Hospital, Capital Medical University, Beijing 100069, China
Author contributions: Lu SC designed the study and prepared the protocol; Zeng DB, Dai CZ, Lu SC, He N, Wang W and Li HJ conducted the study; Zeng DB and Dai CZ contributed important reagents/analytic tools; Zeng DB did the data analysis; Zeng DB and Lu SC wrote the paper.
Supported by Grants from Beijing Municipal Health Bureau, No. 2011-2-18; the Capital Health Development Special Funds, No. 2011-2018-03
Correspondence to: Dr. Shi-Chun Lu, Department of Hepatobiliary Surgery and You-An Liver Transplantation Center, Beijing You-An Hospital, Capital Medical University, No. 8 Xitoutiao Road Youan Men Wai, Beijing 100069, China. lsc620213@yahoo.com.cn
Telephone: +86-10-83997160 Fax: +86-10-83997160
Received: September 5, 2012 Revised: December 24, 2012
Accepted: January 5, 2013
Published online: February 28, 2013

Abstract

AIM: To determine an optimal cutoff value for abnormal splenic artery diameter/proper hepatic artery diameter (S/P) ratio in cirrhosis-induced portal hypertension.

METHODS: Patients with cirrhosis and portal hypertension (n = 770) and healthy volunteers (n = 31) underwent volumetric computed tomography three-dimensional vascular reconstruction to measure the internal diameters of the splenic artery and proper hepatic artery to calculate the S/P ratio. The cutoff value for abnormal S/P ratio was determined using receiver operating characteristic curve analysis, and the prevalence of abnormal S/P ratio and associations between abnormal S/P ratio and major complications of portal hypertension were studied using logistic regression.

RESULTS: The receiver operating characteristic analysis showed that the cutoff points for abnormal splenic artery internal diameter and S/P ratio were > 5.19 mm and > 1.40, respectively. The sensitivity, specificity, positive predictive value, and negative predictive value were 74.2%, 45.2%, 97.1%, and 6.6%, respectively. The prevalence of an abnormal S/P ratio in the patients with cirrhosis and portal hypertension was 83.4%. Patients with a higher S/P ratio had a lower risk of developing ascites [odds ratio (OR) = 0.708, 95%CI: 0.508-0.986, P = 0.041] and a higher risk of developing esophageal and gastric varices (OR = 1.483, 95%CI: 1.010-2.175, P = 0.044) and forming collateral circulation (OR = 1.518, 95%CI: 1.033-2.230, P = 0.034). After splenectomy, the portal venous pressure and maximum and mean portal venous flow velocities were reduced, while the flow rate and maximum and minimum flow velocities of the hepatic artery were increased (P < 0.05).

CONCLUSION: The prevalence of an abnormal S/P ratio is high in patients with cirrhosis and portal hypertension, and it can be used as an important marker of splanchnic hemodynamic disturbances.

© 2013 Baishideng. All rights reserved.

Key words: Portal hypertension; Cirrhosis; Splenic artery internal diameter; Proper hepatic artery internal diameter; Complications; Splenectomy

Zeng DB, Dai CZ, Lu SC, He N, Wang W, Li HJ. Abnormal splenic artery diameter/hepatic artery diameter ratio in cirrhosis-induced portal hypertension. *World J Gastroenterol* 2013; 19(8): 1292-1298 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i8/1292.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i8.1292

INTRODUCTION

Splanchnic and systemic hemodynamic disorders are

common in patients with cirrhosis[1,2]. The systemic hyperdynamic circulation manifests as increased cardiac output and heart rate, and decreased arterial blood pressure and systemic vascular resistance[3,4]. The splanchnic hemodynamic disorder mainly manifests as increased vascular resistance and comparative visceral ischemia, which are associated with hepatic encephalopathy, hepatorenal syndrome, and portal hypertension[5]. Currently, portal hypertension has been found to be attributed to abnormally high portal venous inflow and high resistance in the portal system[6].

The splanchnic hemodynamic disorder in portal hypertension has been studied in a few studies, and some results support the significance of splenic and hepatic artery changes in portal hypertension. As early as in 1970, Yamauchi *et al*[7] observed an increase in the radius of the splenic artery in patients with portal hypertension, and such increase was not present in splenomegaly without portal hypertension. They found an association between the radius of the splenic artery and portal pressure and the degree of reduction in the portal pressure after splenectomy in patients with portal hypertension, and the dilatation of the splenic artery was accompanied by an increase in the diameter of the proper hepatic artery in those patients[7]. Subsequent studies confirmed the increases in the diameters of the splenic and hepatic arteries in patients with liver cirrhosis as compared with healthy controls[8,9], and further found that changes in the splenic artery occur prior to the onset of clinically evident portal hypertension[8].

Splenic and hepatic arterial blood flow distribution abnormalities are also very common in patients who have received liver transplantation. A study by Quintini *et al*[10] confirmed that hepatic artery hypoperfusion in liver transplant recipients can be induced by increased portal venous blood flow because over-perfusion of the portal vein cleared adenosine at the terminal end of the hepatic artery, resulting in hepatic artery contraction, *i.e.*, "hepatic arterial buffer response". Persistence of this response may cause further deterioration of splenic and hepatic arterial blood flow abnormalities, and result in a series of related complications known as splenic artery steal syndrome (SASS) or splenic artery syndrome that includes liver dysfunction, ischemic bile duct injury, and graft dysfunction or loss. Kirbas *et al*[11] performed multi-detector computed tomography (CT) in post-liver transplanted patients, and the authors proposed that a splenic artery diameter > 4 mm or a splenic artery diameter/hepatic artery diameter ratio > 1.5 indicates presence of SASS, thus establishing the importance of splenic artery diameter and the splenic artery diameter/hepatic artery diameter ratio in the clinical diagnosis of splenic and hepatic arterial blood flow distribution abnormalities.

Small-for-size syndrome (SFSS) is a common clinical complication that occurs after segmental liver transplantation[12,13]. Although the pathogenesis of the SFSS is still controversial, it is assumed that portal hypoperfusion with high intravascular shear stress is a causative factor[14-16]. In 2003, a group of researchers reported that portal flow should be modulated perioperatively in adult living-donor liver transplantation if a suboptimal graft-to-recipient body weight ratio is accompanied by high recipient portal inflow[17]. The authors found that splenic artery ligation could decrease recipient portal inflow, increase recipient hepatic arterial flow, and resolve refractory ascites, and neither SFSS nor vascular complications occurred in recipients undergoing splenic artery ligation[17]. Gruttadauria *et al*[18] obtained similar findings when they applied splenic artery embolization (SAE) for the treatment of SFSS after living-related donor liver transplantation. Their results showed that clinical conditions in patients with SFSS rapidly improved after SAE, and they thought that early SAE can protect the segmental graft against the harmful effects of the portal overflow[18]. Other animal and clinical studies of patients with liver cirrhosis and idiopathic portal hypertension showed that splenectomy can reduce portal venous blood flow and portal venous pressure, and increase hepatic arterial blood flow[19,20].

In this study, we examined whether splenic artery diameter and the splenic artery internal diameter/proper hepatic artery internal diameter (S/P) ratio are of clinical significance in patients with cirrhosis and portal hypertension. We determined the optimal cutoff values for abnormal splenic artery diameter and S/P ratio, observed the prevalence of an abnormal S/P ratio, and studied the associations between the S/P ratio and major complications of portal hypertension in patients with cirrhosis. The preoperative and postoperative portal vein hemodynamic indexes were further compared in patients undergoing splenectomy to explore the significance of splenectomy in the treatment of portal hypertension and its complications.

MATERIALS AND METHODS

Participants

A total of 1000 patients with liver cirrhosis and portal hypertension who sought medical treatment at our hospital from January 2011 to May 2011 were recruited. All patients had a pathological diagnosis of liver cirrhosis. Clinical diagnosis of portal hypertension was made in the setting of end-stage liver disease, and in the presence of ascites and/or varices. Of the 1000 patients, 230 were excluded: 109 with hepatic artery variations, 66 with post-splenectomy, 51 with partial splenic artery embolization, and 4 with transjugular intrahepatic portosystemic stent shunting. Thus, a total of 770 patients with liver cirrhosis and portal hypertension were enrolled (554 males and 216 females), of whom 558 had biochemical examination results. Thirty-one healthy volunteers who participated in the donor screening for the living-related liver transplantation were also enrolled. This study was approved by the Institutional Review Boards of our hospital, and all the participants provided written informed consent.

Laboratory tests

All the patients received routine blood, coagulation, and liver function tests in a fasting state. The routine blood

tests were performed with an SYSMES-2100 automatic blood cell analyzer and reagents (SYSMES Co., Japan). Coagulation tests were performed with a CA-7000 automatic coagulation instrument and ancillary reagents (SYSMES Co., Japan). Liver function tests were performed with an AU5400 automatic biochemical analyzer and related reagents (Mishima Olympus Co., Japan).

Measurement of splenic and proper hepatic artery internal diameter

All the patients underwent abdominal enhanced computed tomography (CT) scanning with a General Electric (GE) Lightspeed VCT 64-slice scanner (GE, Milwaukee, WIS, United States). For scanning, the collimator width was 0.625 mm, the tube current was 380 mA, the tube voltage was 120 kV, the slice thickness was 5 mm, the slice interval was 5 mm, and the reconstruction slice thickness was 0.625 mm. A high-pressure syringe was used to inject 100 mL contrast agent iopromide (370 mg/mL) via the antecubital vein at a flow rate of 2.5-3.5 mL/s, and the scan phases were the arterial phase (20-25 s), venous phase (65-70 s), and delayed phase (180 s). The data were input into a GE ADW 4.3 image workstation, and multiplanar reconstruction, maximum intensity projection, and volume rendering post-processing were performed. The splenic and proper hepatic artery internal diameters were measured in the arterial phase. The internal diameter of the proper hepatic artery was measured within 1 cm from its origin. The internal diameter of the splenic artery was measured at 3 locations: within 1 cm from its origin, at the upper edge of the pancreas, and within 1 cm from the point where the splenic artery branches originate. The mean value was calculated and recorded as the internal diameter of the splenic artery. The S/P ratio was then calculated.

Measurement of hemodynamic indexes of portal vein and hepatic artery before and after splenectomy

The free portal venous pressure was measured by catheterization via the right gastroepiploic vein before and after splenectomy. Doppler ultrasound (Esaote, Italy) was used to measure the hemodynamic indexes of the portal vein and hepatic artery within 1 wk prior to splenectomy and within 2 wk after splenectomy. Indexes measured were the portal venous flow rate and the maximum and mean portal venous flow velocities, the flow rate and maximum and minimum flow velocities of the hepatic artery, and the resistance index (RI) of the hepatic artery.

Statistical analysis

Demographic characteristics were presented as number (%) for sex, causes of cirrhosis, and level of liver function, mean ± SD for age, and median [interquartile range (IQR): Q1-Q3] for the internal diameters of the splenic artery and proper hepatic artery and the S/P ratio because data were not normally distributed. Data of patients with cirrhosis and healthy controls were compared using Pearson χ^2 test for sex, two-sample *t* test for age, and Mann-Whitney *U* test for splenic artery diameter, proper hepatic artery diameter, and the S/P ratio, respectively. Receiver operating characteristic (ROC) curve analysis through binary logistic regression was carried out to identify the optimal cutoff values of abnormal internal diameter of the splenic artery and S/P ratio. The prevalence of an abnormal S/P ratio in patients with cirrhosis and portal hypertension was calculated based on the identified cutoff value. Associations between the S/P ratio and complications of cirrhosis and portal hypertension were analyzed using univariate logistic regression. The preoperative and postoperative portal vein hemodynamic indexes and routine blood test data of patients undergoing splenectomy were presented as mean ± SD or median (IQR: Q1-Q3) if data were not normally distributed. The preoperative and postoperative data were compared using paired *t* test or Wilcoxon sign-rank test if data were not normally distributed. All statistical assessments were two-tailed and the significance level α was set at 0.05. All statistical analyses were performed using SPSS 17.0 statistics software (SPSS Inc, Chicago, IL, United States).

RESULTS

Demographic characteristics of participants

A total of 770 patients with cirrhosis and portal hypertension (554 males and 216 females) and 31 healthy controls (16 males and 15 females) were enrolled. Their demographic characteristics are summarized in Table 1. The mean age of patients with cirrhosis and portal hypertension was 53.3 ± 11.3 years, and the mean age of healthy controls was 46.0 ± 12.0 years. Among the patients with cirrhosis and portal hypertension, 622 had cirrhosis due to hepatitis B, 61 had alcoholic cirrhosis, 39 had cirrhosis due to hepatitis C, 24 had autoimmune liver disease, 7 had drug-induced liver damage, 4 had Budd-Chiari syndrome, 4 had cirrhosis caused by hepatitis B and C, 3 had alcoholic cirrhosis with hepatitis B virus infection, 2 had idiopathic portal hypertension, 1 had hepatolenticular degeneration, 1 had cryptogenic cirrhosis, 1 had nonalcoholic fatty liver cirrhosis, and 1 had cirrhosis due to unknown cause.

Internal diameter of splenic artery and S/P ratio

Patients with cirrhosis and portal hypertension had a higher internal diameter of the splenic artery and S/P ratio than healthy controls (both, $P < 0.05$). The median internal diameter of the splenic artery was 5.35 mm (IQR: 4.67-6.18 mm) in patients with cirrhosis and portal hypertension and 4.60 mm (IQR: 4.32-5.32 mm) in healthy controls. The median S/P ratio was 1.48 (IQR: 1.19-1.82) in patients with cirrhosis and portal hypertension and 1.36 (1.13-1.48) in healthy controls (Table 1). The internal diameter of the proper hepatic artery was not significantly different between patients with cirrhosis and portal hypertension and healthy controls.

ROC curve analysis showed that the optimal cutoff values of abnormal internal diameter of the splenic artery and S/P ratio were > 5.19 mm and > 1.40, respectively (Figure 1). The sensitivity, specificity, positive pre-

Table 1 Demographic characteristics of participants *n* (%)

Variables	Patients with cirrhosis and portal hypertension (*n* = 770)	Healthy controls (*n* = 31)	*P* value
Sex			0.025[1]
Male	554 (71.9)	16 (51.6)	
Female	216 (28.1)	15 (48.4)	
Age (yr)	53.3 ± 11.3	46.0 ± 12.0	< 0.001[1]
Causes of cirrhosis			NA
Hepatitis B	622 (80.8)	-	
Hepatitis C	39 (5.1)	-	
Alcoholic cirrhosis	61 (7.9)	-	
Autoimmune liver disease	24 (3.1)	-	
Others	24 (3.1)	-	
Level of liver function			NA
Ⅰ	230 (29.9)	-	
Ⅱ	209 (27.1)	-	
Ⅲ	119 (15.5)	-	
Undefined	212 (27.5)	-	
Splenic artery internal diameter (mm)	5.35 (4.67-6.18)	4.60 (4.32-5.32)	0.002[1]
Proper hepatic artery internal diameter (mm)	3.63 (2.97-4.43)	3.72 (3.14-4.19)	0.830
S/P ratio	1.48 (1.19-1.82)	1.36 (1.13-1.48)	0.012[1]

[1]Indicates significant difference between patients with cirrhosis and portal hypertension and healthy controls. Data were presented as number (%) for sex, cause of cirrhosis, and level of liver function; mean ± SD for age; and median (interquartile range: Q1-Q3) for the internal diameters of the splenic artery and proper hepatic artery and the splenic artery internal diameter/proper hepatic artery internal diameter (S/P) ratio because data were not normally distributed. Data of patients with cirrhosis and healthy controls were compared using Pearson χ^2 for sex, two-sample *t* test for age, and Mann-Whitney *U* test for S/P ratio, respectively. NA: Not assessed.

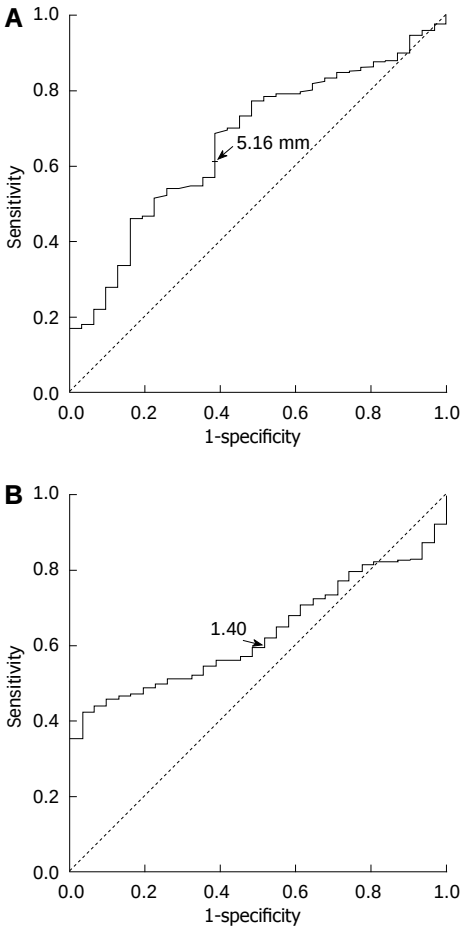


Figure 1 Receiver operating characteristic curve analysis for identifying the optimal cutoff values. A: Abnormal internal diameter of the splenic artery; B: Abnormal splenic artery internal diameter/proper hepatic artery internal diameter (S/P) ratio for patients with cirrhosis and portal hypertension. Binary logistic regression showed that the areas under the receiver operating characteristic curve were 0.667 (95%CI: 0.581-0.753) and 0.633 (95%CI: 0.572-0.694) for the internal diameter of the splenic artery and S/P ratio, respectively (both, $P < 0.05$). The optimal cutoff values were identified to be > 5.19 mm for abnormal internal diameter of the splenic artery and > 1.40 for abnormal S/P ratio.

dictive value, and negative predictive value were 74.2%, 45.2%, 97.1%, and 6.6%, respectively. Using the cutoff value of 1.40, 640 patients (83.4%) with cirrhosis and portal hypertension had an abnormal S/P ratio, and 28 healthy controls (90.3%) had a normal S/P ratio.

Associations between S/P ratio and complications in patients with cirrhosis and portal hypertension

Results of univariate logistic regression are presented in Table 2. The results showed that patients with a higher S/P ratio had a lower risk of developing ascites [odds ratio (OR) = 0.708, 95%CI: 0.508-0.986, $P = 0.041$]. However, patients with a higher S/P ratio had a higher risk of developing esophageal and gastric varices (OR = 1.483, 95%CI: 1.010-2.175, $P = 0.044$) and forming collateral circulation (OR = 1.518, 95%CI: 1.033-2.230, $P = 0.034$).

Portal vein hemodynamic indexes and routine blood test results in patients with cirrhosis and portal hypertension before and after splenectomy

A total of 17 patients with cirrhosis and portal hypertension underwent splenectomy. Table 3 presents the comparison of the preoperative and postoperative portal vein hemodynamic indexes in these patients. After splenectomy, the portal venous pressure and maximum and mean portal venous flow velocities were significantly reduced compared with the preoperative values (all, $P < 0.05$). The postoperative flow rate, maximum flow velocity, and minimum flow velocity of the hepatic artery were significantly higher than the preoperative values (all, $P < 0.05$). The RI of the hepatic artery was significantly reduced after splenectomy ($P < 0.001$).

Table 4 presents the comparison of the results of the preoperative and postoperative routine blood tests in patients with cirrhosis and portal hypertension who underwent splenectomy. After splenectomy, total bilirubin, direct bilirubin, white blood cell (WBC) count, and platelet count were significantly increased (all, $P < 0.05$), and creatinine levels were significantly decreased ($P < 0.05$).

DISCUSSION

The results of this study showed that patients with cirrhosis and portal hypertension had a significantly greater internal diameter of the splenic artery and higher S/P ratio than healthy controls, but not a significantly differ-

Table 2 Univariate logistic regression analyses of associations between splenic artery internal diameter/proper hepatic artery internal diameter ratio and complications in patients with cirrhosis and portal hypertension *n* (%)

Complication	Total (*n* = 770)	Patients with normal S/P ratio (*n* = 130)	Patients with abnormal S/P ratio (*n* = 640)	OR (95%CI)	*P* value
Ascites					
Yes	426 (55.7)	304 (53.5)	122 (61.9)	0.708 (0.508-0.986)	0.041[1]
No	339 (44.3)	264 (46.5)	75 (38.1)	Reference	
Hepatic encephalopathy					
Yes	77 (10.1)	58 (10.2)	19 (9.6)	1.065 (0.617-1.838)	0.820
No	688 (89.9)	510 (89.8)	178 (90.4)	Reference	
Esophageal and gastric varices					
Yes	209 (27.4)	166 (29.3)	43 (21.8)	1.483 (1.010-2.175)	0.044[1]
No	555 (72.6)	401 (70.7)	154 (78.2)	Reference	
Upper gastrointestinal bleeding					
Yes	111 (14.5)	90 (15.9)	21 (10.7)	1.581 (0.954-2.621)	0.076
No	653 (85.5)	477 (84.1)	176 (89.3)	Reference	
Collateral circulation formation					
Yes	611 (79.9)	464 (81.7)	147 (74.6)	1.518 (1.033-2.230)	0.034[1]
No	154 (20.1)	104 (18.3)	50 (25.4)	Reference	

[1]Indicates significant association. The incidences of ascites, hepatic encephalopathy, esophageal and gastric varices, Upper gastrointestinal bleeding, and collateral circulation formation were not available in 5, 5, 6, 6, and 5 patients, respectively. OR: Odds ratio.

Table 3 Comparisons of preoperative and postoperative portal vein hemodynamic indexes in patients with cirrhosis and portal hypertension who received splenectomy (*n* = 17)

Variables	Before splenectomy	After splenectomy	*P* value
Portal vein			
Pressure (cm H_2O)	39.19 ± 5.87	27.04 ± 3.03	< 0.001[1]
Flow rate (mL/min)	1005.50 (833.00-1811.25)	868.00 (539.50-1215.75)	0.116
Maximum flow velocity (cm/s)	23.85 (18.53-31.20)	15.85 (12.68-20.58)	0.009[1]
Mean flow velocity (cm/s)	20.85 (15.90-25.95)	12.25 (10.48-16.40)	0.011[1]
Hepatic artery			
Flow rate (mL/min)	153.50 (128.75-188.77)	365.00 (190.00-511.00)	0.048[1]
Minimum flow velocity (cm/s)	16.25 ± 15.23	32.60 ± 15.23	0.001[1]
Resistance index	0.74 ± 0.05	0.64 ± 0.07	< 0.001[1]

[1]Indicates significant difference between preoperative and postoperative values. Data were presented as mean ± SD, or median (interquartile range: Q1-Q3) if not normally distributed. Preoperative and postoperative data were compared using paired *t*-test or Wilcoxon sign-rank test if data were not normally distributed.

Table 4 Comparisons of preoperative and postoperative routine blood test results in patients with cirrhosis and portal hypertension who received splenectomy (*n* = 17)

Variable	Before splenectomy	After splenectomy	*P* value
ALT (U/L)	29.39 ± 16.86	36.47 ± 28.9	0.439
AST (U/L)	33 (21.9-45.4)	37 (26.65-48.45)	0.653
Total bilirubin (μmol/L)	16.8 (12.95-24.9)	21.4 (16.2-39.55)	0.005[1]
Direct bilirubin (μmol/L)	4.74 ± 2.13	7.56 ± 4.31	0.003[1]
Albumin (g/L)	37.67 ± 3.73	36.74 ± 3.28	0.439
Creatinine (μmol/L)	70.44 ± 22.28	58.69 ± 14.78	0.036[1]
WBC count (E + 09/L)	1.73 (1.26-2.24)	9.05 (6.43-12.84)	< 0.001[1]
RBC count (E + 12/L)	3.45 ± 0.73	3.42 ± 0.47	0.87
Platelet count (E + 09/L)	47.41 ± 24.48	307 ± 132.4	< 0.001[1]

[1]Indicates significant difference between preoperative and postoperative values. Data were presented as mean ± SD, or median (interquartile range: Q1-Q3) if not normally distributed. Preoperative and postoperative data were compared using paired *t* test or Wilcoxon sign-rank test if data were not normally distributed. ALT: Alanine amino transferase; AST: Aspartate amino transferase; WBC: White blood cell; RBC: Red blood cell.

ent internal diameter of the proper hepatic artery. The finding of an increased diameter of the splenic artery in patients with cirrhosis and portal hypertension is consistent with the findings of prior studies[7-9]. The results indicate that excessive perfusion of the splenic artery increases the return blood flow in the splenic vein, thereby

increasing the blood flow in the portal venous system resulting in portal hypertension. However, an increase in the diameter of the hepatic artery was observed in patients with portal hypertension in some early studies[7-9], a finding not observed in our study. A possible reason for this discrepancy is that different imaging methods were used to measure the arterial internal diameter and current enhanced CT scanning is more accurate than the imaging methods used in earlier studies.

The optimal cutoff values for the abnormal internal diameter of the splenic artery and S/P ratio were identified to be > 5.19 mm and > 1.40, respectively, by the ROC curve analysis. When the abnormal S/P ratio > 1.40 was used, 83.4% of patients with cirrhosis and portal hypertension had an abnormal S/P ratio. The prevalence of an abnormal S/P ratio is very high in patients with cirrhosis and portal hypertension, indicating that an abnormal S/P ratio can be used as a marker of splanchnic hemodynamic disturbances in these patients. Machálek *et al*[21] summarized measurements of the splenic artery internal diameter of 30 normal spleens, and found that splenic artery thickening was indicated by an internal diameter of the splenic artery greater than 5.6 ± 1.3 mm. In our study, we found that an internal diameter of the splenic artery greater than 5.19 mm is the best cutoff value to predict cirrhosis and portal hypertension, which is consistent with the finding of Machálek *et al*[21]. Kirbas *et al*[11] proposed that a splenic artery diameter > 4 mm or a splenic artery diameter/hepatic artery diameter ratio > 1.5 indicates presence of SASS after liver transplantation, which is similar to the S/P ratio cutoff point of 1.40 for patients with cirrhosis and portal hypertension determined in our study. However, Kirbas *et al*[11] reported a much lower abnormal value for the internal diameter of the splenic artery than the value reported by Machálek *et al*[21] and our study, which may be due to the a different measurement method used by them.

Our results indicated associations between the S/P ratio and major complications in patients with portal hypertension. Patients with a higher S/P ratio had a lower risk of developing ascites (OR = 0.708, 95%CI: 0.508-0.986, $P = 0.041$) and a higher risk of developing *esophageal* and *gastric* varices (OR = 1.483, 95%CI: 1.010-2.175, $P = 0.044$) and forming collateral circulation (OR = 1.518, 95%CI: 1.033-2.230, $P = 0.034$). The results indicate that the S/P ratio has a great clinical significance for predicting major complications of portal hypertension in patients with cirrhosis.

Studies have also shown that splenectomy can reduce portal venous blood flow and portal venous pressure, increase hepatic arterial blood flow, and improve liver function, thereby decreasing major complications of portal hypertension[22,23]. Our study showed that splenectomy significantly reduced portal venous pressure and *maximum* and mean portal venous flow velocities, and increased the flow rate, maximum flow velocity, and minimum flow velocity of the hepatic artery. The study of SASS after liver transplantation by Mogl *et al*[24] found a significant elevation of the RI of the hepatic artery (0.79 ± 0.14) in patients with SASS, which was significantly reduced (0.65 ± 0.09) after splenic artery embolization. In our study, the preoperative and postoperative RIs of the hepatic artery were 0.74 ± 0.05 and 0.64 ± 0.07, respectively, which is consistent with the findings of Mogl *et al*[24], Our results also showed that postoperative WBC count and platelet levels were significantly increased compared with the preoperative values, indicating that these indexes can be restored to normal values. These results indicate that the major complications of cirrhosis and portal hypertension and splanchnic hemodynamic disturbances can be improved by splenectomy.

The hepatic-venous pressure gradient (HVPG) is the gold standard for the evaluation of the presence and severity of portal hypertension[25]. A limitation of this study is that portal hypertension was diagnosed using clinical criteria, but not based on HVPG results. In addition, the mean age of our healthy controls was younger than that of patients with cirrhosis and portal hypertension. However, the diameter of the splenic artery was not significantly affected by age or gender in adults.

In conclusion, the prevalence of an abnormal S/P ratio is high in patients with cirrhosis and portal hypertension, and it can be used as an important marker of splanchnic hemodynamic disturbances in these patients. The S/P ratio is closely related to major complications of portal hypertension including ascites, esophageal and gastric varices, and collateral circulation formation. Splenectomy is effective for decreasing portal venous pressure and delaying the progress of liver cirrhosis and portal hypertension.

ACKNOWLEDGMENTS

We would like to thank Dr. Mao J for her help in writing and editing this paper.

COMMENTS

Background

The splanchnic hemodynamic disorder in portal hypertension has been studied, and the significance of splenic and hepatic artery changes in portal hypertension has been noted. In patients who have received liver transplantation, the importance of the splenic artery diameter and the splenic artery diameter/hepatic artery diameter ratio in the clinical diagnosis of splenic and hepatic arterial blood flow distribution abnormalities has been established.

Research frontiers

Splenic and hepatic arterial blood flow abnormalities are common in patients with cirrhosis and portal hypertension, but the clinical significance of the splenic artery diameter and splenic artery internal diameter/proper hepatic artery internal diameter (S/P) ratio in those patients has not been clearly elucidated.

Innovations and breakthroughs

For the first time, the authors determined the optimal cutoff values of abnormal internal diameter of the splenic artery and S/P ratio in patients with cirrhosis and portal hypertension, which were > 5.19 mm and > 1.40, respectively. A high prevalence of an abnormal S/P ratio and close associations between the S/P ratio and the major complications of portal hypertension including ascites, *esophageal* and *gastric* varices, and collateral circulation formation were found in patients with cirrhosis and portal hypertension.

Applications

The study results suggest that an abnormal internal diameter of the splenic artery and S/P ratio are of clinical significance in patients with cirrhosis and

portal hypertension, and an abnormal S/P can be used as an important marker of splanchnic hemodynamic disturbances in these patients.

Terminology

In this study, the splenic and proper hepatic artery internal diameters refer to the values measured in the arterial phase of abdominal enhanced computed tomographic (CT) scanning. The internal diameter of the proper hepatic artery was measured within 1 cm from its origin. The internal diameter of the splenic artery was measured at 3 places: within 1 cm from its origin, at the upper edge of the pancreas, and within 1 cm from the point that the splenic artery branches originate. The mean value was calculated and recorded as the internal diameter of the splenic artery. The S/P ratio was then calculated.

Peer review

It is a nice study which sought and found correlations between splenic artery internal diameter/proper hepatic artery internal diameter ratio, calculated by a non-invasive tool (3D CT scan) and clinical complications of portal hypertension. The study is original and the manuscript is well written.

REFERENCES

1 **Møller S**, Bendtsen F, Henriksen JH. Splanchnic and systemic hemodynamic derangement in decompensated cirrhosis. *Can J Gastroenterol* 2001; **15**: 94-106 [PMID: 11240379]

2 **Groszmann RJ**, Abraldes JG. Portal hypertension: from bedside to bench. *J Clin Gastroenterol* 2005; **39**: S125-S130 [PMID: 15758647 DOI: 10.1097/01.mcg.0000155552.14396.3d]

3 **Iwakiri Y**, Groszmann RJ. The hyperdynamic circulation of chronic liver diseases: from the patient to the molecule. *Hepatology* 2006; **43**: S121-S131 [PMID: 16447289 DOI: 10.1002/hep.20993]

4 **Møller S**, Henriksen JH. Cardiovascular complications of cirrhosis. *Gut* 2008; **57**: 268-278 [PMID: 18192456 DOI: 10.1136/gut.2006.112177]

5 **La Villa G**, Gentilini P. Hemodynamic alterations in liver cirrhosis. *Mol Aspects Med* 2008; **29**: 112-118 [PMID: 18177931 DOI: 10.1016/j.mam.2007.09.010]

6 **Sikuler E**, Groszmann RJ. Interaction of flow and resistance in maintenance of portal hypertension in a rat model. *Am J Physiol* 1986; **250**: G205-G212 [PMID: 3953799]

7 **Yamauchi H**, Suda Y, Yamamoto K, Sato T. Arteriographic studies of splenic and hepatic arteries in portal hypertension. *Tohoku J Exp Med* 1970; **101**: 363-374 [PMID: 5482832 DOI: 10.1620/tjem.101.363]

8 **Toni R**, Bolondi L, Gaiani S, Re G, Calabrese L, Cavalli G, Labò G. Accessory ultrasonographic findings in chronic liver disease: diameter of splenic and hepatic arteries, fasting gallbladder volume, and course of left portal vein. *J Clin Ultrasound* 1985; **13**: 611-618 [PMID: 3935674]

9 **Aoki H**, Hasumi A, Hashizume M, Kato H, Moriyasu F, Idezuki Y. Hemodynamic analysis of findings in patients with portal hypertension: multicenter analysis in Japan. Japan Portal Hypertension Study Group. *Hepatogastroenterology* 1995; **42**: 1030-1038 [PMID: 8847016]

10 **Quintini C**, Hirose K, Hashimoto K, Diago T, Aucejo F, Eghtesad B, Vogt D, Pierce G, Baker M, Kelly D, Miller CM. "Splenic artery steal syndrome" is a misnomer: the cause is portal hyperperfusion, not arterial siphon. *Liver Transpl* 2008; **14**: 374-379 [PMID: 18306381 DOI: 10.1002/lt.21386]

11 **Kirbas I**, Tutar NU, Emiroglu FK, Coskun M, Haberal M. Multidetector computed tomography angiography in detection of active bleeding in renal and liver transplant recipients. *Transplant Proc* 2007; **39**: 1111-1115 [PMID: 17524905 DOI: 10.1016/j.transproceed.2007.04.005]

12 **Lo CM**, Liu CL, Fan ST. Portal hyperperfusion injury as the cause of primary nonfunction in a small-for-size liver graft-successful treatment with splenic artery ligation. *Liver Transpl* 2003; **9**: 626-628 [PMID: 12783407 DOI: 10.1053/jlts.2003.50081]

13 **Tucker ON**, Heaton N. The 'small for size' liver syndrome. *Curr Opin Crit Care* 2005; **11**: 150-155 [PMID: 15758596 DOI: 10.1097/01.ccx.0000157080.11117.45]

14 **Dahm F**, Georgiev P, Clavien PA. Small-for-size syndrome after partial liver transplantation: definition, mechanisms of disease and clinical implications. *Am J Transplant* 2005; **5**: 2605-2610 [PMID: 16212618]

15 **Glanemann M**, Eipel C, Nussler AK, Vollmar B, Neuhaus P. Hyperperfusion syndrome in small-for-size livers. *Eur Surg Res* 2005; **37**: 335-341 [PMID: 16465057]

16 **Demetris AJ**, Kelly DM, Eghtesad B, Fontes P, Wallis Marsh J, Tom K, Tan HP, Shaw-Stiffel T, Boig L, Novelli P, Planinsic R, Fung JJ, Marcos A. Pathophysiologic observations and histopathologic recognition of the portal hyperperfusion or small-for-size syndrome. *Am J Surg Pathol* 2006; **30**: 986-993 [PMID: 16861970 DOI: 10.1097/00000478-200608000-00009]

17 **Troisi R**, Cammu G, Militerno G, De Baerdemaeker L, Decruyenaere J, Hoste E, Smeets P, Colle I, Van Vlierberghe H, Petrovic M, Voet D, Mortier E, Hesse UJ, de Hemptinne B. Modulation of portal graft inflow: a necessity in adult living-donor liver transplantation? *Ann Surg* 2003; **237**: 429-436 [PMID: 12616129 DOI: 10.1097/01.SLA.0000055277.78876.B7]

18 **Gruttadauria S**, Mandalà' L, Miraglia R, Caruso S, Minervini MI, Biondo D, Volpes R, Vizzini G, Marsh JW, Luca A, Marcos A, Gridelli B. Successful treatment of small-for-size syndrome in adult-to-adult living-related liver transplantation: single center series. *Clin Transplant* 2007; **21**: 761-766 [PMID: 17988271]

19 **Kawasaki S**, Kidokoro A, Sugiura M, Sanjo K, Idezuki Y. Effects of nonshunting operations on portal venous pressure and hepatic blood flow. *Am J Surg* 1987; **153**: 295-299 [PMID: 3826512 DOI: 10.1016/0002-9610(87)90607-6]

20 **Eipel C**, Abshagen K, Ritter J, Cantré D, Menger MD, Vollmar B. Splenectomy improves survival by increasing arterial blood supply in a rat model of reduced-size liver. *Transpl Int* 2010; **23**: 998-1007 [PMID: 20302595 DOI: 10.1111/j.1432-2277.2010.01079.x]

21 **Machálek L**, Holibková A, Tůma J, Houserková D. The size of the splenic hilus, diameter of the splenic artery and its branches in the human spleen. *Acta Univ Palacki Olomuc Fac Med* 1998; **141**: 45-48 [PMID: 9684482]

22 **Zhang Y**, Wen T, Yan L, Chen Z, Yang H, Deng X, Liang G, Li G, Zhang X, Ran S, Liao Z. The changes of hepatic hemodynamics and functional hepatic reserve after splenectomy with periesophagogastric devascularization. *Hepatogastroenterology* 2009; **56**: 835-839 [PMID: 19621712]

23 **Shi CJ**, Zhang DW, Wang CF, Tian H. Impact of devascularization on liver blood flow dynamics and hepatic function in patients with portal hypertension. *Zhonghua Putong Waike Zazhi* 1999; **3**: 185-187

24 **Mogl MT**, Nüssler NC, Presser SJ, Podrabsky P, Denecke T, Grieser C, Neuhaus P, Guckelberger O. Evolving experience with prevention and treatment of splenic artery syndrome after orthotopic liver transplantation. *Transpl Int* 2010; **23**: 831-841 [PMID: 20180930]

25 **Snowdon VK**, Guha N, Fallowfield JA. Noninvasive evaluation of portal hypertension: emerging tools and techniques. *Int J Hepatol* 2012; **2012**: 691089 [PMID: 22720166]

P- Reviewer Gruttadauria S **S- Editor** Gou SX
L- Editor Ma JY **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i8.1299

World J Gastroenterol 2013 February 28; 19(8): 1299-1305
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Ileal pouch anal anastomosis with modified double-stapled mucosectomy-the experience in China

Ya-Jie Zhang, Yi Han, Mou-Bin Lin, Yong-Gang He, Hao-Bo Zhang, Lu Yin, Liang Huang

Ya-Jie Zhang, Yi Han, Mou-Bin Lin, Yong-Gang He, Hao-Bo Zhang, Lu Yin, Department of General Surgery, RuiJin Hospital Affiliated Shanghai Jiaotong University School of Medicine, Shanghai 200025, China
Liang Huang, Department of Emergency and Trauma Surgery, RuiJin Hospital Affiliated Shanghai Jiaotong University School of Medicine, Shanghai 200025, China
Author contributions: Zhang YJ designed and analysed the data; Han Y studied conception and designed; Lin MB, Zhang HB and He YG contributed to acquisition of data; Yin L studied conception and designed, analysed and interpretated of data; Huang L studied conception and designed acquisition of data; Zhang YJ and Han Y contributed to drafting the article and final approval of the version to be published.
Correspondence to: Liang Huang, MD, Department of Emergency and Trauma Surgery, RuiJin Hospital Affiliated Shanghai Jiaotong University School of Medicine, No. 197, Ruijin No. 2 Road, Shanghai 200025, China. huangliangrj@163.com
Telephone: +86-21-64370045 Fax: +86-21-64333548
Received: August 16, 2012 Revised: October 26, 2012
Accepted: December 15, 2012
Published online: February 28, 2013

Abstract

AIM: To investigate the feasibility and long-term functional outcome of ileal pouch-anal anastomosis with modified double-stapled mucosectomy.

METHODS: From January 2002 to March 2011, fourty-five patients underwent ileal pouch anal anastomosis with modified double-stapled mucosectomy technique and the clinical data obtained for these patients were reviewed.

RESULTS: Patients with ulcerative colitis (n = 29) and familial adenomatous polyposis (n = 16) underwent ileal pouch-anal anastomosis with modified double-stapled mucosectomy. Twenty-eight patients underwent one-stage restorative proctocolectomy, ileal pouch anal anastomosis, protective ileostomy and the ileostomy was closed 4-12 mo postoperatively. Two-stage procedures were performed in seventeen urgent patients, proctectomy and ileal pouch anal anastomosis were completed after previous colectomy with ileostomy. Morbidity within the first 30 d of surgery occurred in 10 (22.2%) patients, all of them could be treated conservatively. During the median follow-up of 65 mo, mild to moderate anastomotic narrowing was occurred in 4 patients, one patient developed persistent anastomotic stricture and need surgical intervention. Thirty-five percent of patients developed at least 1 episode of pouchitis. There was no incontinence in our patients, the median functional Oresland score was 6, 3 and 2 after 1 year, 2.5 years and 5 years respectively. Nearly half patients (44.4%) reported "moderate functioning", 37.7% reported "good functioning", whereas in 17.7% of patients "poor functioning" was observed after 1 year. Five years later, 79.2% of patients with good function, 16.7% with moderate function, only 4.2% of patients with poor function.

CONCLUSION: The results of ileal pouch anal anastomosis with modified double-stapled mucosectomy technique are promising, with a low complication rate and good long-term functional results.

© 2013 Baishideng. All rights reserved.

Key words: Ileal pouch anal anastomosis; Stapled mucosectomy; Ulcerative colitis; Familial adenomatous polyposis; Surgical technique

Zhang YJ, Han Y, Lin MB, He YG, Zhang HB, Yin L, Huang L. Ileal pouch anal anastomosis with modified double-stapled mucosectomy-the experience in China. *World J Gastroenterol* 2013; 19(8): 1299-1305 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i8/1299.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i8.1299

INTRODUCTION

Ileal pouch anal anastomosis (IPAA) is the operative approach for patients requiring restorative proctocolectomy (RPC) due to familial adenomatous polyposis (FAP) and ulcerative colitis (UC). There are two types of IPAA have been performed: a handsewn technique with mucosectomy[1] and a stapling technique with the retention of the mucosa of the rectal stump[2]. Both approaches have advantages and disadvantages. For handsewn technique with mucosectomy, all colorectal mucosa is removed which is thought freeing the patients from the risk of further inflammatory disease, dysplasia or cancer in UC and reducing the risk of polyposis or cancer in FAP, but the manipulation of the anal canal and the excision of the anal transition zone (ATZ) is associated with significant impairment in anal sensation, therefore results in the risk of postoperative problems with continence[3]. For stapling technique with the retention of mucosa which is favoured as it is simpler and provides better functional results, the retention of the potentially diseased rectal mucosa exposes patients not only to disease recurrence but also to malignant degeneration[4,5].

We modify double-stapled techniques by sparing 5 cm short rectal muscular cuff and retaining the mucosa only in ATZ. The aim of this study is to evaluate whether ileal pouch-anal anastomosis with modified double-stapled mucosectomy is a safe and feasible procedure and its long-term functional outcomes.

MATERIALS AND METHODS

Between January 2002 and March 2011, 16 FAP patients and 29 UC patients received IPAA in our hospital. There were 22 males and 23 females with a mean age of 42.4 years (range: 17-67 years). The study is approved by the institutional ethics committee. The main symptoms included diarrhea, mucus or blood in the stool, abdominal pain, and weight loss. Colonoscopic examinations were performed in all patients, and biopsy results were consistent with adenomatous polyposis and ulcerative colitis. Malignant adenomatous polyps were found in three patients diagnosed with FAP.

Surgical procedure and technique

All patients underwent 2-stage procedures. For patients underwent elective surgery, we performed restorative proctocolectomy, ileal J-pouch anal anastomosis and protective ileostomy. Ileostomy was closed 4-12 mo postoperatively.

For patients underwent urgent surgery, we performed colectomy and ileostomy in first stage. Six month later, the proctectomy and ileal J-pouch anal anastomosis were completed. No protective stoma was made.

The operative technique was performed as previously described[6] and is modified. After completing mobilization of the colon, the rectum and the ileocecal region, the end of the ileum (5 cm from the ileocecal region for patient with FAP and more than 15 cm for patient with UC) was cut and closed, a J-shaped pouch with a length of 15 cm was made using a linear stapler (Ethicon, PROXIMATE® Linear Cutter 100). Then, the rectum was resected at the level above the seminal vesicle and the specimen was removed (Figure 1). The mesoileum was trimmed to mobilize the pouch and eliminate any tension on it. A 2 cm incision at the end of pouch was made, the anvil was inserted into it after creating a purse-string suture. The anus was effaced using Lone-Star Retractor, 1:100 000 adrenaline normal saline is injected under the rectal mucosa to separate it from the muscular



Figure 1 Resected specimen.

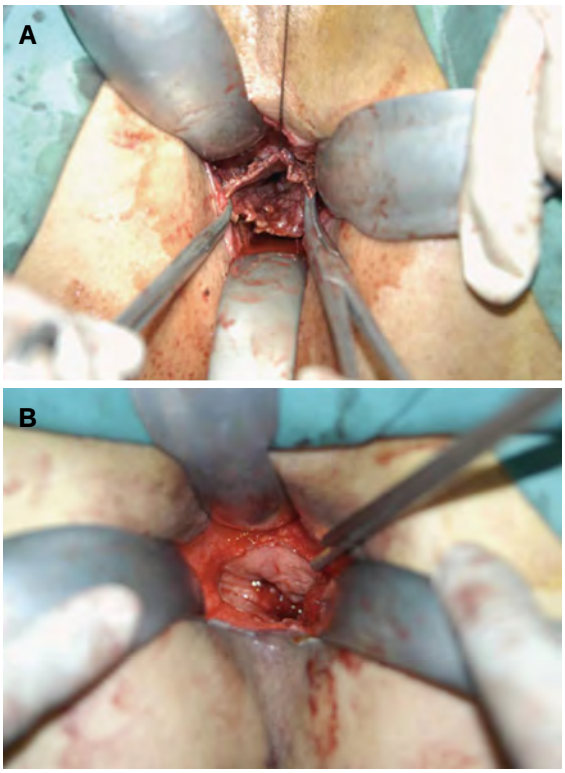


Figure 2 Surgical procedure. A: The mucosal and submucosal tube was incised; B: The end-to-end anastomosis was created by circular stapler.

Table 1 Demographic and perioperative data

Age at operation (yr) (mean ± SD)	42.4 ± 12.5
Gender (male:female)	22:23
Diagnosis (UC:FAP)	29:16:00
Median hemoglobin at the time of admission, g/L [median (range)]	85 (38-148)
Operative time, min [median (range)]	225 (195-325)
Median volume of blood transfusion, mL [median (range)]	600 (0-1600)
Days to regular diet, d [median (range)]	6 (4-14)
Length of postoperative hospitalization, d [median (range)]	12 (9-35)
Interval to closure of the ileostomy, mo [median (range)]	5 (4-12)

UC: Ulcerative colitis; FAP: Familial adenomatous polyposis.

layer. The mucosal and submucosal tube was incised by needle diathermy from 2 cm above the dentate line to the apex of rectal cuff (Figure 2A). We performed transanal purse-string suturing of the rectum 2 cm above the dentate line using 2-0 monofilaments, the J-pouch was pulled down into the muscular cuff. Then, a circular stapler was inserted transanally, and the purse-string sutures were tightened to secure the stapler in place, the anvil was attached in position and the end-to-end anastomosis was created (Figure 2B).

The postoperative anal function was assessed by Kirwan classification[7] and Pouch-specific function score (Oresland score[8], range, 0-15, with a score of 15 denoting the poorest function). The Kirwan classification was graded as full continence, incontinence of gas, occasional minor soiling, frequent major soiling and incontinence. The Oresland score included items on number of daytime and nighttime bowel movements, seepage, pad usage, urgency, diet, medication, and social handicap. Individual items were summed to form an overall pouch-specific functional score (0 being the best score, 15 the worst) which was classified as indicating good functioning (score 0-4), moderate functioning (score 5-7) and poor functioning (score 8-15)[9].

Pouchitis was diagnosed by symptoms of bloody diarrhea, urgent need to pass stool, incontinence, and pain or discomfort while passing stool[10].

Data collected included patient demographics, operative time, operative blood loss, complications, mortality, re-operation rate, length of hospital stay, readmission rates and reasons for readmission, pouchogram details, time to ileostomy closure, follow-up and long-term functional outcome.

RESULTS

The study cohort included 29 UC patients and 16 FAP patients who were treated by ileal pouch anal anastomosis with modified double-stapled mucosectomy. Twenty-eight patients underwent a RPC + IPAA and protective ileostomy in one stage, the ileostomy was closed 4-12 mo postoperatively. Seventeen patients underwent restorative proctectomy and IPAA after previous colectomy with ileostomy, all of whom were UC patients.

Demographic and operative data for the patients are presented in Table 1. The median operative time was 225 min, and in 15 patients, the operative time was more than 4 h. The median estimated blood loss was 550 mL, with a maximum estimated blood loss of 1200 mL, 36 patients requires transfusion and the median volume of blood transfusion was 600 mL. The median postoperative hospitalization was 12 d. The median interval to closure of the ileostomy was 5 mo.

There were no instances of operation-related mortality. Morbidity within the first 30 d of surgery occurred in 10 (22.2%) patients. Three patients developed an intestinal obstruction and could be treated conservatively, but one of the patient had a repeated attack of incomplete intestinal obstruction postoperatively. Two patients developed pelvic abscess and requires percutaneous puncture drainage guided by computed tomography. Six infectious complications occurred in five patients, four wound infection, one urinary tract infection and one pulmonary infection, and all could be treated with prolonged antibiotics or dressing change.

The patients were followed-up for a median period of 65 mo (range, 12-110 mo). After the closure of stoma, mild to moderate anastomotic narrowing was seen in 4 patients (8.8%), the majority being easily managed with digital dilation in the outpatient department. one patient developed persistent anastomotic stricture and require transanal endoscopic microsurgery dilatation. Sixteen patients developed at least 1 episode of pouchitis and the cumulative risk of pouchitis was 35.6%. Two patients with malignant adenomatous polyps died 2 years and 2.5 years after surgery and only 2 patients diagnosed dysplasia by biopsy in the remaining patients.

The median 24 h bowel movements was 7 evacuations per day, 4 evacuations per day and 4 evacuations per day at 1 year, 2.5 years and 5 years after closure of stoma, more than half of the patients reported more than five stools throughout the day 1 year after closure of stoma, but stool frequency decreased gradually, stabilizing at less than five evacuations per day at 2.5 years, and it was five or less in 70% of patients after 5 years. The proportion of patients that had stools every night decreased from 62.2% to 22.8% from 1 to 2.5 years, but this parameter did not improve further after 2.5 years. Urgency of defecation remained unchanged after 2.5 years although there was a tendency towards more patients being able to defer defecation for a longer time. There was no incontinence in our patients. Moreover, impaired continence, a frequent problem initially, also diminished with time, occurring in 33.3% of patients at 1 year. At 2.5 years, however, soiling or seepage occurred in only 5.7% patients during daytime and 20% patients at night, suggesting that improvement of continence can

Table 2 Stool frequency, urgency, pad usage, diet, medication in patients underwent ileal pouch-anal anastomosis at 1 year, 2.5 years and 5 years after closure of stoma *n* (%)

	Score	1 yr (*n* = 45)	2.5 yr (*n* = 35)	5 yr (*n* = 24)	*P* value[3]
24-h stool frequency					0.014
< 5	0	10 (22.2)	19 (54.2)	14 (58.3)	
5-8	1	31 (68.8)	14 (40.0)	8 (33.3)	
> 8	2	4 (8.8)	2 (5.7)	2 (8.3)	
Night time defecation					0.001
Never	0	2 (4.4)	7 (20.0)	6 (25)	
Weekly	1	15 (33.3)	20 (57.1)	13 (54.2)	
Every night	2	28 (62.2)	8 (22.8)	5 (20.8)	
Urgency of defecation[1]					0.382
> 30 min	0	25 (55.5)	25 (71.4)	17 (70.8)	
10-30 min	1	16 (35.5)	8 (22.9)	7 (29.1)	
< 10 min	2	4 (8.8)	2 (5.7)	0 (0)	
Use of pad daytime	1	6 (13.3)	2 (5.7)	1 (4.2)	0.326
Use of pad at night	1	6 (13.3)	3 (8.6)	1 (4.2)	0.454
Soiling or seepage during daytime	1	8 (17.7)	2 (5.7)	2 (8.3)	0.210
Soiling or seepage at night	1	15 (33.3)	7 (20)	5 (20.8)	0.026
Peri-anal soreness					0.989
No	0	38 (84.4)	30 (85.7)	21 (87.5)	
Occasional	1	5 (11.1)	4 (11.4)	2 (8.3)	
Permanent	2	2 (4.4)	1 (2.9)	1 (4.2)	
Dietary restrictions	1	23 (51.1)	8 (22.8)	2 (8.3)	0.001
Regular medication for stool regulation	1	20 (44.4)	6 (17.1)	1 (4.2)	0.000
Social handicap[2]	1	4 (8.8)	2 (5.7)	2 (8.3)	0.862
Functional Oresland score [median (range)]	15	6 (1-12)	3 (0-11)	2 (0-9)	

[1]Urgency of defecation is inability to defer evacuation for more than 30 min; [2]Social handicap is not able to resume full time occupation or to participate in social life; [3]χ^2 test of 45 patients.

be expected to occur even beyond one year. More than half patients mentioned dietary restrictions and 44% patients needed regular medication for stool regulation after 1 year, the rate decreased to 22.8% and 17.1% after 2.5 years and was 8.3% and 4.2% after 5 years. Less than 10% patients felt affected in their everyday life through the functional impairments after 5 years. The difference in 24-h stool frequency, night time defecation, Soiling or seepage at night, dietary restrictions and regular medication for stool regulation has statistical significance. Overall, the median functional Oresland score was 6, 3 and 2 after 1 year, 2.5 years and 5 years respectively. Nearly half patients (44.4%) reported "moderate functioning" (as described in Method), 37.7% reported "good functioning", whereas in 17.7% of patients "poor functioning" was observed after 1 year. Five years later, 79.2% of patients with good function, 4 patients (16.7%) with moderate function, only 4.2% of patients with poor function (Tables 2 and 3). Despite defects in function, patients' satisfaction was generally excellent. So far only one patients have preferred conversion to an ileostomy once more.

DISCUSSION

Restorative proctocolectomy, mucosectomy with ileal pouch-anal anastomosis has become widely accepted and is now considered the standard procedure for patients with ulcerative colitis as well as familial adenomatous polyposis since 1980s[11].

Since both UC and FAP are mucosal diseases, mucosectomy has the advantage of removing the diseased colorectal mucosa, particularly if taken down to the dentate line[12]. In both these conditions an ideal operation would remove all colonic and rectal mucosa thus exempting the patients from the risk of further inflammatory disease, dysplasia or cancer in UC and reducing the risk of polyposis or cancer in FAP. However, it has suggested that mucosectomy with a handsewn anastomosis results in poorer function, more than 50% of patients complained of seepage at night[13] and incontinence were inevitable in some of patients. Moreover, Bullard[14] looked at a 12 years long-term functional outcome using a questionnaire for 235 patients and found that most patients had stable pouch function over time, but a proportion(in 18% patients) suffered measurable deterioration particularly 12 or more years after surgery and improved function was found only in 1% patients. Without prophylactic surgical intervention, malignant transformation into carcinoma occurs in majority FAP patients in the fourth decade of life[15]. Therefore, most patients undergoing pouch surgery are younger and have a long life expectancy. Thus, long-term morbidity and functional outcome is important for their quality of life after surgery.

In order to improve the anal function and prevent incontinence, stapled anastomosis without mucosectomy which confers functional benefit compared with handsewn anastomosis and mucosectomy is performed by many surgeons, but the retained rectal mucosa is

Table 3 Kirwan classification after surgery *n* (%)

Kirwan classification	1 yr (*n* = 45)	2.5 yr (*n* = 35)	5 yr (*n* = 24)	*P* value[1]
Stage I (full continence)	30 (66.7)	28 (80)	19 (79.1)	0.785
Stage II (incontinence of gas)	5 (11.1)	3 (8.6)	2 (8.3)	
Stage III (occasional minor soiling)	9 (20)	4 (11.4)	3 (12.5)	
Stage IV (frequent major soiling)	1 (2.2)	0 (0)	0 (0)	
Stage V (incontinence)	0 (0)	0 (0)	0 (0)	

[1]χ^2 test of 45 patients.

thought a disease-bearing tissue, if left untreated, may present a significant future risks for disease recurrence or malignant transformation[16] and lifelong surveillance is required for these patients[17].

The idea of the modified double-stapled mucosectomy technique was born as a result of the aforementioned disadvantages associated with the previous techniques. The characteristics of our technique is that we retain 5 cm short rectal muscular cuff, remove great part of rectal mucosa excluding the mucosa in ATZ which is usually commenced just above the dentate line and situated a median of 1.05 cm above the lower border of the internal sphincter[18]. Because of the preservation of mucosa in ATZ which is important for anal sensation[19,20], functional results are significantly improved in our series. There was no incontinence in our patients, soiling or seepage occurred in only 5.7% patients during daytime and 20% patients at night after 2.5 years. After 5 years, 24-h stool frequency was five or less in 70% of patients and only 4.2% patients needed regular medication for stool regulation. Most patients can restore normal life and patients' satisfaction was generally excellent. So far only one patients have preferred conversion to an ileostomy once more. Furthermore, retention of 5 cm short rectal muscular cuff has the advantage of making the ileal pouch fully dilate to replace the function of rectum.

The main debate of our technique is regarding the risk of dysplasia and ongoing inflammation in the retained rectum because this method leaves behind intact mucosa of the ATZ. Although residual mucosa can remain inflamed in patients and may become dysplastic, anal transitional zone dysplasia after ileal pouch-anal anastomosis is infrequent, Remzi reported the incidence of dysplasia after stapled RPC was 3%-4.5%[21] and it had not been substantiated that preservation of ATZ lead to the development of cancer in the ATZ. We think it is a inspiring result and take the following measures to minimize the risk. Firstly, cancer risk may be reduced by ensuring that a minimal length of rectal mucosa to be retained. In carrying out our modified double-stapled IPAA, the anastomosis should be performed at a lower level just above the ATZ. Secondly, we recommended that long-term monitoring is necessary and it is easy to find lesions by digital rectal examination when the level of the anastomosis is lower. In such cases, trananal biopsy is convenient. In our study, only 2 patients (4.4%) diagnosed dysplasia by biopsy during the median follow-up period of 65 mo and no patient finds development of adenocarcinoma in the ATZ.

Our study showed a better outcome regarding pelvic-related postoperative complications, only two of the patients (4.4%) developed a peripouch-localized abscess without systemic signs of infection and could be treated by percutaneous puncture drainage which are better than previously reported rates of pelvis sepsis following pouch surgery[22,23]. Thus, we speculate that the retention of rectal muscular cuff has no relation with the pelvic-related infectious complications and retention of short rectal muscular cuff (only 5 cm) can reduce the operative time and the possibility of bleeding. Furthermore, we don't suture the J-pouch with the rectal cuff and the tissue space was blocked out by fibrin glue which can protect against pelvic sepsis, if there is anastomotic leakage, symptoms will be minimized.

On the other hand, the choice of IPAA technique should be based on the situation of patients to make a comprehensive consideration for both oncologic and functional outcome. When the patients at high risk of cuff dysplasia or inflammation, we think it is better to perform a handsewn technique with mucosectomy rather than attempting stapling technique with the retention of partial mucosa of the rectal stump in order to avoid carcinoma formation after IPAA.

Several limitations of our study should be noted. The design was retrospective and our modified technique was not compared with the handsewn technique or the standard stapling technique of the same surgeon. Another study limitation is the small number of patients. Despite being a modified technique of IPAA, small patient numbers precluded robust analysis of the association between clinical and postoperative outcome.

In conclusion, ileal pouch-anal anastomosis with modified double-stapled mucosectomy seems to be a technically feasible and clinically acceptable alternative procedure for the removal of potentially diseased rectal mucosa retained only in ATZ to achieve an improved anal function after surgery.

COMMENTS

Background

Ileal pouch anal anastomosis (IPAA) is the operative approach for patients requiring restorative proctocolectomy due to familial adenomatous polyposis and ulcerative colitis. But the conventional handsewn technique or stapling

technique have their disadvantages such as poor postoperative anal function or disease recurrence. Modify double-stapled techniques by sparing 5 cm short rectal muscular cuff and retaining the mucosa only in anal transition zone is designed to ameliorate aforementioned disadvantages and improve the clinical effects.

Research frontiers

Handsewn technique with mucosectomy and stapling technique with the retention of the mucosa of the rectal stump are the routine methods of IPAA. In the area of improving anal function and reducing the risk of disease recurrence, the research hotspot is to deeply understand the anatomy of anus and modify the surgical technique.

Innovations and breakthroughs

In the previous reports of IPAA, the most applicable methods are handsewn technique with mucosectomy and stapling technique with the retention of the mucosa of the rectal stump. The authors modify double-stapled techniques by sparing 5 cm short rectal muscular cuff and retaining the mucosa only in anal transition zone. This is the first study to report the long-term functional outcomes with this technique in China. Furthermore, the results of the modified technique are promising, with a low complication rate and good long-term anal function.

Applications

The study results suggest that ileal pouch anal anastomosis with modified double-stapled mucosectomy is a feasible surgical technique for patients with familial adenomatous polyposis and ulcerative colitis.

Terminology

Ileal pouch-anal anastomosis: IPAA is constructed for people who have had their large intestine surgically removed due to disease or injury. It is formed by folding loops of small intestine (the ileum) back on themselves and stitching or stapling them together. The internal walls are then removed thus forming a reservoir. The reservoir is then stitched or stapled with the anal mucosa. Familial adenomatous polyposis: Familial adenomatous polyposis is an inherited condition in which numerous polyps form mainly in the epithelium of the large intestine. While these polyps start out benign, malignant transformation into colon cancer occurs when not treated; Ulcerative colitis: Ulcerative colitis is a form of inflammatory bowel disease, a disease of the colon, that includes characteristic ulcers, or open sores. The main symptom of active disease is usually constant diarrhea mixed with blood, of gradual onset; Anal transition zone: Anal transition zone is usually commenced just above the dentate line and situated a median of 1.05 cm above the lower border of the internal sphincter. It is important for anal sensation.

Peer review

Interesting paper, some fine tuning is needed but certainly adds to the knowledge.

REFERENCES

1 **Parks AG**, Nicholls RJ. Proctocolectomy without ileostomy for ulcerative colitis. *Br Med J* 1978; **2**: 85-88 [PMID: 667572 DOI: 10.1136/bmj.2.6130.85]

2 **Heald RJ**, Allen DR. Stapled ileo-anal anastomosis: a technique to avoid mucosal proctectomy in the ileal pouch operation. *Br J Surg* 1986; **73**: 571-572 [PMID: 3730791 DOI: 10.1002/bjs.1800730719]

3 **Saigusa N**, Kurahashi T, Nakamura T, Sugimura H, Baba S, Konno H, Nakamura S. Functional outcome of stapled ileal pouch-anal canal anastomosis versus handsewn pouch-anal anastomosis. *Surg Today* 2000; **30**: 575-581 [PMID: 10930221 DOI: 10.1007/s005950070095]

4 **Silvestri MT**, Hurst RD, Rubin MA, Michelassi F, Fichera A. Chronic inflammatory changes in the anal transition zone after stapled ileal pouch-anal anastomosis: is mucosectomy a superior alternative? *Surgery* 2008; **144**: 533-537; discussion 533-537 [PMID: 18847636 DOI: 10.1016/j.surg.2008.06.003]

5 **Lovegrove RE**, Constantinides VA, Heriot AG, Athanasiou T, Darzi A, Remzi FH, Nicholls RJ, Fazio VW, Tekkis PP. A comparison of hand-sewn versus stapled ileal pouch anal anastomosis (IPAA) following proctocolectomy: a meta-analysis of 4183 patients. *Ann Surg* 2006; **244**: 18-26 [PMID: 16794385 DOI: 10.1097/01.sla.0000225031.15405.a3]

6 **Kmiot WA**, Keighley MR. Totally stapled abdominal restorative proctocolectomy. *Br J Surg* 1989; **76**: 961-964 [PMID: 2804600 DOI: 10.1002/bjs.1800760931]

7 **Vernava AM**, Robbins PL, Brabbee GW. Restorative resection: coloanal anastomosis for benign and malignant disease. *Dis Colon Rectum* 1989; **32**: 690-693 [PMID: 2752856 DOI: 10.1007/BF02555775]

8 **Oresland T**, Fasth S, Nordgren S, Hultén L. The clinical and functional outcome after restorative proctocolectomy. A prospective study in 100 patients. *Int J Colorectal Dis* 1989; **4**: 50-56 [PMID: 2708883 DOI: 10.1007/BF01648551]

9 **Berndtsson IE**, Carlsson EK, Persson EI, Lindholm EA. Long-term adjustment to living with an ileal pouch-anal anastomosis. *Dis Colon Rectum* 2011; **54**: 193-199 [PMID: 21228668 DOI: 10.1007/DCR.0b013e3181ff42d8]

10 **Cheifetz A**, Itzkowitz S. The diagnosis and treatment of pouchitis in inflammatory bowel disease. *J Clin Gastroenterol* 2004; **38**: S44-S50 [PMID: 15115932 DOI: 10.1097/01.mcg.0000124001.93146.ef]

11 **Biondi A**, Zoccali M, Costa S, Troci A, Contessini-Avesani E, Fichera A. Surgical treatment of ulcerative colitis in the biologic therapy era. *World J Gastroenterol* 2012; **18**: 1861-1870 [PMID: 22563165 DOI: 10.3748/wjg.v18.i16.1861]

12 **Régimbeau JM**, Panis Y, Pocard M, Hautefeuille P, Valleur P. Handsewn ileal pouch-anal anastomosis on the dentate line after total proctectomy: technique to avoid incomplete mucosectomy and the need for long-term follow-up of the anal transition zone. *Dis Colon Rectum* 2001; **44**: 43-50; discussion 50-1 [PMID: 11805562 DOI: 10.1007/BF02234819]

13 **Taylor BA**, Wolff BG, Dozois RR, Kelly KA, Pemberton JH, Beart RW. Ileal pouch-anal anastomosis for chronic ulcerative colitis and familial polyposis coli complicated by adenocarcinoma. *Dis Colon Rectum* 1988; **31**: 358-362 [PMID: 2835217 DOI: 10.1007/BF02564884]

14 **Bullard KM**, Madoff RD, Gemlo BT. Is ileoanal pouch function stable with time? Results of a prospective audit. *Dis Colon Rectum* 2002; **45**: 299-304 [PMID: 12068184 DOI: 10.1007/s10350-004-6171-7]

15 **Wolf ND**, Kadmon M, Wolf RC, Brechtel A, Keller M. Quality of life after restorative proctocolectomy and ileal pouch-anal anastomosis in patients with familial adenomatous polyposis: a matter of adjustment. *Colorectal Dis* 2011; **13**: e358-e365 [PMID: 21801297 DOI: 10.1111/j.1463-1318.2011.02737.x]

16 **Litzendorf ME**, Stucchi AF, Wishnia S, Lightner A, Becker JM. Completion mucosectomy for retained rectal mucosa following restorative proctocolectomy with double-stapled ileal pouch-anal anastomosis. *J Gastrointest Surg* 2010; **14**: 562-569 [PMID: 19937191 DOI: 10.1007/s11605-009-1099-9]

17 **Vrouenraets BC**, Van Duijvendijk P, Bemelman WA, Offerhaus GJ, Slors JF. Adenocarcinoma in the anal canal after ileal pouch-anal anastomosis for familial adenomatous polyposis using a double-stapled technique: report of two cases. *Dis Colon Rectum* 2004; **47**: 530-534 [PMID: 14978621 DOI: 10.1007/s10350-003-0073-y]

18 **Thompson-Fawcett MW**, Warren BF, Mortensen NJ. A new look at the anal transitional zone with reference to restorative proctocolectomy and the columnar cuff. *Br J Surg* 1998; **85**: 1517-1521 [PMID: 9823914 DOI: 10.1046/j.1365-2168.1998.00875.x]

19 **Miller R**, Lewis GT, Bartolo DC, Cervero F, Mortensen NJ. Sensory discrimination and dynamic activity in the anorectum: evidence using a new ambulatory technique. *Br J Surg* 1988; **75**: 1003-1007 [PMID: 3219524 DOI: 10.1002/bjs.1800751018]

20 **Holder-Murray J**, Fichera A. Anal transition zone in the surgical management of ulcerative colitis. *World J Gastroenterol* 2009; **15**: 769-773 [PMID: 19230038 DOI: 10.3748/wjg.15.769]

21 **Remzi FH**, Fazio VW, Delaney CP, Preen M, Ormsby A, Bast J, O'Riordain MG, Strong SA, Church JM, Petras RE, Gramlich T, Lavery IC. Dysplasia of the anal transitional zone after ileal pouch-anal anastomosis: results of prospective evaluation after a minimum of ten years. *Dis Colon Rectum* 2003; **46**: 6-13 [PMID: 12544515 DOI: 10.1007/s10350-004-6488-2]

22 **Fukushima T**, Sugita A, Koganei K, Shinozaki M. The incidence and outcome of pelvic sepsis following handsewn and stapled ileal pouch anal anastomoses. *Surg Today* 2000; **30**: 223-227 [PMID: 10752773 DOI: 10.1007/s005950050049]

23 **Hueting WE**, Buskens E, van der Tweel I, Gooszen HG, van Laarhoven CJ. Results and complications after ileal pouch anal anastomosis: a meta-analysis of 43 observational studies comprising 9,317 patients. *Dig Surg* 2005; **22**: 69-79 [PMID: 15838175 DOI: 10.1159/000085356]

P- Reviewer Lakatos PL **S- Editor** Gou SX **L- Editor** A
E- Editor Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i8.1306

World J Gastroenterol 2013 February 28; 19(8): 1306-1313
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

BRIEF ARTICLE

Tumor suppressor function of ezrin-radixin-moesin-binding phosphoprotein-50 through β-catenin/E-cadherin pathway in human hepatocellular cancer

Xiu-Lan Peng, Meng-Yao Ji, Zi-Rong Yang, Jia Song, Wei-Guo Dong

Xiu-Lan Peng, Meng-Yao Ji, Zi-Rong Yang, Jia Song, Wei-Guo Dong, Department of Gastroenterology, Renmin Hospital of Wuhan University, Wuhan 430060, Hubei Province, China
Author contributions: Peng XL and Dong WG contributed equally to this work; Peng XL, Ji MY, Yang ZR and Song J designed research; Peng XL, Ji MY, Yang ZR and Song J performed research; Yang ZR, Peng XL and Song J analyzed data; Peng XL and Dong WG wrote the paper.
Supported by Fundamental Research Funds for the Central Universities of China, No. 201130202020005
Correspondence to: Wei-Guo Dong, MD, Department of Gastroenterology, Renmin Hospital of Wuhan University, 238 Jiefang Road, Wuhan 430060, Hubei Province, China. dongwg66@163.com
Telephone: +86-27-88041911 Fax: +86-27-88041911
Received: November 9, 2012 Revised: January 1, 2013
Accepted: January 23, 2013
Published online: February 28, 2013

Abstract

AIM: To determine the effect and molecular mechanism of ezrin-radixin-moesin-binding phosphoprotein-50 (EBP50) in hepatocellular carcinoma (HCC).

METHODS: Three human HCC cell lines, *i.e.*, SMMC7721, HepG2 and Hep3B, were used. We transfected the Pbk-CMV-HA-EBP50 plasmid into SMMC7721 cells with Lipofectamine 2000 to overexpress EBP50. Western blotting were performed to determine the effects of the plasmid on EBP50 expression and to detect the expression of β-catenin and E-cadherin before and after the transfection of the plasmid into SMMC7721 cells. *In vitro* cell proliferation was assessed with a Cell Counting Kit-8 (CCK-8) assay. Cell cycle distribution was assessed with flow cytometry. Invasion and migration ability of before and after the transfection were determined with a transwell assay. Cell apoptosis was demonstrated with Annexin V-FITC. The effect of EBP50 overexpressing on tumor growth *in vivo* was performed with a xenograft tumor model in nude mice.

RESULTS: The transfection efficiency was confirmed with Western blotting (1.36 ± 0.07 *vs* 0.81 ± 0.09, $P < 0.01$). The CCK8 assay demonstrated that the growth of cells overexpressing EBP50 was significantly lower than control cells ($P < 0.01$). Cell cycle distribution showed there was a G0/G1 cell cycle arrest in cells overexpressing EBP50 (61.3% ± 3.1% *vs* 54.0% ± 2.4%, $P < 0.05$). The transwell assay showed that cell invasion and migration were significantly inhibited in cells overexpressing EBP50 compared with control cells (5.8 ± 0.8 *vs* 21.6 ± 1.3, $P < 0.01$). Annexin V-FITC revealed that apoptosis was significantly increased in cells overexpressing EBP50 compared with control cells (14.8% ± 2.7% *vs* 3.4% ± 1.3%, $P < 0.05$). The expression of β-catenin was downregulated and E-cadherin was upregulated in cells overexpressing EBP50 compared with control cells (0.28 ± 0.07 *vs* 0.56 ± 0.12, $P < 0.05$; 0.55 ± 0.08 *vs* 0.39 ± 0.07, $P < 0.05$). *In vivo* tumor growth assay confirmed that up-regulation of EBP50 could obviously slow the growth of HCC derived from SMMC7721 cells (28.9 ± 7.2 *vs* 70.1 ± 7.2, $P < 0.01$).

CONCLUSION: The overexpression of EBP50 could inhibit the growth of SMMC7721 cells and promote apoptosis by modulating β-catenin, E-cadherin. EBP50 may serve asa potential therapeutic target in HCC.

© 2013 Baishideng. All rights reserved.

Key words: Hepatocellular carcinoma; Ezrin-radixin-moesin-binding phosphoprotein-50; Growth; Migration; Invasion

Peng XL, Ji MY, Yang ZR, Song J, Dong WG. Tumor suppressor function of ezrin-radixin-moesin-binding phosphoprotein-50 through β-catenin/E-cadherin pathway in human hepatocellular

cancer. *World J Gastroenterol* 2013; 19(8): 1306-1313 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i8/1306.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i8.1306

INTRODUCTION

Hepatocellular carcinoma (HCC) is the third most common cause of mortality due to cancer in the world[1]. The low rate of early diagnosis renders the tumors unresectable and results in metastasis to the lymph nodes or distant organs. In addition, HCC cells are relatively resistant to chemotherapy; thus, the overall survival rate is poor. Recently, targeted molecular therapy has been shown to be effective in treating HCC, which encouraged us to study the cellular and molecular mechanisms of HCC and identify more effective treatments.

The etiology of hepatocarcinogenesis is still not fully understood, the activation of oncogenes, dysfunction of tumor suppressor genes, and aberrant activation of signal transduction pathways have been described. β-catenin is a key effector molecule in the Wnt signaling pathway and also plays an important role in cell-cell adhesion[2,3]. E-cadherin is a member of the cadherin family and is a classical type I cadherin. Both β-catenin and E-cadherin are membrane-associated proteins that are essential for regulating and providing cellular adhesion. Membrane-associated adhesion proteins regulate cell motility, including metastasis and invasion, and they link the extracellular matrix and actin cytoskeleton, which is critical in many biological signal transduction pathways. β-catenin and E-cadherin dysfunction has been described in several tumors, such as breast[4], prostate[5], gastric[6], colorectal[7], lung[8], oral[9] and hepatocellular carcinoma[10].

Ezrin-radixin-moesin-binding phosphoprotein-50 (EBP50, also known as NHERF1) is a 55-kDa phosphoprotein, and it belongs to the family of PDZ scaffolding proteins. Previous studies suggest that EBP50 is a potential tumor suppressor in several types of tumors[11-15]. Our previous studies also showed that EBP50 can inhibit the growth of gastric and pancreatic cancer cells[16,17]. EBP50 is mainly localized at the apical plasma membrane in human epithelial tissues[18]. The loss of normal apical membrane expression and/or distribution to the cytoplasm and nuclear overexpression was found in several human cancers[12-15,18]. Shibata *et al*[19] found that 45% of HCC cases expressed high levels of EBP50 mRNA, and 55% of HCC cases showed no difference. The cytoplasmic and nuclear accumulation of the EBP50 protein was present in 55% of HCC tissues compared with the surrounding noncancerous liver tissue, and EBP50 interacts with β-catenin to participate in hepatocarcinogenesis. The role of EBP50 as an oncogene or a tumor suppressor in HCC remains unclear.

In this study, we examined the effects of EBP50 upregulation on cell growth, migration, invasion, and apoptosis in the HCC cell line SMMC7721 and determined the expression of β-catenin and E-cadherin to elucidate the role of EBP50 in HCC.

MATERIALS AND METHODS

Cell lines and plasmids

The human HCC cell lines Hep3B, SMMC7721, HepG2 were obtained from the Cell Bank of Shanghai Institute for Biological Sciences (Shanghai, China). Of the three cells lines, the SMMC7721 cell line has relatively low EBP50 protein expression levels. These cells were cultured in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum at 37 ℃ in a humidified atmosphere of 5% CO_2. The pBK-CMV-HA-EBP50 plasmid was kindly provided by Dr. Randy Hall from Emory University (Atlanta, GA, United States).

Stable transfection

The pBK-CMV-HA-EBP50 plasmid or the pBK-CMV-HA vector was transfected into SMMC7721 cells with Lipofectamine^{TM} 2000 (Invitrogen, CA, United States) according to the manufacturer's protocol. G418 (350 μg/mL) was used to select stably transfected cells. Single-cell clones were isolated for clonal expansion. The transfection efficiency was determined with Western blotting. Subsequently, stably transfected cell clones were cultured and amplified in culture medium supplemented with 350 μg/mL G418.

Cell proliferation assay

The cell-counting kit-8 (CCK-8, Dojindo, Japan) colorimetric assay was used to measure cell proliferation and viability with triplicate experiments for each set of conditions. We seeded 5×10^3 cells per well in a 96-well plate. Cell viability was assessed using the Cell Counting Kit after culture for 24 h. The absorbance at 450 nm wasmeasured with a plate reader.

Western blotting

Western blotting was performed as described previously[20]. The specific antibodies were rabbit anti-EBP50 antibody (1:1000, Abcam; Pierce, GA, United States), rabbit anti-β-catenin antibody (1:1000, Abcam), mouse anti-E-cadherin antibody (1:1000, Abcam), and mouse anti-β-actin antibody (1:1000, Abcam).

Transwell assay

Invasion through a Matrigel-coated filter was measured in transwell chambers (Corning, New York, NY, United States) as described previously[14]. Cells (1×10^4) were trypsinized and suspended in the upper chambers. After incubation for 24 h, the transwell chambers were fixed with 4% paraformaldehyde and stained with Giemsa (Sigma). Cells in five randomly selected fields were photographed, and the number of trans-membrane cells wascounted.

Cell cycle assay

Cells were trypsinized and fixed in 70% ethanol on

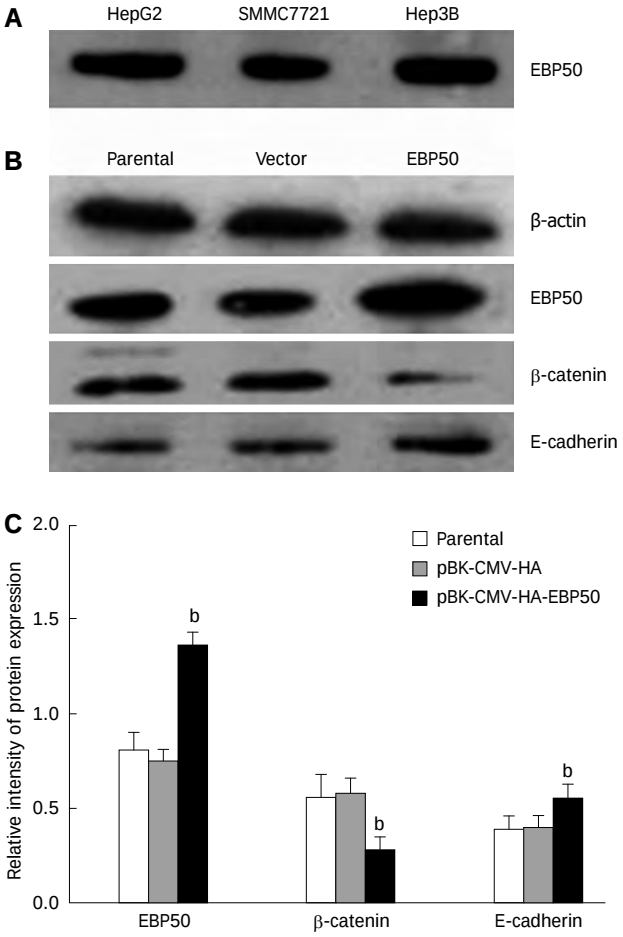


Figure 1 Ezrin-radixin-moesin-binding phosphoprotein-50, β-catenin, and E-cadherinprotein levels were detected with Western blotting. A: The expression of ezrin-radixin-moesin-binding phosphoprotein-50 (EBP50) protein in Hep3B, SMMC7721, HepG2; B: Western blotting analysis of EBP50, β-catenin, and E-cadherinprotein protein before and after transfection; C: Analysis of the expression of proteins. $^{b}P < 0.01$ *vs* parental or pBK-CMV-HA.

ice and then stained with 50 μg/mL propidium iodide (Sigma) and 0.1 μg/mL RNase A (Sigma). The cells were then analyzed with flow cytometry using a FACStar Plus, and Cell Quest software was used to calculate the percentage of the cell population in each phase.

Cellular apoptosis assay

Apoptosis was assessed with the Annexin V-FITC kit according to the manufacturer's protocol. A total of 1×10^6 cells/mL were washed twice with cold PBS and resuspended in binding buffer. A total of 5 mL of Annexin V-FITC and 10 mL of propidium iodide (PI) were added (BioVision, Mountain New, CA, United States), and the cells were incubated for 10 min at room temperature in the dark. At the end of the incubation, 200 μL of binding buffer was added, and the cells were immediately analyzed with flow cytometry. Cell Quest software was used to analyze flow cytometry data.

Ex vivo tumor growth assay

Male nude mice (4-6 wk old, $n = 6$) were (Beijing HFK Biosciencs Co., Ltd) used in the experiments. After alcohol preparation of the skin, 1×10^6 SMMC cells (pBK-CMV-HA-EBP50, pBK-CMV-HA vector and control cells), suspended in 100 μL PBS, were subcutaneously inoculated into the dorsal area of the nude mice. Six weeks after injection, At the end of the experiment, tumors were harvested and weighed. All experiments were performed according to the recommendations of the Institutional Animal Care and Use Committee, and the study protocol was approved by the Ethics Committee for Animal Research of Wuhan University, China.

Statistical analysis

The results were analyzed using SPSS 13.0 statistical software. All of the data are presented as the means ± SD. Statistically significant differences between groups in each assay were compared with a one-way analysis of variance (ANOVA), and $P < 0.05$ was considered to be statistically significant.

RESULTS

EBP50 expression in HCC cells

We used Western blotting to evaluate the expression of EBP50 in human HCC cell lines, with SMMC7721 expressing the lowest of the three cell lines (Figure 1). To investigate the role of EBP50 in HCC, we overexpressed EBP50 in SMMC7721 cells, which expressed relatively low levels of endogenous EBP50. SMMC7721 cells were transfected with pBK-CMV-HA-EBP50 or the pBK-CMV-HA vector to establish vector control cells. To evaluate the transfection efficiency, we determined the protein expression of EBP50 with Western blotting (Figure 1). The data showed that after transfection for 48 h, the protein levels of EBP50 were upregulated in pBK-CMV-HA-EBP50-transfected cells compared with pBK-CMV-HA vector-transfected cells and parental cells (1.36 ± 0.07 *vs* 0.81 ± 0.09 or 0.75 ± 0.06, $P < 0.01$).

Expression of β-catenin and E-cadherin in HCC cells

To investigate the biological effect of EBP50 in HCC and the potential signaling pathway through which it acts, we analyzed the expression of β-catenin and E-cadherin in pBK-CMV-HA-EBP50-and pBK-CMV-HA-transfected cells and untransfected cells. The protein levels of β-catenin was downregulated (0.28 ± 0.07 *vs* 0.56 ± 0.12 or 0.58 ± 0.08, $P < 0.05$) and E-cadherin (0.55 ± 0.08 *vs* 0.39 ± 0.07 or 0.40 ± 0.06, $P < 0.05$) was upregulated in pBK-CMV-HA-EBP50-transfected cells (Figure 1). The expression level was the same as previously reported by Hayashi *et al*[12].

Overexpression of EBP50 suppresses cancer cell growth

To assess the potential effects of EBP50 overexpression on cell proliferation and survival, we measured the number of viable cells at different times in vitro with CCK-8 assays. The pBK-CMV-HA vector had no effect on the proliferative ability of SMMC7721 cells, whereas pBK-

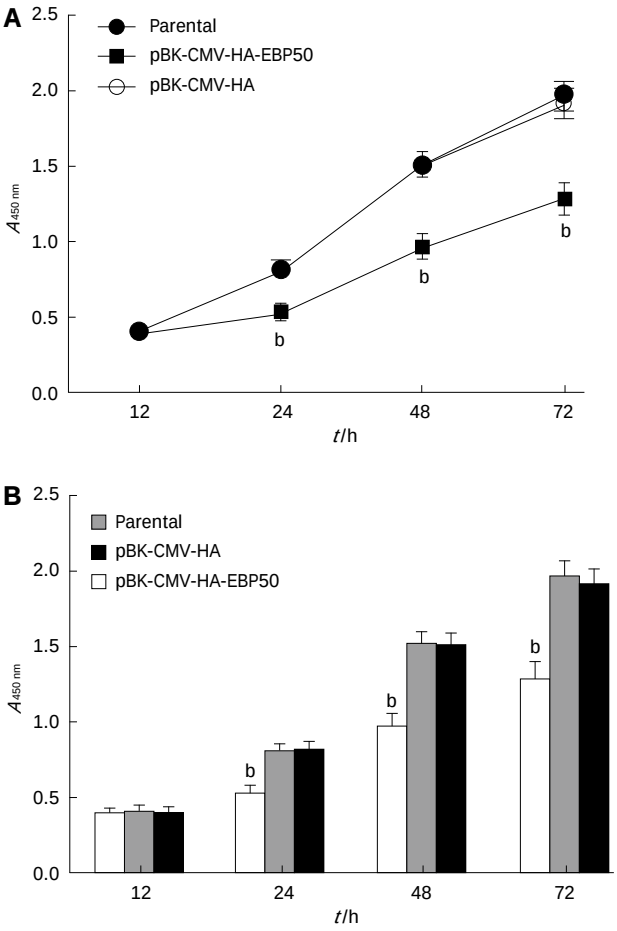


Figure 2 Growth rate comparison of parental, pBK-CMV-HA-EBP50, pBK-CMV-HA cells with a Cell Counting Kit-8 assay. A: Growth rate comparison of parental, pBK-CMV-HA-EBP50, pBK-CMV-HA cells; B: Analysis of the growth rate. $^{b}P < 0.01$ *vs* parental or pBK-CMV-HA.

CMV-HA-EBP50-transfected cells showed a dramatic reduction in proliferation ($P < 0.01$, Figure 2).

The cell cycle distribution was measured to determine whether the growth-inhibitory activity of EBP50 on SMMC7721 cell proliferation was due to cell cycle alterations. Compared with the control cells, the percentage of pBK-CMV-HA-EBP50 cells in G0/G1 phase was increased to 61.3% ± 3.1% from 54.0% ± 2.4% in parental cells and 54.5% ± 1.9% in mock cells ($P < 0.05$, Figure 3).

Overexpression of EBP50 suppresses cancer cell migration and invasion

We explored whether the over-expression of EBP50 resulted in decreased invasion and migration in cancer cells with a transwell assay. As shown in Figure 4A-D, the number of migrated pBK-CMV-HA-EBP50 cells (5.8 ± 0.8 cells/HP) was lower than the pBK-CMV-HA vector cells (20.4 ± 1.1 cells/HP) and parental cells (21.6 ± 1.3 cells/HP) ($P < 0.01$), which indicates that EBP50 could significantly suppress the invasive ability of SMMC7721 cells. No significant difference was found between SMMC7721 parental cells and the pBK-CMV-HA vector cells. The result showed that upregulating EBP50 expression in SMMC7721 cells inhibited tumor cell invasion and migration.

Overexpression of EBP50 promotes cell apoptosis

Apoptosis was investigated with an annexin V/propidium iodide assay. The pBK-CMV-HA-EBP50 cells showed an approximately fourfold increase in apoptosis ($P < 0.05$) compared with the parental group and the pBK-CMV-HA vector group (Figure 4E), suggesting that the pBK-CMV-HA-EBP50 plasmid enhances SMMC7721 apoptosis.

Overexpression of EBP50 decreased the growth of the tumor ex vivo

The cell growth response as result of NHERF1 overexpression was also examined in the nude mice model. We compared the growth of SMMC7721 cells infected with pBK-CMV-HA-EBP50, pBK-CMV-HA vector and control cells *ex vivo*. At 6 wk after inoculation, obvious tumors were formed in the two control groups of mice inoculated with SMMC7721 cells or the pBK-CMV-HA vector, while tumor growth was obviously suppressed in mice inoculated with pBK-CMV-HA-EBP50 cells. As shown in Figure 5, tumors formed from pBK-CMV-HA-EBP50 (28.9 ± 7.2 mg) were significantly smaller than those from pBK-CMV-HA and control cells (68.9 ± 7.9 mg, 70.1 ± 7.2 mg, $n = 6$) ($P < 0.01$), suggesting that expression of EBP50 suppressed tumor growth *ex vivo*.

DISCUSSION

In the present study, to determine the role of EBP50 in HCC as an oncogene or a tumor suppressor, we used the pBK-CMV-HA-EBP50 plasmid to increase the expression of EBP50 in SMMC7721 cells. Western blotting analysis proved that EBP50 protein levels were significantly upregulated in pBK-CMV-HA-EBP50-transfected SMMC7721 cells. The proliferation rate of cells over-expressing EBP50 was lower than the pBK-CMV-HA vector and parental SMMC7721 cells (Figure 2). In a transwell assay, the number of migrated cells over-expressing EBP50 was significantly lower than control cells, indicating that the over-expression of EBP50 can inhibit the anchorage-independent growth of HCC cells (Figure 4). Cellular apoptosis analysis showed that EBP50 overexpression could increase apoptosis and induce G0/G1 cell cycle arrest in HCC cells (Figures 3 and 4). *Ex vivo* tumor growth assay confirmed that cells overexpressing EBP50 could obviously slow the growth of HCC cells (Figure 5). Zheng *et al*[11] reported that EBP50 overexpression could inhibit breast cancer cell proliferation and promote cell apoptosis through decreasing ERK activation. The current study also suggests EBP50 as a potential tumor suppressor in HCC.

The molecular mechanisms through which EBP50 exerts its tumor suppressor functions are diverse. EBP50 contains two PDZ domains and an ERM domain. PDZ domains have been implicated in protein-protein interac-

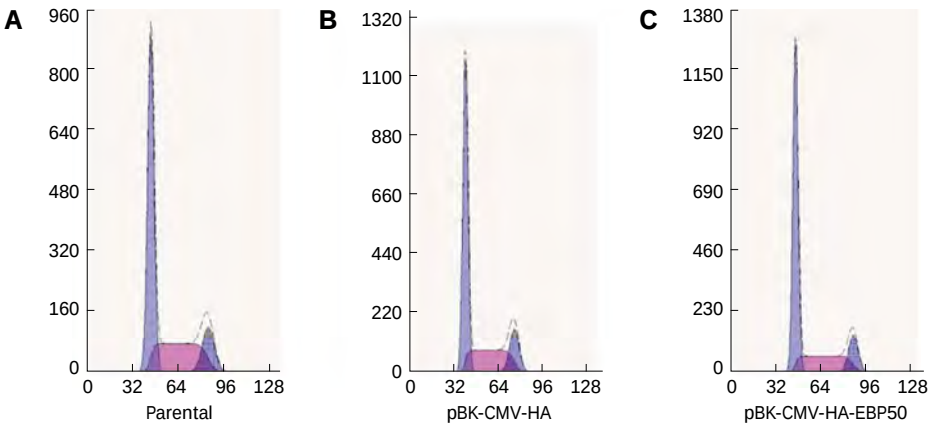


Figure 3 Effect of ezrin-radixin-moesin-binding phosphoprotein-50 over-expression on cell cycle distribution. A: Cell cycle analysis of parental (G0/G1, 54.2%); B: pBK-CMV-HA (G0/G1, 56.2%); C: pBK-CMV-HA-EBP50 (G0/G1, 61.4%) cells with flowcytometry.

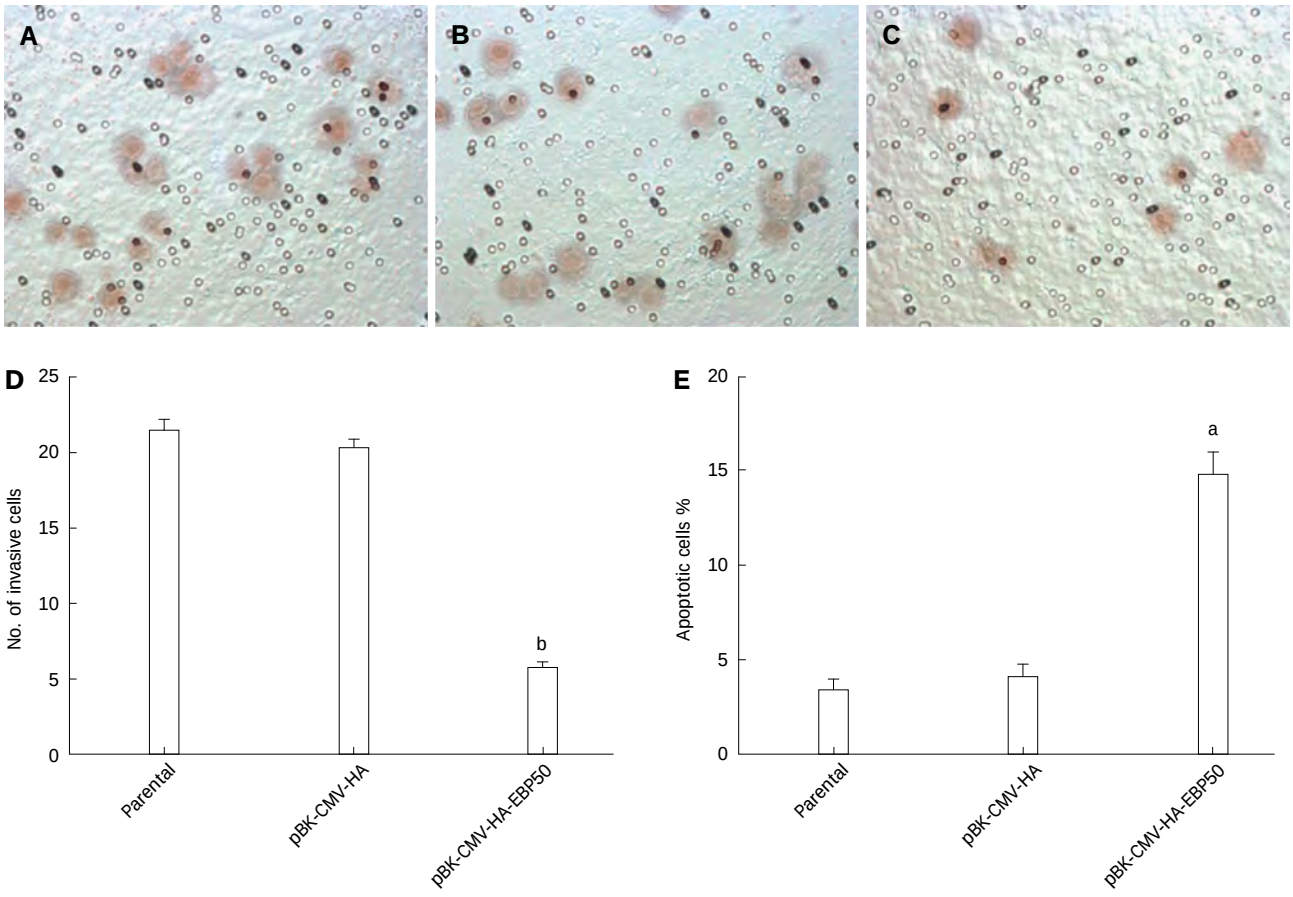


Figure 4 Ezrin-radixin-moesin-binding phosphoprotein-50 over-expression suppressed the migration and invasion of cancer cells. A: Migration and invasion analysis of parental (21.6 ± 1.3 cells/HP); B: pBK-CMV-HA (20.4 ± 1.1 cells/HP); C: pBK-CMV-HA-EBP50 (5.8 ± 0.8 cells/HP) cells with transwell assey; D: Analysis of the number of invasive cells; E: Apoptotic analysis of parental (3.4% ± 1.3%), pBK-CMV-HA (4.1% ± 1.5%) and pBK-CMV-HA-EBP50 (14.8% ± 2.7%) cells with flowcytometry. $^{a}P < 0.05$, $^{b}P < 0.01$ *vs* parental or pBK-CMV-HA.

tions, and the ERM domain binds to ezrin through its ERM-binding region[21]. In normal cells, EBP50 interact with cyclin dependent kinase and PP2A through its reversible phosphorylation of the protein to regulate cell division[22]. EBP50 interacts with many tumor-related proteins through these domains, including PTEN, PDGFR, EGFR, podocalyxin,and GPCRs, to participate in tumorigenesis[23-29]. EBP50 forms a complex with beta-catenin to promote Wnt signaling and may participate in the development of HCC[19].

Both β-catenin and E-cadherin play an essential role in cell-cell junctions in Wnt signal pathway. β-catenin binds directly to the cytoplasmic tail of E-cadherin to form a bridge between E-cadherin and the actin cyto-

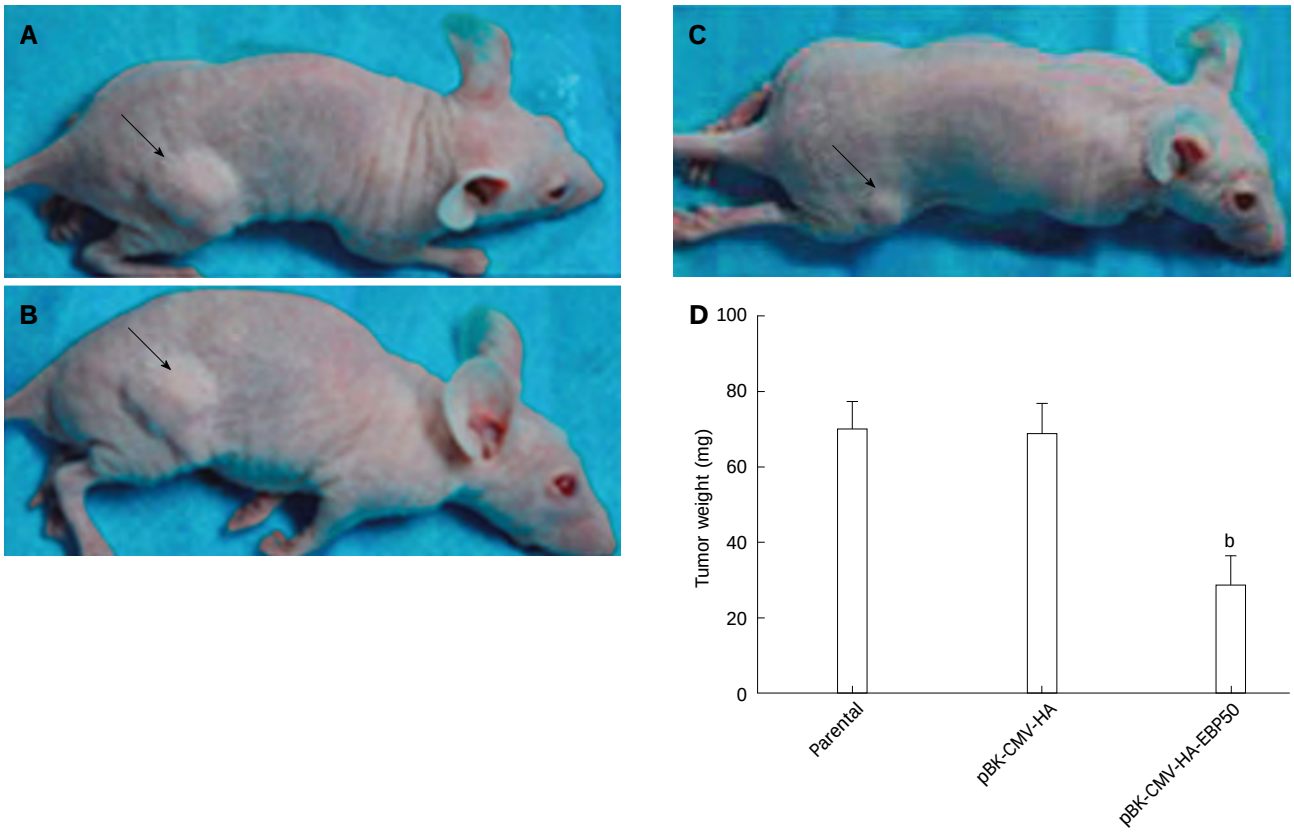


Figure 5 Tumorigenicity was inhibited by overexpression of ezrin-radixin-moesin-binding phosphoprotein-50 *ex vivo* (arrow). A: Tumor weight in nude mice of parental (70.1 ± 7.2 mg); B: pBK-CMV-HA (68.9 ± 7.9 mg); C: pBK-CMV-HA-EBP50 (28.9 ± 7.2 mg); D: Tumor weight of the nude mice in each group. $^{b}P < 0.01$ *vs* parental or pBK-CMV-HA.

skeleton and stabilizes adherens junctions[30]. The plasma membrane localization of β-catenin allows it to function as a tumor suppressor by interacting with E-cadherin, and the phosphorylation of β-catenin results in its accumulation in the cytoplasm and translocation to the nucleus, which allows it to act as an oncogenic protein by increasing the transcription of genes involved in growth control[31]. The loss of membranous E-cadherin expression was found in highly aggressive tumors including HCC and contributed to their high metastatic potential[32-34]. Aberrant activation of Wnt/β-catenin signaling has been reported in a wide range of HCC[35].

In our study, EBP50 upregulation resulted in an increase in E-cadherin levels and a decrease in β-catenin levels. EBP50 is required to maintain a fraction of the cortical β-catenin protein at the membrane; EBP50 binds to the C-terminal PDZ motif of β-catenin through its PDZ2 domain, and this association is required for β-catenin localization at cell-cell junctions[36]. In EBP50-depleted cells, there was a shift of β-catenin to the nucleus and an increased invasiveness of cells[12]. In EBP50-deficient MEFs, disorganization of E-cadherin-mediated adherens junctions and an increase in β-catenin transcriptional activity was observed[26], suggesting that the depletion of EBP50 decreased the interaction between β-catenin and E-cadherin. Because of the contributing role of β-catenin and E-cadherin in HCC hyperplasia and tumorigenesis[10,19], it is conceivable that the regulation of β-catenin/E-cadherin as a result of EBP50 overexpression contributes to HCC initiation or progression.

Our results suggest a tumor-suppressing role of EBP50 and establish that it may be a promising target for therapeutic intervention in HCC. Additionally, the interactions of EBP50 with β-catenin/E-cadherin enhance the tumor suppressor properties of EBP50 in HCC.

ACKNOWLEDGMENTS

The Pbk-CMV-HA-EBP50 plasmid was kindly provided by Dr. Randy Hall from Emory University.

COMMENTS

Background

Hepatocellular carcinoma (HCC) is the fifth most common cancer globally and relatively resistant to chemotherapy, it encouraged us to study the cellular and molecular mechanisms of HCC targeted molecular therapy. ezrin-radixin-moesin-binding phosphoprotein-50 (EBP50) is a PDZ scaffolding protein, previous studies suggest that EBP50 is a potential tumor suppressor in colorectal, prostatic, biliary, breast, gastric and pancreatic cancer, and it has an abnormal expression in HCC. But EBP50 is an oncogene or a tumor suppressor in HCC remains unclear. The authors aimed to examine the role of EBP50 and whether it interact with the β-catenin/E-cadherin pathway in HCC.

Research frontiers

Targeted molecular therapy is a new effective treatment for cancer including HCC. EBP50 is a potential tumor suppressor in several cancers, but the role of EBP50 is an oncogene or a tumor suppressor remains controversial and its exact role in HCC remains unknown.

Innovations and breakthroughs

Authors used a pBK-CMV-HA-EBP50 plasmid to overexpress EBP50 in SMMC7721 cells. The results showed that overexpression of EBP50 could inhibit the growth, migration, invasion of SMMC7721 cells and promote cell apoptosis, and modulate the expression of beta-catenin, E-cadherin. EBP50 may serve as a potential therapeutic target in HCC. It suggested that EBP50 is a tumor suppressor and interact with β-catenin/E-cadherin in HCC.

Applications

The results showed that EBP50 is a tumor suppressor and interact with β-catenin/E-cadherin in HCC. It may contribute to the future research of HCC and be a promising target for therapeutic intervention in HCC.

Terminology

Ezrin-radixin-moesin-binding phosphoprotein-50: EBP50 (also known as NHERF1) is a 55-kDa phosphoprotein; β-catenin: A adhesion protein, a key mediator in the canonical Wnt signal pathway; E-cadherin: A member of the cadherin family and is a classical type I cadherin.

Peer review

The function of EBP50 as a tumor suppressor has been studied in other cancers, but not in HCC. The expression in HCC was not significantly different between patient cases reported by Shibata *et al*. Thus they examined the effects of EBP50 up-regulation on cell growth, migration, invasion, and apoptosis in the HCC cell line SMMC7721 and determined the conclusion. This conclusion is meaningful in the sense characterized the function of EBP in HCC cells.

REFERENCES

1 **El-Serag HB**. Hepatocellular carcinoma. *N Engl J Med* 2011; **365**: 1118-1127 [PMID: 21992124]

2 **Tsanou E**, Peschos D, Batistatou A, Charalabopoulos A, Charalabopoulos K. The E-cadherin adhesion molecule and colorectal cancer. A global literature approach. *Anticancer Res* 2008; **28**: 3815-3826 [PMID: 19189669]

3 **Kikuchi A**. Regulation of beta-catenin signaling in the Wnt pathway. *Biochem Biophys Res Commun* 2000; **268**: 243-248 [PMID: 10679188 DOI: 10.1006/bbrc.1999.1860]

4 **Morrogh M**, Andrade VP, Giri D, Sakr RA, Paik W, Qin LX, Arroyo CD, Brogi E, Morrow M, King TA. Cadherin-catenin complex dissociation in lobular neoplasia of the breast. *Breast Cancer Res Treat* 2012; **132**: 641-652 [PMID: 22080244 DOI: 10.1007/s10549-011-1860-0]

5 **Lee J**, Ju J, Park S, Hong SJ, Yoon S. Inhibition of IGF-1 signaling by genistein: modulation of E-cadherin expression and downregulation of β-catenin signaling in hormone refractory PC-3 prostate cancer cells. *Nutr Cancer* 2012; **64**: 153-162 [PMID: 22098108]

6 **Czyzewska J**, Guzińska-Ustymowicz K, Ustymowicz M, Pryczynicz A, Kemona A. The expression of E-cadherin-catenin complex in patients with advanced gastric cancer: role in formation of metastasis. *Folia Histochem Cytobiol* 2010; **48**: 37-45 [PMID: 20529814 DOI: 10.2478/v10042-010-0017-z]

7 **Aamodt R**, Bondi J, Andersen SN, Bakka A, Bukholm G, Bukholm IR. The Prognostic Impact of Protein Expression of E-Cadherin-Catenin Complexes Differs between Rectal and Colon Carcinoma. *Gastroenterol Res Pract* 2010; **2010**: 616023 [PMID: 20814557]

8 **Shapiro M**, Akiri G, Chin C, Wisnivesky JP, Beasley MB, Weiser TS, Swanson SJ, Aaronson SA. Wnt pathway activation predicts increased risk of tumor recurrence in patients with stage I nonsmall cell lung cancer. *Ann Surg* 2013; **257**: 548-554 [PMID: 23011390 DOI: 10.1097/SLA.0b013e31826d81fd]

9 **González-Moles MA**, Bravo M, Ruiz-Avila I, Gil-Montoya JA, Acebal F, Esteban F. E-cadherin in non-tumor epithelium adjacent to oral cancer as risk marker for the development of multiple tumors. *Br J Oral Maxillofac Surg* 2013; **51**: 157-163 [PMID: 22658605 DOI: 10.1016/j.bjoms.2012.05.001]

10 **Wang XQ**, Zhang W, Lui EL, Zhu Y, Lu P, Yu X, Sun J, Yang S, Poon RT, Fan ST. Notch1-Snail1-E-cadherin pathway in metastatic hepatocellular carcinoma. *Int J Cancer* 2012; **131**: E163-E172 [PMID: 22052196 DOI: 10.1002/ijc.27336]

11 **Zheng JF**, Sun LC, Liu H, Huang Y, Li Y, He J. EBP50 exerts tumor suppressor activity by promoting cell apoptosis and retarding extracellular signal-regulated kinase activity. *Amino Acids* 2010; **38**: 1261-1268 [PMID: 20012548 DOI: 10.1007/s00726-009-0437-2]

12 **Hayashi Y**, Molina JR, Hamilton SR, Georgescu MM. NHERF1/EBP50 is a new marker in colorectal cancer. *Neoplasia* 2010; **12**: 1013-1022 [PMID: 21170265]

13 **Bartholow TL**, Becich MJ, Chandran UR, Parwani AV. Immunohistochemical analysis of ezrin-radixin-moesin-binding phosphoprotein 50 in prostatic adenocarcinoma. *BMC Urol* 2011; **11**: 12 [PMID: 21672215 DOI: 10.1186/1471-2490-11-12]

14 **Lin YY**, Hsu YH, Huang HY, Shann YJ, Huang CY, Wei SC, Chen CL, Jou TS. Aberrant nuclear localization of EBP50 promotes colorectal carcinogenesis in xenotransplanted mice by modulating TCF-1 and β-catenin interactions. *J Clin Invest* 2012; **122**: 1881-1894 [PMID: 22466651 DOI: 10.1172/JCI45661]

15 **Clapéron A**, Guedj N, Mergey M, Vignjevic D, Desbois-Mouthon C, Boissan M, Saubaméa B, Paradis V, Housset C, Fouassier L. Loss of EBP50 stimulates EGFR activity to induce EMT phenotypic features in biliary cancer cells. *Oncogene* 2012; **31**: 1376-1388 [PMID: 21822312 DOI: 10.1038/onc.2011.334]

16 **Lv XG**, Ji MY, Dong WG, Lei XF, Liu M, Guo XF, Wang J, Fang C. EBP50 gene transfection promotes 5-fluorouracil-induced apoptosis in gastric cancer cells through Bax- and Bcl-2-triggered mitochondrial pathways. *Mol Med Report* 2012; **5**: 1220-1226 [PMID: 22366766]

17 **Ji MY**, Fan DK, Lv XG, Peng XL, Lei XF, Dong WG. The detection of EBP50 expression using quantum dot immunohistochemistry in pancreatic cancer tissue and down-regulated EBP50 effect on PC-2 cells. *J Mol Histol* 2012; **43**: 517-526 [PMID: 22622406 DOI: 10.1007/s10735-012-9424-0]

18 **Stemmer-Rachamimov AO**, Wiederhold T, Nielsen GP, James M, Pinney-Michalowski D, Roy JE, Cohen WA, Ramesh V, Louis DN. NHE-RF, a merlin-interacting protein, is primarily expressed in luminal epithelia, proliferative endometrium, and estrogen receptor-positive breast carcinomas. *Am J Pathol* 2001; **158**: 57-62 [PMID: 11141479 DOI: 10.1016/S0002-9440(10)63944-2]

19 **Shibata T**, Chuma M, Kokubu A, Sakamoto M, Hirohashi S. EBP50, a beta-catenin-associating protein, enhances Wnt signaling and is over-expressed in hepatocellular carcinoma. *Hepatology* 2003; **38**: 178-186 [PMID: 12830000 DOI: 10.1053/jhep.2003.50270]

20 **Liu QS**, Zhang J, Liu M, Dong WG. Lentiviral-mediated miRNA against liver-intestine cadherin suppresses tumor growth and invasiveness of human gastric cancer. *Cancer Sci* 2010; **101**: 1807-1812 [PMID: 20500517 DOI: 10.1111/j.1349-7006.2010.01600.x]

21 **Weinman EJ**, Steplock D, Tate K, Hall RA, Spurney RF, Shenolikar S. Structure-function of recombinant Na/H exchanger regulatory factor (NHE-RF). *J Clin Invest* 1998; **101**: 2199-2206 [PMID: 9593775 DOI: 10.1172/JCI204]

22 **Boratkó A**, Gergely P, Csortos C. Cell cycle dependent association of EBP50 with protein phosphatase 2A in endothelial cells. *PLoS One* 2012; **7**: e35595 [PMID: 22523604 DOI: 10.1371/journal.pone.0035595]

23 **Takahashi Y**, Morales FC, Kreimann EL, Georgescu MM. PTEN tumor suppressor associates with NHERF proteins to attenuate PDGF receptor signaling. *EMBO J* 2006; **25**: 910-920 [PMID: 16456542 DOI: 10.1038/sj.emboj.7600979]

24 **Pan Y**, Weinman EJ, Dai JL. Na+/H+ exchanger regulatory factor 1 inhibits platelet-derived growth factor signaling in breast cancer cells. *Breast Cancer Res* 2008; **10**: R5 [PMID: 18190691 DOI: 10.1186/bcr1846]

25 **Curto M**, McClatchey AI. Nf2/Merlin: a coordinator of re-

ceptor signalling and intercellular contact. *Br J Cancer* 2008; **98**: 256-262 [PMID: 17971776 DOI: 10.1038/sj.bjc.6604002]
26 **Lazar CS**, Cresson CM, Lauffenburger DA, Gill GN. The Na+/H+ exchanger regulatory factor stabilizes epidermal growth factor receptors at the cell surface. *Mol Biol Cell* 2004; **15**: 5470-5480 [PMID: 15469991 DOI: 10.1091/mbc.E04-03-0239]
27 **James MF**, Beauchamp RL, Manchanda N, Kazlauskas A, Ramesh V. A NHERF binding site links the betaPDGFR to the cytoskeleton and regulates cell spreading and migration. *J Cell Sci* 2004; **117**: 2951-2961 [PMID: 15161943 DOI: 10.1242/jcs.01156]
28 **Rochdi MD**, Parent JL. Galphaq-coupled receptor internalization specifically induced by Galphaq signaling. Regulation by EBP50. *J Biol Chem* 2003; **278**: 17827-17837 [PMID: 12626493 DOI: 10.1074/jbc.M210319200]
29 **Hsu YH**, Lin WL, Hou YT, Pu YS, Shun CT, Chen CL, Wu YY, Chen JY, Chen TH, Jou TS. Podocalyxin EBP50 ezrin molecular complex enhances the metastatic potential of renal cell carcinoma through recruiting Rac1 guanine nucleotide exchange factor ARHGEF7. *Am J Pathol* 2010; **176**: 3050-3061 [PMID: 20395446]
30 **Provost E**, Rimm DL. Controversies at the cytoplasmic face of the cadherin-based adhesion complex. *Curr Opin Cell Biol* 1999; **11**: 567-572 [PMID: 10508647 DOI: 10.1016/S0955-0674(99)00015-0]
31 **Yang SZ**, Kohno N, Yokoyama A, Kondo K, Hamada H, Hiwada K. Decreased E-cadherin augments beta-catenin nuclear localization: studies in breast cancer cell lines. *Int J Oncol* 2001; **18**: 541-548 [PMID: 11179484]
32 **Da Silva L**, Parry S, Reid L, Keith P, Waddell N, Kossai M, Clarke C, Lakhani SR, Simpson PT. Aberrant expression of E-cadherin in lobular carcinomas of the breast. *Am J Surg Pathol* 2008; **32**: 773-783 [PMID: 18379416 DOI: 10.1097/PAS.0b013e318158d6c5]
33 **Humar B**, Blair V, Charlton A, More H, Martin I, Guilford P. E-cadherin deficiency initiates gastric signet-ring cell carcinoma in mice and man. *Cancer Res* 2009; **69**: 2050-2056 [PMID: 19223545 DOI: 10.1158/0008-5472.CAN-08-2457]
34 **Vasioukhin V**. Adherens junctions and cancer. *Subcell Biochem* 2012; **60**: 379-414 [PMID: 22674080 DOI: 10.1007/978-94-007-4186-7_16]
35 **Nejak-Bowen KN**, Monga SP. Beta-catenin signaling, liver regeneration and hepatocellular cancer: sorting the good from the bad. *Semin Cancer Biol* 2011; **21**: 44-58 [PMID: 21182948 DOI: 10.1016/j.semcancer.2010.12.010]
36 **Kreimann EL**, Morales FC, de Orbeta-Cruz J, Takahashi Y, Adams H, Liu TJ, McCrea PD, Georgescu MM. Cortical stabilization of beta-catenin contributes to NHERF1/EBP50 tumor suppressor function. *Oncogene* 2007; **26**: 5290-5299 [PMID: 17325659 DOI: 10.1038/sj.onc.1210336]

P- Reviewer Yu DY **S- Editor** Gou SX **L- Editor** A
E- Editor Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i8.1314

World J Gastroenterol 2013 February 28; 19(8): 1314-1317
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Gastric adenocarcinoma arising in gastritis cystica profunda presenting with selective loss of KCNE2 expression

Natsumi Kuwahara, Riko Kitazawa, Koto Fujiishi, Yusa Nagai, Ryuma Haraguchi, Sohei Kitazawa

Natsumi Kuwahara, Riko Kitazawa, Koto Fujiishi, Yusa Nagai, Ryuma Haraguchi, Sohei Kitazawa, Department of Molecular Pathology, Ehime University, Graduate School of Medicine, Ehime 791-0295, Japan
Author contributions: Kuwahara N, Kitazawa R, Fujiishi K, Nagai Y, Haraguchi R and Kitazawa S contributed to the manuscript writing and revision.
Supported by The Grant-in-Aid from Ministry of Education, Culture, Sports, Science and Technology, Japan
Correspondence to: Sohei Kitazawa, MD, PhD, Department of Molecular Pathology, Ehime University, Graduate School of Medicine, Shitsukawa 454, Toon City, Ehime 791-0295, Japan. kitazawa@m.ehime-u.ac.jp
Telephone: +81-89-9605264 Fax: +81-89-9605267
Received: November 2, 2012 Revised: December 19, 2012
Accepted: January 17, 2013
Published online: February 28, 2013

Abstract

Gastritis cystica profunda (GCP) is a rare condition caused by ectopic entrapment of gastric glands, probably secondary to the disruption of muscularis mucosae. GCP is often associated with gastric adenocarcinoma, and loss of the KCNE2 subunit from potassium channel complexes is considered a common primary target molecule leads to both GCP and malignancy. In this study, we, for the first time, analyzed the expression of KCNE2 in surgically excised tissue from human gastric cancer associated with GCP and confirmed that reduced KCNE2 expression correlates with disease formation.

© 2013 Baishideng. All rights reserved.

Key words: KCNE2; Gastritis cystica profunda; Immunohistochemistry

Kuwahara N, Kitazawa R, Fujiishi K, Nagai Y, Haraguchi R, Kitazawa S. Gastric adenocarcinoma arising in gastritis cystica profunda presenting with selective loss of KCNE2 expression. *World J Gastroenterol* 2013; 19(8): 1314-1317 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i8/1314.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i8.1314

INTRODUCTION

Gastritis cystica profunda (GCP) is a rare condition with nonspecific symptoms and radiographic images, making its diagnosis difficult without definitive surgical resection[1]. Clinically, GCP can be misdiagnosed as gastric lymphoma, stromal tumors, gastric cancer, or Menetrier disease. Histopathologically, GCP shows disruption of the integrity of muscularis mucosa that leads to cystically dilated submucosal glands with superficial inflammation in the lamina propria[1,2]. Since the majority of GCP cases are seen secondary to prolonged chronic inflammation, ischemia, gastric surgery and suturing material, an injury of the muscularis mucosae is assumed to trigger the ectopic entrapment of gastric glands in the submucosa, the muscularis mucosae or serosa and to lead to GCP[1,2]. Moreover, GCP is often associated with gastric adenocarcinoma indicates that it can lead to a secondary malignancy[2]. Indeed, experiments have shown that animals predisposed to Helicobacter infection develop not only secondary GCP but also subsequent gastric carcinoma[3]. This close association between GCP and malignancy has been interpreted as concurrent sharing of causative factors common to both disease conditions[1,3]. Recently, with the use of the KCNE2 (also known as MiRP1) deficient mouse model, loss of the KCNE2 subunit from potassium channel complexes is considered a common primary target molecule that gives rise to both GCP and malignancy[4].

Here, the expression of KCNE2 in surgically excised tissue from human gastric cancer associated with GCP is, for the first time, analyzed to confirm that its reduced level correlates with disease formation.

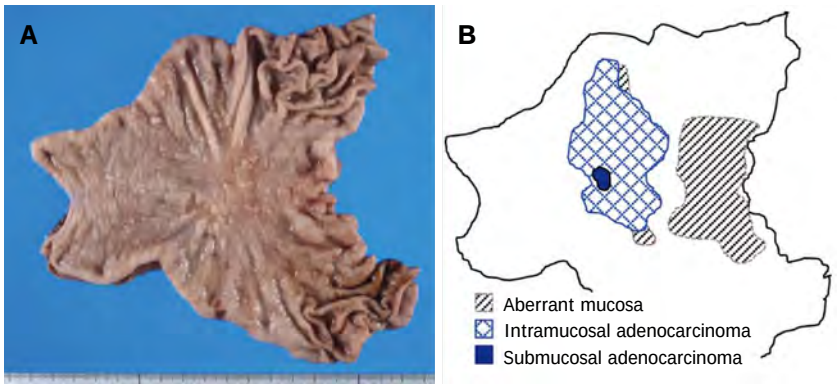


Figure 1 Macroscopic finding and distribution of ectopic cystic lesion and cancer in surgically excised stomach. Surgically excised stomach (A) and schematic distribution of the lesions (B). Ectopic cystic mucosa is located mainly on the oral side and partly in the body part overlapped with a cancer lesion (shaded area). A well- to moderately-differentiated tubular adenocarcinoma in the intramucosal layer (checked area) and part of a poorly-differentiated component infiltrating the submucosal layer (blue area) are located mainly in the body part of the stomach.

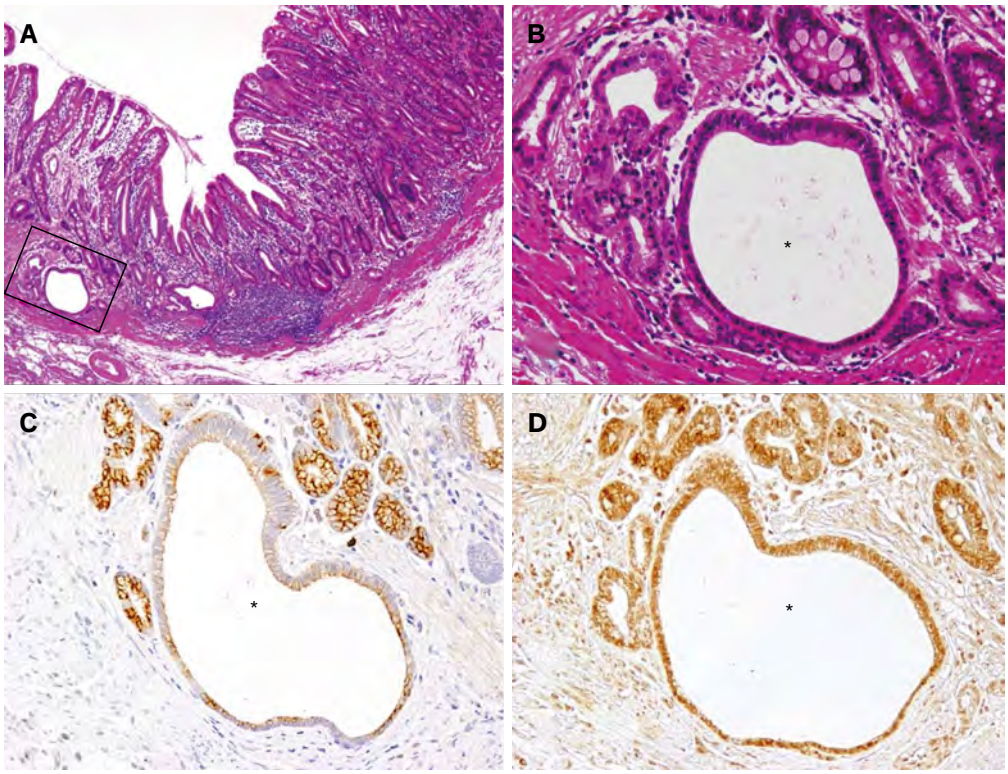


Figure 2 Expression of KCNE2 and estrogen receptor in non-neoplastic cystic lesion by immunohistochemistry. A: Low power magnification of a transitional area from the non-neoplastic mucosal layer with intramucosal cystic lesions (left) to the intramucosal adenocarcinoma (right, HE, × 40); B: High power magnification (A, squared area) around the intramucosal cystic lesion (asterisk, HE, × 200); C: KCNE2 immunostaining of a serial section of (B) shows that KCNE2 is almost negative in the dilated cystic gland (asterisk), while the surrounding non-cystic glands are positive (× 200); D: Estrogen receptor immunostaining of serial sections of (B) and (C) show that ER is equally expressed in both cystic (asterisk) and non-cystic glands (× 200).

CASE REPORT

A 63-year-old man was admitted to the hospital with the chief complain of abdominal pain. The patient's past medical and family history was unremarkable. An endoscopic and other examinations revealed the presence of multiple erosive lesions, and a biopsy demonstrated only severe superficial gastritis with erosion. Under the diagnosis of benign erosive gastritis, the patient was treated with medication and followed up monthly. After one year, however, a follow-up endoscopic examination revealed an irregularly-shaped ulcerating lesion, and the pathological diagnosis of adenocarcinoma was made through biopsy analysis. The patient underwent distal partial gastrectomy, and pathological examination of the excised stomach (Figure 1A) revealed the presence of ectopic cystic mucosa with intestinal metaplasia, especially in the oral side (Figure 1B, dotted area, Figure 2B: HE, × 200). Overlaying the ectopic cystic mucosa, a well- to moderately-differentiated tubular adenocarcinoma was

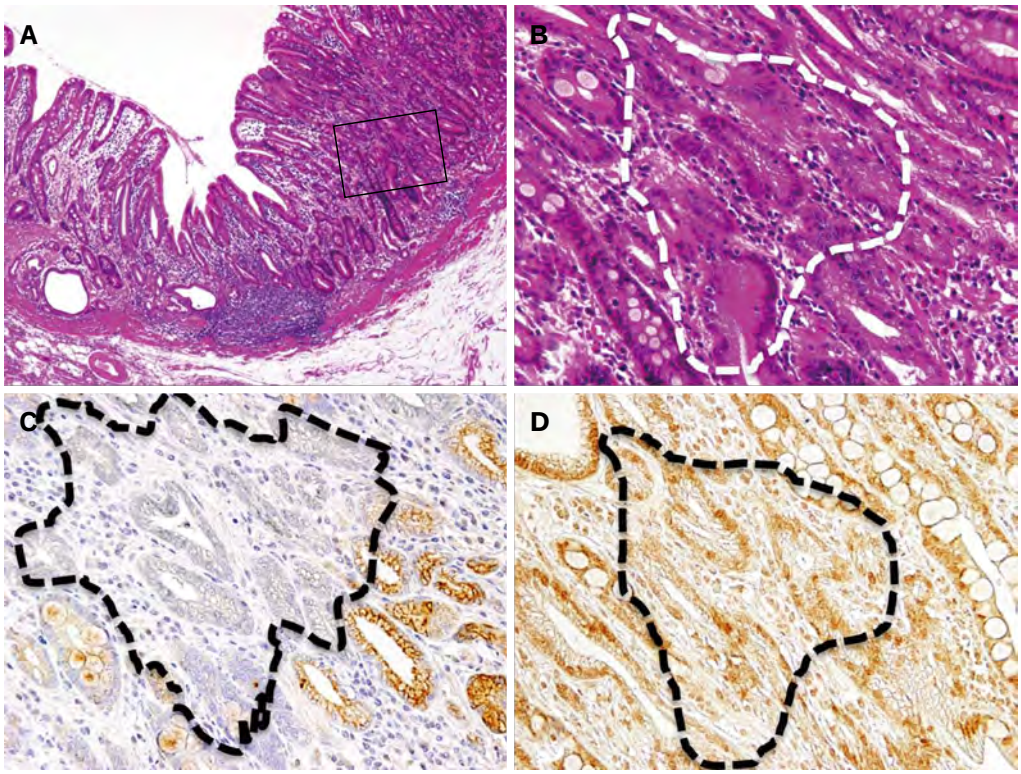


Figure 3 Expression of KCNE2 and estrogen receptor in adenocarcinoma area. A: Low power magnification of a transitional area from the non-neoplastic mucosal layer with intramucosal cystic lesions (left) to the intramucosal adenocarcinoma (right, HE, × 40); B: High power magnification (A, squared area) around the intramucosal adenocarcinoma (circled by white-hatched line, HE, × 200); C: KCNE2 immunostaining of a serial section of (B) shows that KCNE2 expression is almost negative in adenocarcinoma (circled by black-hatched line), while the surrounding non-neoplastic glands are positive (× 200); D: Estrogen receptor immunostaining of serial sections of (B) and (C) show that estrogen receptor is equally expressed in both cancerous (circled by black-hatched line) and non-neoplastic glands (× 200).

observed in the superficial proper mucosal layer (M), with part of a poorly-differentiated component infiltrating the submucosal layer (SM) in the body of the stomach (Figure 1B, hatched area, Figure 3B: HE, × 200). The pathological diagnosis of gastric adenocarcinoma arising in gastritis cystica profunda was made.

Immunohistochemistry

Since targeted deletion of *KCNE2* in mice causes gastric lesions resembling gastritis cystica profunda and gastric neoplasia[4], we examined the expression of KCNE2 in the current case immunohistochemically. Also, since signaling through the estrogen receptor (ER) modulates KCNE2 expression[5], we additionally examined the expression of ER. To determine the localization of KCNE2 and ER, rabbit polyclonal anti-KCNE2 antibody (ABCAM, Cambridge, United Kingdom) and rabbit monoclonal anti-ER (EST) antibody (EPIOTOMICS, United States) were used as primary antibodies. Tissue sections were deparaffinized in xylene for 20 min (solvent refreshed at 10 and 5 min) and immersed in absolute ethanol for 10 min (solvent refreshed at 5 min), then rehydrated in 90% and 70% ethanol (5 min each), and finally placed in distilled water for 15 min (solvent refreshed every 5 min). The samples were inactivated in 1 mmol/L EDTA (pH 8.0) plus distilled water in a microwave for 15 min (high heat for 5 min and low heat for 10 min), and then cooled to approximately 25 ℃ over 1 h. After being undergoing liquid block treatment, the samples were immersed in PBS for 15 min (solvent refreshed every 5 min). After adding blocking buffer to the moisture chamber and incubating the samples with primary antibodies at room temperature for 1 h, they were washed 3 times for 5 min each in PBS, incubated in the moisture chamber at room temperature for 30 min with the secondary antibody diluted at 1:200 with PBS, immersed in PBS for 15 min (solvent refreshed every 5 min), then incubated with DAB for 15 min and washed in PBS.

Immunohistochemical analyses revealed that KCNE2 was universally and strongly expressed on the surface of the cells at the bottom of the cryptic glands, while its expression was diminished in the cystic or dilated lesions (Figure 2C). ER expression was observed in both non-cystic and cystic glands (Figure 2D). In adenocarcinoma, KCNE2 expression was significantly reduced compared with the surrounding non-cancerous gastric mucosa with intestinal metaplasia (Figure 3C), while ER expression was observed in both cancerous and non-cancerous glands (Figure 3D).

DISCUSSION

In this study the expression status of KCNE2 in surgically excised gastric adenocarcinoma coexisting with GCP was examined in light of that the KCNE2-deficient mouse model develops both GCP and gastric cancer.

Since KCNE2 expression is modulated by ER, and since ER expression status per se is related to carcinogenesis and progression stages of gastric cancer[6], we additionally examined the immunohistochemical expression of ER in in the excised tissue.

KCNE2 has originally been identified as a potassium channel protein, and in the stomach, it is expressed mainly in the cytoplasm of parietal cells[5,7,8]. Reduction of KCNE2 in experimental animal models results in profoundly reduced proton secretion, abnormal parietal cell morphology, achlorhydria, hypergastrinemia[5,8], and striking gastric glandular hyperplasia arising from an increase in the number of non-acid-secretory cells[4]. Functionally, KCNE2 also exerts anti-proliferative effects on gastric cancer cells by down-regulating Cyclin D1 and restricting cell growth[5,8]. Indeed, reflecting anti-proliferative or tumor-suppressor functions of KCNE2, long-term observation of *KCNE2* -/- mice has revealed that reduced KCNE2 expression causes diffuse hyperplasia in gastric mucosa, resulting in a pathologic condition similar to gastric cancer associated with GCP[4].

We examined here, for the first time, the expression of KCNE2 in surgically excised stomach tissue demonstrating both GCP and adenocarcinoma. While KCNE2 expression in both GCP and adenocarcinoma areas was diminished, that in surrounding non-neoplastic and non-cystic cells was clearly maintained, as determined by immunohistochemical analysis. These data, albeit from a single case study, suggest that selective loss of KCNE2 expression is related to the development and clinical manifestation of GCP with subsequent occurrence of cancer. Furthermore, because selective loss of KCNE2 expression is seen at the level of a single cystic gland and cancer cell nest unit, silencing KCNE2 expression may occur at the level of a single tissue progenitor cell. Although the precise molecular mechanism of such selective loss of KCNE2 expression is largely unknown, it is at least not by the loss of estrogen receptor signaling. We believe that these new data are important because they confirm previous experimental information, and should be assessed in other similar cases. Further genetical and epigenetics studies based on a cumulative case study are needed to elucidate the role of KCNE2 expression in the development of GCP.

ACKNOWLEDGMENTS

We appreciate Ms. Yuki Takaoka for technical assistance.

REFERENCES

1 **Fonde EC**, Rodning CB. Gastritis cystica profunda. *Am J Gastroenterol* 1986; **81**: 459-464 [PMID: 3706265]
2 **Mitomi H**, Iwabuchi K, Amemiya A, Kaneda G, Adachi K, Asao T. Immunohistochemical analysis of a case of gastritis cystica profunda associated with carcinoma development. *Scand J Gastroenterol* 1998; **33**: 1226-1229 [PMID: 9867104 DOI: 10.1080/00365529850172610]
3 **Wang TC**, Dangler CA, Chen D, Goldenring JR, Koh T, Raychowdhury R, Coffey RJ, Ito S, Varro A, Dockray GJ, Fox JG. Synergistic interaction between hypergastrinemia and Helicobacter infection in a mouse model of gastric cancer. *Gastroenterology* 2000; **118**: 36-47 [PMID: 10611152 DOI: 10.1016/S0016-5085(00)70412-4]
4 **Roepke TK**, Purtell K, King EC, La Perle KM, Lerner DJ, Abbott GW. Targeted deletion of Kcne2 causes gastritis cystica profunda and gastric neoplasia. *PLoS One* 2010; **5**: e11451 [PMID: 20625512 DOI: 10.1371/journal.pone.0011451]
5 **Kundu P**, Ciobotaru A, Foroughi S, Toro L, Stefani E, Eghbali M. Hormonal regulation of cardiac KCNE2 gene expression. *Mol Cell Endocrinol* 2008; **292**: 50-62 [PMID: 18611433 DOI: 10.1016/j.mce.2008.06.003]
6 **Hogan AM**, Collins D, Baird AW, Winter DC. Estrogen and gastrointestinal malignancy. *Mol Cell Endocrinol* 2009; **307**: 19-24 [PMID: 19524122 DOI: 10.1016/j.mce.2009.03.016]
7 **Yanglin P**, Lina Z, Zhiguo L, Na L, Haifeng J, Guoyun Z, Jie L, Jun W, Tao L, Li S, Taidong Q, Jianhong W, Daiming F. KCNE2, a down-regulated gene identified by in silico analysis, suppressed proliferation of gastric cancer cells. *Cancer Lett* 2007; **246**: 129-138 [PMID: 16677757 DOI: 10.1016/j.canlet.2006.02.010]
8 **Lee MP**, Ravenel JD, Hu RJ, Lustig LR, Tomaselli G, Berger RD, Brandenburg SA, Litzi TJ, Bunton TE, Limb C, Francis H, Gorelikow M, Gu H, Washington K, Argani P, Goldenring JR, Coffey RJ, Feinberg AP. Targeted disruption of the Kvlqt1 gene causes deafness and gastric hyperplasia in mice. *J Clin Invest* 2000; **106**: 1447-1455 [PMID: 11120752 DOI: 10.1172/JCI10897]

P- Reviewers Bordas JM, Leitman M
S- Editor Wen LL **L- Editor** A **E- Editor** Zhang DN

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i8.1318

World J Gastroenterol 2013 February 28; 19(8): 1318-1321
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Hepatitis B reactivation in chronic myeloid leukemia patients receiving tyrosine kinase inhibitor

Guan-Min Lai, Sheng-Lei Yan, Cheng-Shyong Chang, Chien-Yu Tsai

Guan-Min Lai, Cheng-Shyong Chang, Chien-Yu Tsai, Division of Hemato-Oncology, Department of Internal Medicine, Changhua Christian Hospital, Changhua 500, Taiwan
Sheng-Lei Yan, Division of Gastroenterology, Department of Internal Medicine, Chang-Bing, Show Chwan Memorial Hospital, Changhua 500, Taiwan
Author contributions: Lai GM and Yan SL contributed equally to this work; Lai GM and Yan SL contributed to the drafting the article; Chang CS, Yan SL and Tsai CY were responsible for the analysis and interpretation of the data; Chang CS was responsible for the critical revision and final approval of the article.
Correspondence to: Cheng-Shyong Chang, MD, Division of Hemato-Oncology, Department of Internal Medicine, Changhua Christian Hospital, Changhua 500, Taiwan. 15120@cch.org.tw
Telephone: +886-4-7238595 Fax: +886-4-7232946
Received: December 20, 2012 Revised: January 9, 2013
Accepted: January 23, 2013
Published online: February 28, 2013

Abstract

Hepatitis B virus (HBV) reactivation is a well-recognized complication in patients with chronic HBV infection receiving cytotoxic or immunosuppressive chemotherapy. Imatinib mesylate and nilotinib are selective Bcr/Abl tyrosine kinase inhibitors, which are now widely used in the treatment of patients with chronic myeloid leukemia. Although HBV reactivation induced by imatinib mesylate has been reported, nilotinib-related HBV reactivation has not been reported in the English literature. We report here 2 cases of HBV reactivation in chronic myeloid leukemia patients receiving imatinib mesylate and a novel case of nilotinib related HBV reactivation.

© 2013 Baishideng. All rights reserved.

Key words: Hepatitis B virus; Chronic myeloid leukemia; Imatinib mesylate; Nilotinib; Tyrosine kinase inhibitor

Lai GM, Yan SL, Chang CS, Tsai CY. Hepatitis B reactivation in chronic myeloid leukemia patients receiving tyrosine kinase inhibitor. *World J Gastroenterol* 2013; 19(8): 1318-1321 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i8/1318.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i8.1318

INTRODUCTION

Hepatitis B virus (HBV) reactivation is a well-recognized complication in patients with chronic HBV infection receiving cytotoxic or immunosuppressive chemotherapy for hematologic malignancies[1-4]. Imatinib mesylate (IM) (Glivec; Novartis, Basel, Switzerland), a selective Bcr/Abl tyrosine kinase inhibitor (TKI), is now widely used in the treatment of chronic myeloid leukemia (CML) and gastrointestinal stromal tumors[5,6]. Nilotinib (Tasigna; Novartis, Basel, Switzerland), a second generation TKI, was approved in 2007 for CML patients with resistance or intolerance to IM[5]. Although HBV reactivation induced by IM has been reported[7-12], nilotinib-related HBV reactivation has not been documented in current literature. We report here 2 cases of HBV reactivation in CML patients receiving IM and a novel case of nilotinib related Hepatitis B virus reactivation. Possible mechanism of TKI related HBV reactivation is discussed in this manuscript.

CASE REPORT

Case 1

A 43-year-old man was a HBV carrier who received regular follow-ups at our institution. On December 2011, he was diagnosed with CML, which was confirmed by the presence of Philadelphia chromosome (Ph+) in cytogenetic study. He started to receive IM 400 mg once daily. The pretreatment liver panel were alanine transaminase (ALT) = 40 U/L (normal 11-40 U/L), total bilirubin = 0.8 mg/dL (normal 0.3-1.2 mg/dL), albumin = 3.5 g/dL (normal 3.5-5.5 g/dL) and prothrombin time = 11 s (normal 10-12 s). Major molecular response (MMR, < 0.1% Bcr-Abl/Abl ratio according to the international scale (IS)

was achieved after 3.5 mo of IM treatment. His liver tests were within normal limits until 6 mo after IM treatment, when he started to feel easy fatigue. The patient denied usage of other medications such as acetaminophen or herbs. Laboratory investigation revealed an increased aspartate aminotransferase (AST) level of 273 U/L and an increased ALT level of 1086 U/L (Figure 1A). Hepatitis B surface antigen (HBsAg) and hepatitis B e-antigen (HBeAg) were positive. HBV DNA was positive at a concentration of 229 254 031 IU/mL. Results for hepatitis A immunoglobulin M (IgM) antibody, hepatitis C antibody, cytomegalovirus (CMV), Epstein Barr virus (EBV) and herpes simples 1/2 (HSV) were negative. Antinuclear antibodies (ANA) and immunoglobulins were within normal limits. Other biochemical studies such as bilirubin, alkaline phosphatase, and albumin were normal. Liver ultrasonography showed coarse liver parenchyma and normal biliary tract. Because HBV reactivation was considered, he started to receive entecavir 0.5 mg once daily. IM was not discontinued. After 1-mo treatment with entecavir, his ALT level fell to 117 IU/L and HBV DNA level was 11 IU/mL, as demonstrated in Figure 1A. Clinical symptoms resolved 6 wk later with entecavir. Complete molecular response (CMR, < 0.0001% Bcr-Abl/Abl ratio according to the IS) was achieved 9 mo after IM treatment. The clinical course of this patient is summarized in Figure 1A.

Case 2

A 67-year-old man with history of diabetes mellitus and HBV carrier received regular follow-ups at our hospital. He was diagnosed with CML with initial presentation of leukocytosis and thrombocytosis. He started to receive IM 400 mg once daily after bone marrow biopsy and cytogenetic study. The pretreatment liver panel were ALT = 26 U/L, total bilirubin = 1.1 mg/dL, albumin = 4.8 g/dL and prothrombin time = 12.6 s. Complete cytogenetic response (CCyR, < 1% Bcr-Abl/Abl ratio according to the IS) was achieved 7 mo after IM treatment. However, jaundice and anorexia developed 53 mo after IM treatment. Laboratory studies revealed an increased AST level of 110 U/L and an increased ALT level of 374 U/L (Figure 1B). Total bilirubin level was 2.74 mg/dL. HBsAg and HBeAg were positive. HBV DNA was positive at a concentration of 12 165 714 IU/mL. Results for hepatitis A IgM antibody, hepatitis C antibody, HSV, EBV, CMV, ANA were within normal limits. Liver ultrasonography showed no biliary tract dilatation. Entecavir 0.5 mg once daily was prescribed under the consideration of HBV reactivation. IM was not discontinued. After 1-mo treatment with entecavir, his ALT level fell to 41 IU/L, as demonstrated in Figure 1B. HBV DNA level was 10 IU/mL 12 mo after entecavir administration. Status of CMR was achieved on April, 2011. The clinical course of this patient is summarized in Figure 1B.

Case 3

A 50-year-old woman was referred to our institution

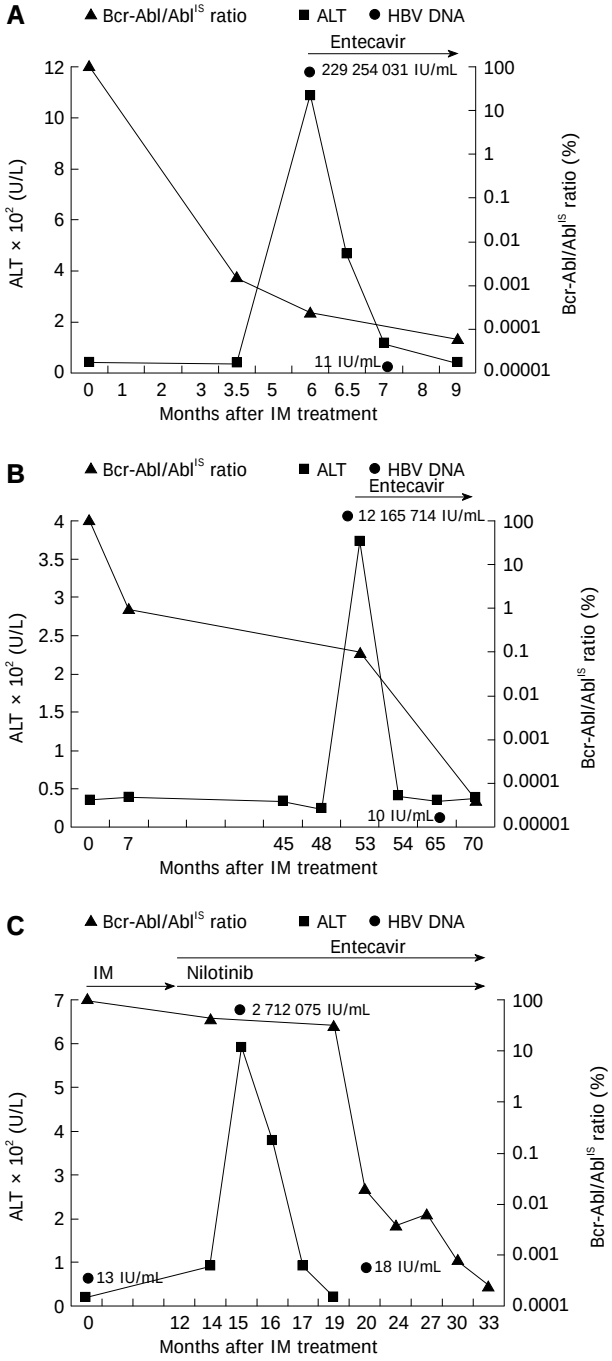


Figure 1 Clinical course of cases. A: Case 1, showing alanine transaminase (ALT) and Bcr-Abl/Abl^{IS} ratio over time; B: Case 2, showing ALT and Bcr-Abl/Abl^{IS} ratio over time; C: Case 3, showing ALT and Bcr-Abl/Abl^{IS} ratio over time. IM: Imatinib mesylate; IS: International scale.

on November 2009 for evaluation of leukocytosis, anemia, and thrombocytopenia. She was a HBV carrier. A diagnosis of CML was made based on the findings of proliferation of myeloid lineage cells and presence of Ph+ in bone marrow biopsy. IM 400 mg daily was used after diagnosis of CML. The pretreatment liver panel were ALT = 23 U/L, total bilirubin = 0.4 mg/dL, albumin = 4.1 g/dL and prothrombin time = 11 s. Because Bcr-Abl/Abl ratio was not significantly reduced 12 mo later with IM, treatment regimen was shifted to nilotinib

400 mg twice daily. However, 3 mo after treatment with nilotinib, the patient experienced tea-colored urine and easy fatigue. The patient denied usage of acetaminophen or herbs. Laboratory studies revealed an increased AST level of 346 U/L and an increased ALT level of 592 U/L (Figure 1C). Total-bilirubin level was 2.54 mg/dL. Coagulation profiles were normal. HBsAg was positive, whereas HBeAg was negative. Results for hepatitis A, hepatitis C, HSV, EBV, and CMV were negative. HBV DNA was positive at a concentration of 27 120 705 IU/mL. Liver ultrasonography showed normal results. Because HBV reactivation was considered, entecavir 0.5 mg once daily was prescribed. Nilotinib was not discontinued. After 2-mo treatment with entecavir, ALT level fell to 92 IU/L, as demonstrated in Figure 1C. HBV DNA was at a lower level of 18 IU/mL 6 mo after treatment with entecavir. Status of MMR was achieved 6 mo after entecavir treatment. The clinical course of this patient is summarized in Figure 1C.

DISCUSSION

HBV reactivation is characterized by an abrupt rise of HBV DNA in patients with previously inactive or resolved HBV infection during or closely after chemotherapy. The current generally accepted definition of HBV reactivation following chemotherapy is the development of hepatitis with a serum ALT greater than three times the upper limit of normal or an absolute increase of 100 IU/L, associated with a demonstrable increase in HBV DNA by at least a 10-fold[1,2]. HBV reactivation not only occurred in HBsAg-positive patients but also in HBsAg-negative patients with anti-hepatitis B core antibody positivity and/or anti-hepatitis B surface antibody positivity[1-4]. In a prospective study of 626 cancer patients undergoing chemotherapy, HBV reactivation occurred in nearly 20% of them[1]. The risk factors of HBV reactivation included male gender, younger age, HBeAg seropositivity, high pre-chemotherapy HBV DNA above 3×10^5 copies/mL, use of corticosteroids and anthracyclines, and duration of chemotherapy[1,4,13]. The pathogenesis of chemotherapy-induced HBV reactivation is not clear but may involve a 2-stage process, an initial immunosuppressive stage and an immune-restoration stage[3,4]. The initial immunosuppressive stage is characterized by marked increase in serum levels of HBV DNA and HBeAg. This stage is probably related to the suppression of immune mechanism that serves to control HBV replication. The immune-restoration stage occurs after subsequent withdrawl of immunosuppressive drugs, resulting in rapid destruction of infected hepatocyte.

IM, a rationally designed TKI that blocks the ATP-binding site of Bcr/Abl, is currently recommended as the first line therapy for CML[5,6]. However, resistance to IM may occur. Nilotinib was designed to overcome IM resistance with a better efficacy and mild adverse effects[5]. Hepatotoxicity has been reported in 1%-4% of CML patients treated with IM; however, liver dysfunction may resolve with either dose reduction or discontinuation of IM[14]. In the present study, the TKIs were not discontinued after hepatic flare. Hepatic dysfunction improved in three cases after receiving entecavir, thus excluding the possibility of considering IM or nilotinib related hepatotoxicity as the cause of hepatic flare in our study. In the third case of our study, the diagnosis of HBV reactivation was established by low pretreatment HBV load and high level at the time of hepatic flare. Although the HBV load was not examined before IM treatment in the first and second cases, HBsAg and HBeAg were positive before treatment and high level of HBV load was detected at the time of hepatic flare. It is reasonable to consider HBV reactivation as the cause of hepatic flare in these two cases of our study[15-18].

The mechanism of TKI-induced HBV reactivation remains unclear due to limited case reports. *In vitro* studies have shown that IM can inhibit T-cell activation[19] and proliferation[20]. Nilotinib inhibited the Src-family kinase LCK and T-cell function and hampered the proliferation and function of CD8+ T lymphocytes[21,22]. It is worth noting that HBV reactivation occurred after achieving MMR in the first case (Figure 1A) and after CCyR in the second case (Figure 1B), a similar condition as that in the report of Ikeda *et al*[7]. In the study of Mohamad *et al*[23], CML patients who were in complete molecular or cytogentic response after IM treatment restored function of plasmacytoid dendritic cells, which are crucial effectors in innate immunity. Therefore, the finding of hepatic flare after complete molecular or cytogenetic responses in the first two cases may provide evidence to support the hypothesis of immune-restoration stage of HBV reactivation. Furthermore, in the third case of our study, the hepatic flare occurred 5 mo before achieving CCyR (Figure 1C). This finding suggests that nilotinib treatment might provoke a different pathway other than that of IM to achieve immune restoration. Further studies may be needed to explain this observation. An important issue that should be addressed is the preemptive therapy with nucleoside/nucleotide analogues (NAs) in patients undergoing TKI therapy. Although preemptive treatment with NAs before immunosuppressive chemotherapy is recommended in European Association for the Study of the Liver Clinical Practice guidelines[24], there is no recommendation on whether NAs should be given in patients undergoing TKI therapy due to lack of prospective studies.

In conclusion, this case report highlights the importance that HBV reactivation may occur in hematologic patients undergoing TKI therapy. Once HBV reactivation is suspected during TKI treatment, early detection of HBV load and utilization of antiviral agent are suggested to achieve better clinical outcome.

REFERENCES

1 **Yeo W**, Chan PK, Zhong S, Ho WM, Steinberg JL, Tam JS, Hui P, Leung NW, Zee B, Johnson PJ. Frequency of hepatitis

B virus reactivation in cancer patients undergoing cytotoxic chemotherapy: a prospective study of 626 patients with identification of risk factors. *J Med Virol* 2000; **62**: 299-307 [PMID: 11055239 DOI: 10.1002/1096-9071(200011)62:]

2 **Yeo W**, Zee B, Zhong S, Chan PK, Wong WL, Ho WM, Lam KC, Johnson PJ. Comprehensive analysis of risk factors associating with Hepatitis B virus (HBV) reactivation in cancer patients undergoing cytotoxic chemotherapy. *Br J Cancer* 2004; **90**: 1306-1311 [PMID: 15054446 DOI: 10.1038/sj.bjc.6601699]

3 **Xunrong L**, Yan AW, Liang R, Lau GK. Hepatitis B virus (HBV) reactivation after cytotoxic or immunosuppressive therapy--pathogenesis and management. *Rev Med Virol* 2001; **11**: 287-299 [PMID: 11590667 DOI: 10.1002/rmv.322]

4 **Firpi RJ**, Nelson DR. Management of viral hepatitis in hematologic malignancies. *Blood Rev* 2008; **22**: 117-126 [PMID: 18343002 DOI: 10.1016/j.blre.2008.02.001]

5 **Jabbour E**, Fava C, Kantarjian H. Advances in the biology and therapy of patients with chronic myeloid leukaemia. *Best Pract Res Clin Haematol* 2009; **22**: 395-407 [PMID: 19959090 DOI: 10.1016/j.beha.2009.09.002]

6 **Druker BJ**, Tamura S, Buchdunger E, Ohno S, Segal GM, Fanning S, Zimmermann J, Lydon NB. Effects of a selective inhibitor of the Abl tyrosine kinase on the growth of Bcr-Abl positive cells. *Nat Med* 1996; **2**: 561-566 [PMID: 8616716 DOI: 10.1038/nm0596-561]

7 **Ikeda K**, Shiga Y, Takahashi A, Kai T, Kimura H, Takeyama K, Noji H, Ogawa K, Nakamura A, Ohira H, Sato Y, Maruyama Y. Fatal hepatitis B virus reactivation in a chronic myeloid leukemia patient during imatinib mesylate treatment. *Leuk Lymphoma* 2006; **47**: 155-157 [PMID: 16321842 DOI: 10.1080/14639230500236818]

8 **Thia TJ**, Tan HH, Chuah TH, Chow WC, Lui HF. Imatinib mesylate-related fatal acute hepatic failure in a patient with chronic myeloid leukaemia and chronic hepatitis B infection. *Singapore Med J* 2008; **49**: e86-e89 [PMID: 18362995]

9 **Lakhani S**, Davidson L, Priebat DA, Sherker AH. Reactivation of chronic hepatitis B infection related to imatinib mesylate therapy. *Hepatol Int* 2008; **2**: 498-499 [PMID: 19669326 DOI: 10.1007/s12072-008-9099-5]

10 **Kang BW**, Lee SJ, Moon JH, Kim SN, Chae YS, Kim JG, Hwang YJ, Sohn SK. Chronic myeloid leukemia patient manifesting fatal hepatitis B virus reactivation during treatment with imatinib rescued by liver transplantation: case report and literature review. *Int J Hematol* 2009; **90**: 383-387 [PMID: 19641858 DOI: 10.1007/s12185-009-0386-2]

11 **Kim SG**, Chun JM, Jin R, Kim JY, Won DI, Hwang YJ. Living donor liver transplantation for acute hepatic failure caused by reactivation of hepatitis B virus infection after chemotherapy for hematologic malignancy: case reports. *Transplant Proc* 2010; **42**: 843-845 [PMID: 20430187 DOI: 10.1016/j.transproceed.2010.02.038]

12 **Wang YD**, Cui GH, Li M, Gowrea B, Xia J, Hu Y. Hepatitis B virus reactivation in a chronic myeloid leukemia patient treated with imatinib mesylate. *Chin Med J (Engl)* 2012; **125**: 2636-2637 [PMID: 22882953]

13 **Yeo W**, Johnson PJ. Diagnosis, prevention and management of hepatitis B virus reactivation during anticancer therapy. *Hepatology* 2006; **43**: 209-220 [PMID: 16440366 DOI: 10.1002/hep.21051]

14 **Cohen MH**, Williams G, Johnson JR, Duan J, Gobburu J, Rahman A, Benson K, Leighton J, Kim SK, Wood R, Rothmann M, Chen G, U KM, Staten AM, Pazdur R. Approval summary for imatinib mesylate capsules in the treatment of chronic myelogenous leukemia. *Clin Cancer Res* 2002; **8**: 935-942 [PMID: 12006504]

15 **Ohyashiki K**, Kuriyama Y, Nakajima A, Tauchi T, Ito Y, Miyazawa H, Kimura Y, Serizawa H, Ebihara Y. Imatinib mesylate-induced hepato-toxicity in chronic myeloid leukemia demonstrated focal necrosis resembling acute viral hepatitis. *Leukemia* 2002; **16**: 2160-2161 [PMID: 12357373 DOI: 10.1038/sj.leu.2402702]

16 **James C**, Trouette H, Marit G, Cony-Makhoul P, Mahon FX. Histological features of acute hepatitis after imatinib mesylate treatment. *Leukemia* 2003; **17**: 978-979 [PMID: 12750713 DOI: 10.1038/sj.leu.2402910]

17 **Ayoub WS**, Geller SA, Tran T, Martin P, Vierling JM, Poordad FF. Imatinib (Gleevec)-induced hepatotoxicity. *J Clin Gastroenterol* 2005; **39**: 75-77 [PMID: 15599217]

18 **Kikuchi S**, Muroi K, Takahashi S, Kawano-Yamamoto C, Takatoku M, Miyazato A, Nagai T, Mori M, Komatsu N, Ozawa K. Severe hepatitis and complete molecular response caused by imatinib mesylate: possible association of its serum concentration with clinical outcomes. *Leuk Lymphoma* 2004; **45**: 2349-2351 [PMID: 15512829 DOI: 10.1080/10428190412331272721]

19 **Seggewiss R**, Loré K, Greiner E, Magnusson MK, Price DA, Douek DC, Dunbar CE, Wiestner A. Imatinib inhibits T-cell receptor-mediated T-cell proliferation and activation in a dose-dependent manner. *Blood* 2005; **105**: 2473-2479 [PMID: 15572591 DOI: 10.1182/blood-2004-07-2527]

20 **Cwynarski K**, Laylor R, Macchiarulo E, Goldman J, Lombardi G, Melo JV, Dazzi F. Imatinib inhibits the activation and proliferation of normal T lymphocytes in vitro. *Leukemia* 2004; **18**: 1332-1339 [PMID: 15190258 DOI: 10.1038/sj.leu.2403401]

21 **Liang R**, Lau GK, Kwong YL. Chemotherapy and bone marrow transplantation for cancer patients who are also chronic hepatitis B carriers: a review of the problem. *J Clin Oncol* 1999; **17**: 394-398 [PMID: 10458258]

22 **Ridruejo E**, Cacchione R, Villamil AG, Marciano S, Gadano AC, Mandó OG. Imatinib-induced fatal acute liver failure. *World J Gastroenterol* 2007; **13**: 6608-6111 [PMID: 18161937]

23 **Mohty M**, Jourdan E, Mami NB, Vey N, Damaj G, Blaise D, Isnardon D, Olive D, Gaugler B. Imatinib and plasmacytoid dendritic cell function in patients with chronic myeloid leukemia. *Blood* 2004; **103**: 4666-4668 [PMID: 14715630 DOI: 10.1182/blood-2003-09-3220]

24 **European Association For The Study Of The Liver**. EASL clinical practice guidelines: Management of chronic hepatitis B virus infection. *J Hepatol* 2012; **57**: 167-185 [PMID: 22436845 DOI: 10.1016/j.jhep.2012.02.010]

P- Reviewer Piekarska A **S- Editor** Gou SX **L- Editor** A **E- Editor** Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i8.1322

World J Gastroenterol 2013 February 28; 19(8): 1322-1326
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Multiple endocrine neoplasia type 1 with upper gastrointestinal hemorrhage and perforation: A case report and review

Ying-Ying Lu, Feng Zhu, Da-Dao Jing, Xie-Ning Wu, Lun-Gen Lu, Gen-Quan Zhou, Xing-Peng Wang

Ying-Ying Lu, Feng Zhu, Da-Dao Jing, Xie-Ning Wu, Lun-Gen Lu, Xing-Peng Wang, Department of Gastroenterology, Shanghai First People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200080, China
Gen-Quan Zhou, Department of Radiology, Shanghai First People's Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200080, China
Author contributions: Lu YY and Zhu F wrote the manuscript; Lu LG, Wu XN, Jing DD and Wang XP revised the manuscript; Zhou GQ contributed to the image revision.
Correspondence to: Da-Dao Jing, Professor, Department of Gastroenterology, Shanghai First People's Hospital, Shanghai Jiao Tong University School of Medicine, No. 100, Haining Rd, Shanghai 200080, China. jingdadao@medmail.com.cn
Telephone: +86-21-63240090-3061 Fax: +86-21-63241377
Received: November 11, 2012 Revised: January 9, 2013
Accepted: January 23, 2013
Published online: February 28, 2013

Abstract

Multiple endocrine neoplasia type 1 (MEN1) is a rare hereditary syndrome known to predispose subjects to endocrine neoplasms in a variety of tissues such as the parathyroid glands, pituitary gland, pancreas and gastrointestinal tract. We herein report a patient with a past history of pituitary adenoma, presenting with symptoms of chronic diarrhea for nearly one year and a sudden upper gastrointestinal hemorrhage as well as perforation without signs. Nodules in the duodenum and in the uncinate process and tail of pancreas and enlargement of the parathyroid glands were detected on preoperative imaging. Gastroscopy revealed significant ulceration and esophageal reflux diseases. The patient underwent subtotal parathyroidectomy and autotransplantation, pylorus-preserving pancreaticoduodenectomy and pancreatic tail resection and recovered well. The results observed in our patient suggest that perforation and bleeding of intestine might be symptoms of Zollinger-Ellison Syndrome in patients with MEN1.

© 2013 Baishideng. All rights reserved.

Key words: Pituitary adenoma; Hyperparathyroidism; Gastrinoma; Gastrointestinal hemorrhage; Perforation

Lu YY, Zhu F, Jing DD, Wu XN, Lu LG, Zhou GQ, Wang XP. Multiple endocrine neoplasia type 1 with upper gastrointestinal hemorrhage and perforation: A case report and review. *World J Gastroenterol* 2013; 19(8): 1322-1326 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i8/1322.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i8.1322

INTRODUCTION

Multiple endocrine neoplasia type 1 (MEN1) syndrome is transmitted as an autosomal dominant mutation of a suppressor gene located on chromosome 11q13 and it is a rare congenital disease. The classical clinical manifestation of MEN1 is composed of parathyroid hyperplasia, pancreatic endocrine tumor and pituitary adenoma[1]. The definition of MEN1 is the coincidence of at least two of the above mentioned tumors[1].

We herein report a case of MEN1 with sudden upper gastrointestinal hemorrhage and perforation 14 years after surgery for pituitary adenoma.

CASE REPORT

A 42-year-old man was hospitalized with symptoms of nausea, vomiting and diarrhea for more than one year. The patient, presenting with decreased vision, diminished interest in sex, fatigue, weakness and polyuria, was diagnosed with pituitary adenoma in 1998. His blood prolactin level was high (more than 200 ng/mL, reference range, 2-23 ng/mL). The patient underwent removal of the tumor *via* the trans-sphenoidal approach in February 1998. Histological examination of the surgical specimen

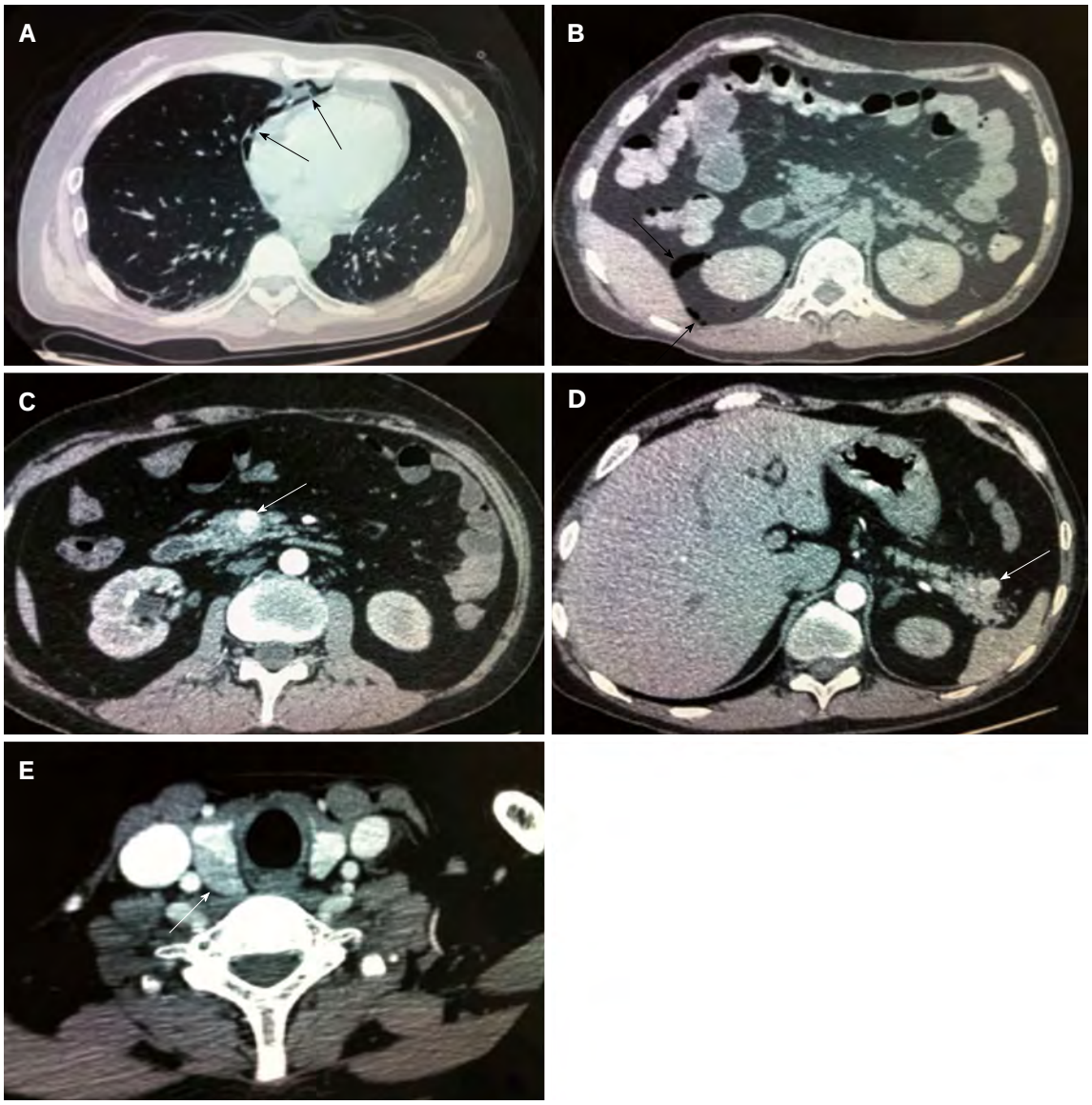


Figure 1 Computed tomography scan of the case. A: Chest Computed tomography (CT) shows a mediastinal emphysema (arrows); B: Epigastric CT shows accumulation of gas and fluid (arrows) in the anterior pararenal space just adjacent to the thickened wall of the horizontal part of the duodenum as well as accumulation of gas in the right perirenal space; C, D: A small intestine CT shows bowel wall thickening and strong enhancement of the horizontal part of the duodenum and a nodular mass (arrows) with a rich blood supply in the uncinate process (C) and tail (D) of the pancreas; E: Thyroid gland CT shows mild nodular goiter with nodules (arrow) posterior and lateral to the thyroid gland.

showed a chromophore cell adenoma. The patient's family history was notable for his brother also having a pituitary adenoma. In 2009, the patient underwent surgical procedures to remove a kidney stone. Recently, the patient had diarrhea, nausea and vomiting lasting for more than one year while gastroscopy and colonoscopy were performed prior to admission. Gastroscopy showed reflux esophagitis, esophageal protrusive lesion, gastric polyp and duodenal ulcer. Colonoscopy was normal. The patient was admitted on March 18, 2012. Physical examination revealed hypopigmentation of skin, sparse hair with normal blood pressure, body temperature, breath sounds and heart rhythms. The abdomen was prominently tender with normal bowel sounds and there was no shifting dullness found on the mass. Rectal examination was normal. Laboratory tests showed the following data: leukocytosis (22.40×10^9), mildly low hemoglobin level (115 g/L), elevated serum calcium (3.05 mmol/L, reference range, 2.1-2.55 mmol/L), low phosphorus level (0.57 μmol/L, reference range, 0.81-1.45 μmol/L), extremely high level of parathyroid hormone (PTH) (627 pg/mL, reference range, 15-65 pg/mL). Serum magnesium, calcitonin, electrolytes, glucose, liver and renal function were normal. Computed tomography (CT) scan of the chest revealed mediastinal emphysema with hypostasis in the inferior lobe of the lung (Figure 1A). Epigastric CT scan showed accumulation of gas and fluid in the anterior pararenal space just adjacent to the thickened wall of horizontal duodenum as well as accumulation of gas in the right perirenal space, which implied the possibility of duodenal perforation. CT imaging also found renal and hepatic cysts (Figure 1B). The patient complained of melena

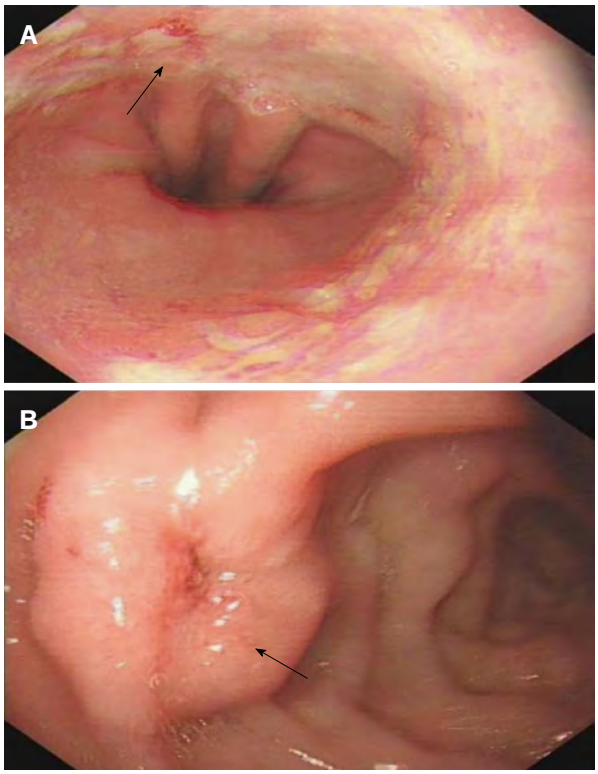


Figure 2 Gastroscopy reveals reflux esophagitis LA grade C and multiple deep ulcers in the descending part of duodenum, arrows indicate the erosion and ulcer respectively.

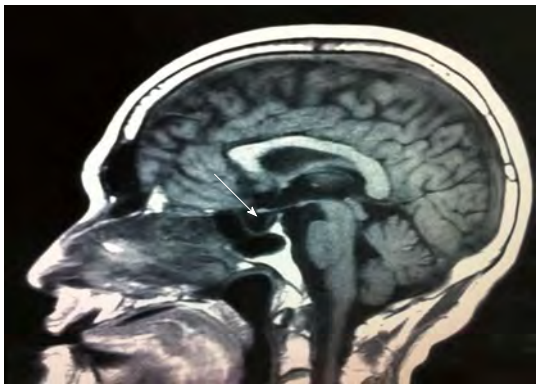


Figure 3 Pituitary Magnetic resonance imaging shows no space-occupying lesion in the sellar region (arrow).

two days after admission and the complete blood count showed medium anemia (hemoglobin 86 g/L) and positive stool occult blood (OB). Emergency gastroscopy was performed and revealed chronic superficial gastritis with erosion, reflux esophagitis LA grade C and multiple deep ulcers in the descending part of the duodenum (Figure 2). Pathological analysis showed chronic inflammation of duodenal mucosa. CT scan of the small intestine showed bowel wall thickening and strong enhancement of the horizontal part of the duodenum. A nodular mass with rich blood supply in the uncinate process and tail of pancreas led to a diagnosis highly suspicious for Zollinger-Ellison syndrome (ZES), and an adenoma on the left

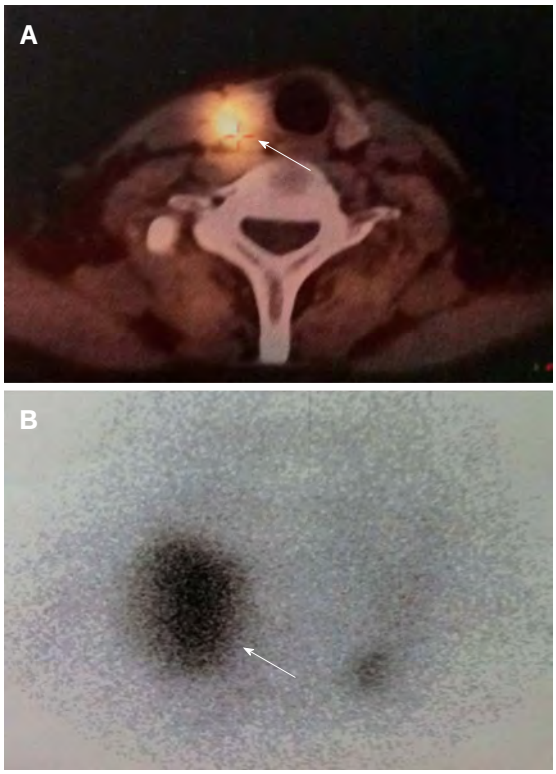


Figure 4 Radio-isotope scan reveals soft tissue masses (arrows) posterior to the thyroid gland which displayed abnormal uptake of 99mTc-MIBI.

adrenal gland and multiple liver cysts were also found in this CT image (Figure 1C-D). Magnetic resonance imaging (MRI) of the pituitary showed no space-occupying lesion in the sellar region (Figure 3). PTH was re-examined and the result was 401 pg/mL, which was still significantly higher than the normal value. Laboratory tests also revealed elevated serum gastrin (342.27 pg/mL, reference range, 0-108 pg/mL), elevated prolactin (127.50 ng/mL, reference range, 4.97-23.3 ng/mL) and low testosterone level (0.19 ng/mL, reference range, 2.8-8 ng/mL). Serum progesterone, follicle-stimulating hormone (FSH), luteotropic hormone, estradiol, cortisol, adrenocorticotropic hormone (ACTH), aldosterone and thyroid hormones were normal. CT scan of the thyroid gland showed a mild nodular goiter with nodules posterior and lateral to the thyroid gland, which might originate from an enlargement of the parathyroid glands (Figure 1E). A radioisotope scan revealed soft tissue masses posterior to the thyroid gland and abnormal uptake of 99mTc-MIBI, considered to be parathyroid adenoma or hyperplasia (Figure 4). The patient was diagnosed with MEN1 presenting as hyperparathyroidism, gastrinoma and prolactinoma. He underwent subtotal parathyroidectomy and autotransplantation on April 9, 2012. The pathology of all parathyroid glands was consistent with chief cell hyperplasia with immunohistochemical expression of CK19, CK8, CgA, Syn and NSE and negative expression of TTF-1, TG and Ki67 (Figure 5A). After operation, the levels of serum calcium and PTH fell to normal. The patient had no evidence of metastatic disease on preoperative studies. With the

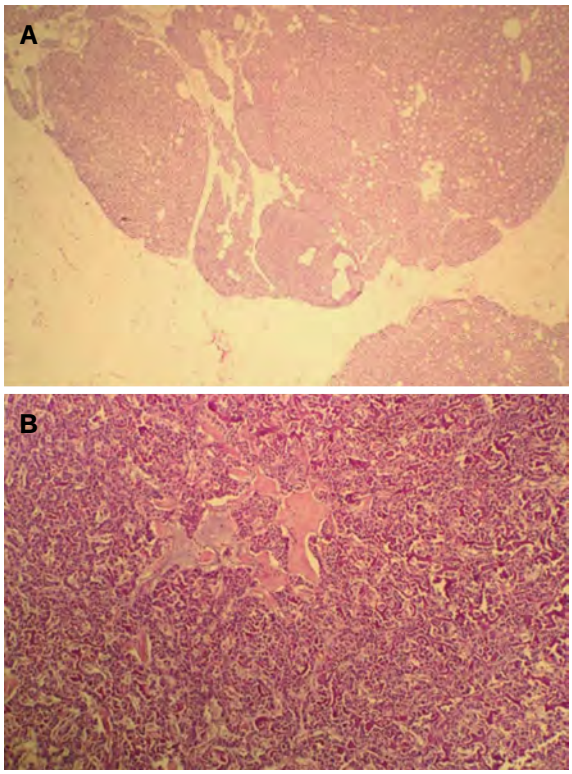


Figure 5 Histological and Pathological analysis of the case (hematoxylin and eosin staining, × 100). A: Histological analysis shows chief cell hyperplasia of parathyroid gland; B: Pathological analysis shows that tumor cells had acidophilic cytoplasm and round nucleoli which were uniform in size and shape, arranged in tubular, organoid and gyriform patterns.

pancreatic endocrine tumors being well-located in the CT image, pylorus-preserving pancreaticoduodenectomy and pancreatic tail resection were performed on April 24, 2012 for removing gastrinoma and cure of ZES. Pathological analysis showed that tumor cells had an acidophilic cytoplasm and round nucleoli which were uniform in size and shape, arranging in tubular, organoid and gyriform patterns (Figure 5B). The pathological diagnosis was a well-differentiated neuroendocrine tumor, infiltrating the muscular layer of the duodenal bowel wall, with no blood vessels and nerves involved and a well-differentiated neuroendocrine tumor also formed in the tail of pancreas, with immunohistochemical expression of NSE, Syn, CK8, CgA and α-AT, weakly positive expression of CK, CD56, Vim, CK19 and Ki67, and negative expression of 5-HT, insulin and ACTH. Pathologic examination of three lymph nodes near the duodenum and the head of pancreas showed chronic lymphadenitis. The postoperative level of serum gastrin was 39.34 pg/mL, and was returning to normal. The patient was discharged two weeks after the successful surgical resection of the tumor and is currently doing well but requires careful follow-up.

DISCUSSION

MEN1 can cause combinations of more than 20 different endocrine and nonendocrine tumors. Pituitary adenomas affect approximately 30% of patients and usually they are prolactin-secreting micro-adenomas or "nonfunctional" tumors[2]. Fasting prolactin concentration exceeding the upper limit of normal by 20-fold allows the diagnosis of prolactinoma[3]. In our case, the high prolactin level and CT imaging led to a diagnosis of pituitary adenoma which was further confirmed by histological analysis after operation. The patient had no other symptoms and was then considered to be cured. After a period of time, he suffered from chronic diarrhea lasting for nearly one year. There was little information regarding the cause of diarrhea until CT scan showed mediastinal emphysema and accumulation of gas and fluid in the anterior pararenal and right perirenal space which strongly implied the possibility of intestinal perforation. The patient had no positive abdominal physical signs that caused our bewilderment. While we were looking for the possible site of bowel perforation, the patient complained of melena unexpectedly and stool OB was positive, which led to the diagnosis of acute upper gastrointestinal bleeding. Emergency gastroscopy was performed and revealed multiple deep ulcers in the descending part of the duodenum, leading to the suspicion of gastrinoma, which was further confirmed by the elevated serum gastrin level and contrast-enhanced CT; and treatment with high-dose proton pump inhibitors was given. It is now evident that many affected patients have adrenal lesions. A non-functional adrenal cortical tumor was described in up to 40% of MEN1 cases and diagnosed by radiological imaging[4]. Our patient had an adenoma of the left adrenal gland but with normal testosterone, progesterone, FSH, ACTH, LH and aldosterone levels.

The patient had a significantly high serum calcium and PTH level which led to the diagnosis of hyperparathyroidism. Imaging showed parathyroid adenoma or hyperplasia. Hypercalcemia reminded us that the patient underwent a surgical procedure to remove a kidney stone three years ago. The patient stayed asymptomatic then and no further testing was performed, which suggested that clinical manifestation of MEN-1 is mostly mild for a long period of time and a lack of regular screening might result in numerous complications[5].

The fraction of MEN-1 in patients with primary hyperparathyroidism (HPT) is estimated at 1%-5%[6]. As is known, calcium is a secretagogue for gastrin, so HPT may exacerbate gastric acid secretion in concomitant ZES[7]. In practical terms, when serum calcium approaches or exceeds 2.75 mmol/L, surgery is recommended. The most common options for initial surgical management are either total parathyroidectomy and cervical thymectomy with heterotopic parathyroid autotransplantation or subtotal parathyroidectomy and cervical thymectomy. Subtotal parathyroidectomy is preferred because of the advantage of a lower risk of permanent hypoparathyroidism and an acceptable risk of recurrent HPT. In our patient, subtotal parathyroidectomy and autotransplantation were performed and the level of serum calcium decreased immediately. No hypoparathyroidism was detected post-operatively.

In MEN1, pancreatic neuroendocrine tumors occur

in 40%-80% of patients and are mostly non-functioning tumors or gastrinomas. The treatment of pancreaticoduodenal disease is quite controversial because of the inability to achieve biochemical cure consistently. In one retrospective analysis, surgical treatment for MEN-1-associated pancreatic tumors < 2 cm showed no advantage over conservative treatment[8]. However, analysis of another cohort of patients revealed that early detection and surgery are beneficial for MEN-1-associated pancreatic tumors[9]. Gastrinomas are the most common functional gastrointestinal neuroendocrine tumors, presenting in up to 54% of MEN1 patients. The diagnosis of ZES depends on proving elevated gastrin levels in the presence of high gastric acidity. Approximately one-third of patients with ZES can be diagnosed by a serum gastrin > 1000 pg/mL with a gastric pH < 3.0. The other two-thirds of patients require provocative tests. Our patient had a moderate increase in serum gastrin but serum gastrin remained < 1000 pg/mL and might require provocative tests to confirm the diagnosis. As the secretin supply was limited, nodules of our patient detected on preoperative imaging that caused significant ulceration and perforation, esophageal reflux symptoms and chronic diarrhea made the diagnosis valid. Prior to surgery, all MEN1 patients should be evaluated for coexisting neuroendocrine and other tumors as well as for the presence of metastases. Preoperative imaging is selected to define the extent of resection, and to identify tumors that would be outside the scope of resection. In our case, contrast-enhanced CT scan of the small intestine showed bowel wall thickening and strong enhancement of the horizontal part of the duodenum, a nodular mass with a rich blood supply in the uncinate process and tail of pancreas, but with no regional lymph nodes and hepatic metastases found. The lesions were well-located in CT imaging, hence no further imaging techniques, such as endoscopic ultrasound or somatostatin receptor scintigraphy, were necessary. The goals of surgical resection of pancreatic endocrine tumors are complete removal of the gross tumor burden, and preservation of pancreatic function. This often results in the subtotal resection of the distal pancreas, and enucleation of tumors in the head of the pancreas and duodenum. In all patients with gastrinoma, the duodenum should be opened and submucosal tumors resected. Total pancreatectomy is rarely indicated. According to all the information collected pre-operatively, our patient received pylorus-preserving pancreaticoduodenectomy and pancreatic tail resection. Postoperative complications were carefully monitored and an optimistic outcome was acquired. The level of serum gastrin returned to normal soon after surgery and the patient recovered well. We hope the patient is completely cured with no requirement for medication, but continued surveillance of all MEN1 patients is necessary and this patient is now under our careful follow-up.

ACKNOWLEDGMENTS

We wish to thank Xiang-Jun Sun from the Pathology department of our hospital for providing pathological results.

REFERENCES

1 **Brandi ML**, Gagel RF, Angeli A, Bilezikian JP, Beck-Peccoz P, Bordi C, Conte-Devolx B, Falchetti A, Gheri RG, Libroia A, Lips CJ, Lombardi G, Mannelli M, Pacini F, Ponder BA, Raue F, Skogseid B, Tamburrano G, Thakker RV, Thompson NW, Tomassetti P, Tonelli F, Wells SA, Marx SJ. Guidelines for diagnosis and therapy of MEN type 1 and type 2. *J Clin Endocrinol Metab* 2001; **86**: 5658-5671 [PMID: 11739416]

2 **Corbetta S**, Pizzocaro A, Peracchi M, Beck-Peccoz P, Faglia G, Spada A. Multiple endocrine neoplasia type 1 in patients with recognized pituitary tumours of different types. *Clin Endocrinol (Oxf)* 1997; **47**: 507-512 [PMID: 9425388]

3 **Tortosa F**, Chico A, Rodriguez-Espinosa J, Ruscalleda J, de Leiva A. Prevalence of MEN 1 in patients with prolactinoma. MEN1 Study Group of the Hospital de la Santa Creu i Sant Pau of Barcelona. *Clin Endocrinol (Oxf)* 1999; **50**: 272 [PMID: 10396374]

4 **Skogseid B**, Larsson C, Lindgren PG, Kvanta E, Rastad J, Theodorsson E, Wide L, Wilander E, Oberg K. Clinical and genetic features of adrenocortical lesions in multiple endocrine neoplasia type 1. *J Clin Endocrinol Metab* 1992; **75**: 76-81 [PMID: 1352309]

5 **Chudek J**, Piecha G, Nieszporek T, Marini F, Brandi ML, Wiecek A. Novel 1113delC menin gene mutation in a Polish family with multiple endocrine neoplasia type 1 syndrome. *Eur J Intern Med* 2006; **17**: 447-449 [PMID: 16962958]

6 **Uchino S**, Noguchi S, Sato M, Yamashita H, Yamashita H, Watanabe S, Murakami T, Toda M, Ohshima A, Futata T, Mizukoshi T, Koike E, Takatsu K, Terao K, Wakiya S, Nagatomo M, Adachi M. Screening of the Men1 gene and discovery of germ-line and somatic mutations in apparently sporadic parathyroid tumors. *Cancer Res* 2000; **60**: 5553-5557 [PMID: 11034102]

7 **Gogel HK**, Buckman MT, Cadieux D, McCarthy DM. Gastric secretion and hormonal interactions in multiple endocrine neoplasia type I. *Arch Intern Med* 1985; **145**: 855-859 [PMID: 2859842]

8 **Triponez F**, Goudet P, Dosseh D, Cougard P, Bauters C, Murat A, Cadiot G, Niccoli-Sire P, Calender A, Proye CA. Is surgery beneficial for MEN1 patients with small (& lt; or = 2 cm), nonfunctioning pancreaticoduodenal endocrine tumor? An analysis of 65 patients from the GTE. *World J Surg* 2006; **30**: 654-62; discussion 663-4 [PMID: 16680582]

9 **Kouvaraki MA**, Shapiro SE, Cote GJ, Lee JE, Yao JC, Waguespack SG, Gagel RF, Evans DB, Perrier ND. Management of pancreatic endocrine tumors in multiple endocrine neoplasia type 1. *World J Surg* 2006; **30**: 643-653 [PMID: 16680581]

P- Reviewer Loffroy R **S- Editor** Jiang L **L- Editor** A
E- Editor Zhang DN



World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i8.1327

World J Gastroenterol 2013 February 28; 19(8): 1327-1329
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Glomus tumor in the stomach: Computed tomography and endoscopic ultrasound findings

Min Tang, Jun Hou, Dong Wu, Xin-Ye Han, Meng-Su Zeng, Xiu-Zhong Yao

Min Tang, Dong Wu, Xin-Ye Han, Meng-Su Zeng, Xiu-Zhong Yao, Department of Radiology, Zhongshan Hospital of Fudan University, Shanghai 200032, China
Min Tang, Dong Wu, Xin-Ye Han, Meng-Su Zeng, Xiu-Zhong Yao, Department of Medical Image, Shanghai Medical College of Fudan University, Shanghai 200032, China
Jun Hou, Department of Pathology, Zhongshan Hospital of Fudan University, Shanghai 200032, China
Author contributions: Wu D and Zeng MS approved the final version to be published; Han XY revised the overall language; Hou J provided the figures and discussion of the pathology; Yao XZ revised the report critically for important intellectual content; and Tang M designed and wrote the case report.
Supported by National Natural Science Foundation of China, No. 30901228
Correspondence to: Meng-Su Zeng, MD, PhD, Department of Radiology, Zhongshan Hospital of Fudan University, No. 138, Fenglin Road, Xuhui District, Shanghai 200032, China. zeng.mengsu@zs-hospital.sh.cn
Telephone: +86-21-64041990 Fax: +86-21-64439906
Received: September 26, 2012 Revised: January 21, 2013
Accepted: January 23, 2013
Published online: February 28, 2013

Abstract

A 57-year-old man presented with intermittent dull abdominal pain after a period of 1 year. Abdominal computed tomography (CT) was performed. Except for the endoscopy, the work-up for possible medical causes remained inconclusive. An open-abdomen, partial surgical excision of the stomach was performed after the unsuccessful endoscopic resection. The pathology report revealed a glomus tumor of the stomach. Importantly, glomus tumors of the stomach are rare and are almost always benign. Therefore, the most important current role of imaging associated with the diagnostic approach and therapeutic plan for a glomus tumor is to differentiate it from other gastric submucosal tumors (SMTs). We report this case with representative radiologic findings, including CT and endoscopic ultrasound (EUS) reports, and also correlate them with clinical and pathologic presentations that can help in the early detection and differentiation of gastric SMTs from other SMTs. As such, the purpose of this report is to provide a better understanding of relevant CT and EUS features. Alternative treatments should be considered carefully according to the imaging results.

© 2013 Baishideng. All rights reserved.

Key words: Glomus tumor; Stomach; Diagnosis; Therapy

Tang M, Hou J, Wu D, Han XY, Zeng MS, Yao XZ. Glomus tumor in the stomach: Computed tomography and endoscopic ultrasound findings. *World J Gastroenterol* 2013; 19(8): 1327-1329 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i8/1327.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i8.1327

INTRODUCTION

Glomus tumors of the stomach are rare; predominantly benign; and commonly described as solitary, well-defined gastric submucosal tumors (SMTs) in the antrum with non-specific clinical manifestations[1-4]. Gastrointestinal stromal tumors (GISTs), undoubtedly the most common malignant SMTs of the stomach, have the capacity to become malignant and later metastasize. Therefore, the most important and plausible current role of imaging in the diagnostic approach and therapeutic plan for a glomus tumor is to differentiate it from other SMTs[1,4]. We report this case with representative radiologic findings, including computed tomography (CT) and endoscopic ultrasound (EUS) reports, and also correlate them with clinical pathologic presentations that can help in the early detection and differentiation of GISTs from other SMTs. The diagnosis was surgically confirmed. Contrast-enhanced CT showed peripheral nodular or homogeneous strong enhancement in the arterial phase and prolonged enhancement in the delayed phase[1-6].

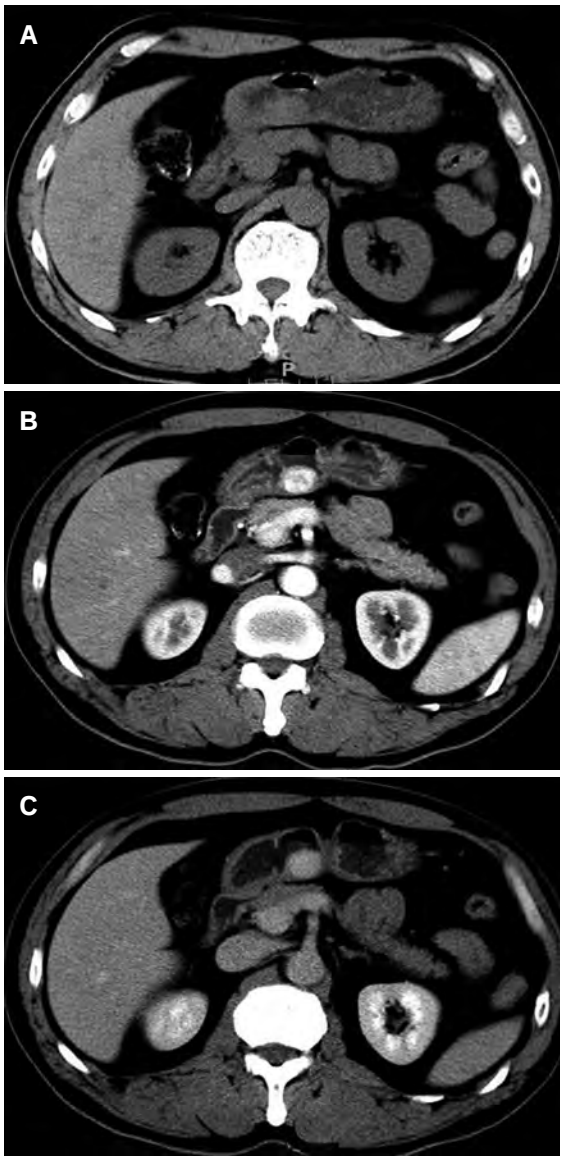


Figure 1 A 57-year-old man presented with intermittent dull abdominal pain after a duration of 1 year with a glomus tumor in the lesser curvature. A: A plain computed tomography (CT) scan of the tumor revealing a clear regular contour and an isodensity mass; B: The mass shows dense homogeneous enhancement with an intact overlying mucosa on early-phase contrast-enhanced CT; C: The mass shows continuous homogeneous enhancement on delay-phase contrast-enhanced CT.

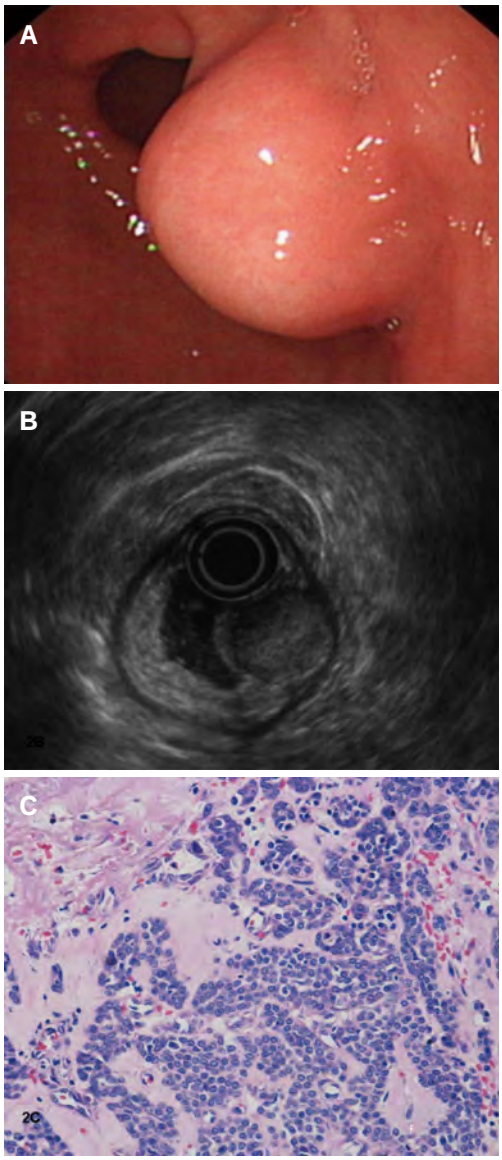


Figure 2 Gastroscopy shows a submucosa eminent lesion in the stomach wall. A: Endoscopic ultrasonic shows a solid mass originating from the superficial layer of the muscular propria. It is 20 mm × 16 mm in size, and a marginal halo can be observed; B: The mass appears as a round, hypoechoic lesion with homogenous echogenicity. After a failed endoscopic submucosal dissection with ligation for the treatment of the small tumor, a subtotal gastrectomy was performed; C: Microscopic examination shows numerous dilated, thin-walled vascular spaces surrounded by uniform glomus cells (hematoxylin and eosin stain, × 50).

CASE REPORT

One year ago, a 57-year-old man presented to the local primary hospital with intermittent dull abdominal pain, especially when hungry. At that time, an upper gastrointestinal endoscope revealed mild diffuse gastritis. The patient underwent conservative medical management for 8 mo, during which the abdominal pain disappeared. Over a recent 3-wk period, however, the pain reappeared at levels higher than observed previously. The patient was transferred to our hospital and subjected to further investigation. A plain CT scan of the tumor revealed a clear regular contour and an isodensity mass (Figure 1A). The mass showed dense homogeneous enhancement, suggesting a lesion with an abundant blood supply and an intact overlying mucosa on an early- (Figure 1B) and delayed-phase (Figure 1C) contrast-enhanced CT. The endoscope examination showed a submucosal gastric mass that was approximately 2.0 cm and was identified at the lesser curvature of the antrum (Figure 2A). A solid mass originated from the superficial layer of the muscular propria, measuring 20 mm × 16 mm, with a marginal halo. The mass appeared to be a round, hypoechoic lesion with homogenous echogenicity (Figure 2B).

Endoscopic submucosal dissection (ESD) was initially planned, although it was unsuccessful due to significant bleeding. As such, the patient was treated via open-abdomen partial surgical excision of the stomach. After 7 mo, an abdominal CT follow-up showed no sign of tumor recurrence.

Pathologic findings indicated that this was a glomus tumor, which is characterized by many vessels that are lined with endothelial cells and surrounded by a solid proliferation of round or cuboidal cells with a round nucleus and eosinophilic cytoplasm (Figure 2C). Immunohistochemical studies revealed that the tumor cells were positive for smooth muscle actin (40%) and muscle-specific actin (100%++) but negative for desmin and CD34/CD31.

DISCUSSION

Gastric tumors are either epithelial or mesenchymal in origin. Radiologic findings enable a straightforward differentiation between mucosal or submucosal tumors. However, the identification of benign or malignant tumors depends on their biological behavior. Approximately 85% to 90% of all gastric tumors are benign, and 50% of these are mesenchymal in origin. This subgroup of gastric tumors can be further divided into two groups. The first group arises from the somatic tissue, which includes smooth muscle tumors, neural tumors, lipocystic tumors, and tumors originating from vascular or perivascular tissues (*e.g.*, glomus tumors). In contrast, more than 95% of all malignant tumors of the stomach are adenocarcinomas. The remaining malignant tumor types include lymphomas, sarcomas (*e.g.*, malignant gastrointestinal stromal tumors), and carcinoid tumors, among others. The second group is larger and includes GISTs[1]. Glomus tumors in the stomach are rare and are almost always benign; therefore, the most important role of imaging in diagnostic and therapeutic approaches is the differentiation of GISTs from other gastric submucosal tumors.

In 1935, Masson published a monograph entitled "Les glomus cutanes del'Homme." The classic location of a glomus tumor is the subungual region of a digit, although it may occur anywhere in the body, including the skin, soft tissues, nerves, stomach, nasal cavity, trachea, and liver. Glomus tumors of the stomach typically appear as a submucosal nodule or mass on the antrum[2]. Although a glomus tumor of the stomach is a pathologic diagnosis, it should be included as a differential diagnosis if a solitary, hypervascular submucosal tumor is present in the stomach. Other mesenchymal tumors, such as carcinoid tumors, GISTs, neurilemmomas, and hemangiomas, may show a similar pattern. The overlap of radiologic findings in many gastric tumors makes their differentiation difficult. However, some unusual gastric tumors have characteristic radiologic features that may suggest a specific diagnosis. The incidence of gastric carcinoid tumors is increasing, and multiple tumors may occur from the fundus to the antrum. The imaging features of GISTs are variable, ranging from a small nodule with signal changes similar to the gastric muscle to a large mass with a cystic change, an intratumoral hemorrhage, necrosis, or ulceration with air-fluid levels. Hemangiomas are characterized by color changes in the vessels that appear blue or red in an endoscopy and may contain phleboliths that can be detected by CT. Neurilemmomas have a moderately or markedly higher hyperintensity with T2-weighted imaging. In combination with tumor size, location, enhanced pattern, and the intrinsic soft-tissue content, the high resolution provided by an magnetic resonance imaging may constitute one the noninvasive diagnostic or therapeutic options for gastric glomus tumors, in addition to surgery, endoscopic mucosal resectioning, and fine-needle aspiration biopsy[1,3,4].

Our report describes a method of discerning between gastric glomus tumors and other SMTs *via* CT and EUS imaging findings. Endoscopic submucosal enucleation is a feasible and safe procedure for the treatment of this lesion. However, further investigation and comparative studies are required to confirm the safety and efficacy of this method. Radiologically, glomus tumors appear as smooth submucosal masses with or without ulceration and may contain tiny flecks of calcification. Imaging results may suggest suspicious malignant findings, such as a large lesion measuring more than 3-5 cm, a lesion with irregular margins, a cystic space, a heterogeneous echo, or an interval change on follow-up. However, in the absence of such findings, close surveillance is recommended along with an operation and postoperative follow-ups at undefined time intervals[4-6].

In conclusion, CT and EUS findings that correlate with clinical presentations can contribute to the early detection and identification of gastric glomus tumors, particularly in terms of assessing the tumor blood supply. Endoscopists prefer ESD to open surgery, although the former necessitates caution due to complications that may occur due to significant bleeding.

REFERENCES

1 **Park SH**, Han JK, Kim TK, Lee JW, Kim SH, Kim YI, Choi BI, Yeon KM, Han MC. Unusual gastric tumors: radiologic-pathologic correlation. *Radiographics* 1999; **19**: 1435-1446 [PMID: 10555667 DOI: 10.1109/12.600830]

2 **Murray MR**, Stout AP. The glomus tumor: Investigation of its distribution and behavior, and the identity of its "epithelioid" cell. *Am J Pathol* 1942; **18**: 183-203 [PMID: 19970623]

3 **Maffei M**, Andereggen E, Genevay M, Huber O, Dumonceau JM. Clinical challenges and images in GI. A gastric glomus tumor. *Gastroenterology* 2006; **131**: 1380, 1660 [PMID: 17128505 DOI: 10.1053/j.gastro.2006.09.031]

4 **Kang JH**, Lim JS, Kim JH, Hyung WJ, Chung YE, Choi JY, Park MS, Kim MJ, Kim KW. Role of EUS and MDCT in the diagnosis of gastric submucosal tumors according to the revised pathologic concept of gastrointestinal stromal tumors. *Eur Radiol* 2009; **19**: 924-934 [PMID: 19023576 DOI: 10.1007/s00330-008-1224-2]

5 **Canzonieri V**, Bidoli E. Differential diagnosis of malignant gastric tumors. A pathological appraisal with reference to locally advanced gastric carcinoma. *Suppl Tumori* 2003; **2**: S5-S9 [PMID: 12914380]

6 **Ferrozzi F**, Tognini G, Marchesi G, Spaggiari E, Pavone P. Gastric tumors with fatty components. CT findings and differential diagnosis. *Radiol Med* 2000; **100**: 343-347 [PMID: 11213412]

P- Reviewer Kita H **S- Editor** Wen LL **L- Editor** A **E- Editor** Li JY

World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/esps/
wjg@wjgnet.com
doi:10.3748/wjg.v19.i8.1330

World J Gastroenterol 2013 February 28; 19(8): 1330-1332
ISSN 1007-9327 (print) ISSN 2219-2840 (online)
© 2013 Baishideng. All rights reserved.

CASE REPORT

Phlegmonous gastritis after esophagectomy: A case report

Jun-Qiang Fan, Da-Ren Liu, Chao Li, Gang Chen

Jun-Qiang Fan, Gang Chen, Department of Thoracic Surgery, Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310009, Zhejiang Province, China
Da-Ren Liu, Chao Li, Department of General Surgery, Second Affiliated Hospital, School of Medicine, Zhejiang University, Hangzhou 310009, Zhejiang Province, China
Author contributions: The authors contributed equally to this work.
Correspondence to: Jun-Qiang Fan, MD, Department of Thoracic Surgery, Second Affiliated Hospital, School of Medicine, Zhejiang University, 88 Jiefang St, Hangzhou 310009, Zhejiang Province, China. fanfun@126.com
Telephone: +86-571-87783570 Fax: +86-571-87783570
Received: November 4, 2012 Revised: January 3, 2013
Accepted: January 23, 2013
Published online: February 28, 2013

Abstract

Phlegmonous gastritis is an unusual infection of the gastric wall, which is extremely rare and associated with a poor prognosis. Here, we report the case of a 65-year-old male patient with a history of splenectomy, who had phlegmonous gastritis after esophagectomy. Computed tomography revealed a remarkably distended thoracic stomach, and the gastric wall was locally thickened. Gastric mucosa was red and white in color and significantly edematous on gastroscopy. He was successfully treated with a combination of antibiotics and povidone-iodide intraluminal lavage. In addition to this case, the clinical presentations, imaging examinations as well as treatments of phlegmonous gastritis are discussed.

© 2013 Baishideng. All rights reserved.

Key words: Phlegmonous gastritis; Esophagectomy; Splenectomy; Computed tomography

Fan JQ, Liu DR, Li C, Chen G. Phlegmonous gastritis after esophagectomy: A case report. *World J Gastroenterol* 2013; 19(8): 1330-1332 Available from: URL: http://www.wjgnet.com/1007-9327/full/v19/i8/1330.htm DOI: http://dx.doi.org/10.3748/wjg.v19.i8.1330

INTRODUCTION

Phlegmonous gastritis (PG) is an unusual infection of the gastric wall caused by suppurative bacteria. Mortality due to PG approached 92% before the advent of antibiotics and was as high as 64% even after antibiotics became available[1]. Clinical presentations of PG are generally nonspecific and include epigastric pain, vomiting and infection. Therefore, it is extremely difficult to make a correct diagnosis at the early onset, and many patients undergo gastrectomy due to the severity of the disease in the acute phase. Herein, we report a rare PG after esophagectomy which was successfully treated with conservative treatment.

CASE REPORT

A 65-year-old male patient was admitted with the complaint of epigastric discomfort for 1 wk. He had a history of splenectomy 18 mo previously due to a splenic rupture after a traffic accident. Esophageal erosions located 34 cm below the incisor teeth were revealed by preoperative gastroscopy. Pathological examination of the esophageal lesion indicated adenocarcinoma. Therefore, an exploratory thoracotomy was performed. During surgery, a 3 cm solid mass without well-defined borders was found in the lower esophagus. An esophagus resection followed by supra-arch esophagogastric anastomosis was performed.

However, on the first postoperative night, the patient became febrile with a fever of 39 ℃. The next morning, the high temperature continued and the patient suddenly developed dyspnea with SaO_2 continuously below 90% after oxygen *via* a face mask (5 L/min). Physical examination revealed weakened breath sounds in the left lower lobe and laboratory examinations showed decreased PO_2 (63.8 mmHg) and elevated leukocytes (21.3×10^4) and neutrophils (90.4%). Chest X-ray showed a large consolidation shadow of the left lung, and both costophrenic



Figure 1 Postoperative chest X-ray. A large consolidation shadow of the left lung, low density radiolucent areas on the left upper lobe and high density fragment images on the right lung are shown. Both costophrenic angles were obscure.

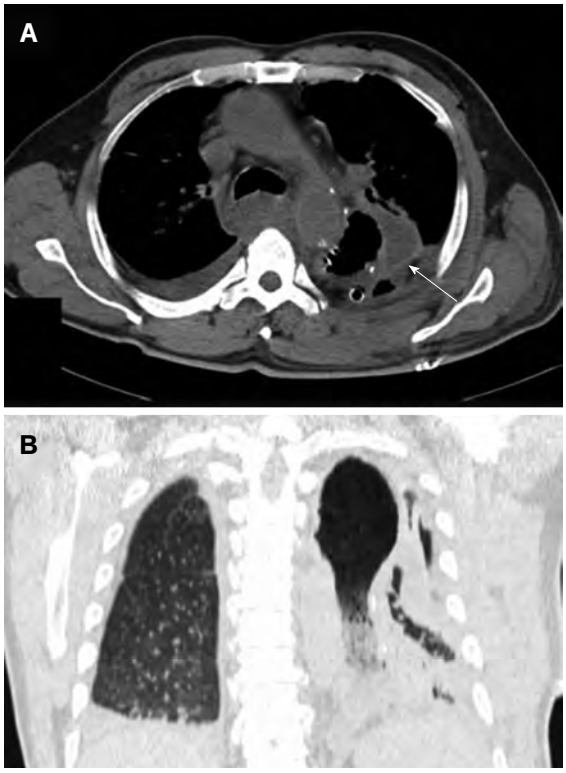


Figure 2 Computed tomography scan. A: Axial computed tomography (CT) imaging of the chest: The CT scan showed a remarkably distended thoracic stomach and a locally thickened gastric wall (arrow); B: Coronal CT scan of the chest: Imaging revealed a thoracic stomach with a large consolidation shadow and in air bronchograms of the left lung, pleural and pericardial effusions were also suggested.

angles were obscure (Figure 1). Therefore, tracheal intubation was performed based on the intended diagnosis of respiratory failure.

Over the next 5 d, the patient's body temperature remained at 39 ℃. Chest computed tomography (CT) revealed a remarkably distended thoracic stomach, and the gastric wall was locally thickened, pleural and pericardial effusions were also suggested (Figure 2). Gastrointestinal endoscopy revealed congestion and edema of the gastro-



Figure 3 Endoscopic examination of the postoperative stomach. The gastric mucosa was red and white in color, significantly edematous, and scattered erosions covered by purulent exudate were observed. No anastomotic leak was noted.

esophageal anastomosis, the gastric mucosa was red and white in color and significantly edematous, a large number of scattered erosions covered by a layer of purulent exudates were also observed (Figure 3). Thus, the diagnosis of PG, which was suspected on CT, was confirmed. The sputum culture revealed *Staphylococcus aureus*, whereas the bacterial culture was negative for the gastric aspirate.

Following the results of the sputum culture, the patient was treated with a combination of teicoplanin and linezolid (Zyvox), as well as intraluminal lavage with 2.5% povidone-iodine. After 3 d, his temperature decreased, PO_2, and PCO_2 levels returned to normal, and the patient was successfully extubated. Antibiotics were continued for two weeks and no evidence of gastric mucosa inflammation was detected by gastroscopy. The patient was discharged on the 30th day after surgery.

DISCUSSION

PG is a rare condition caused by various organisms, affects both sexes and occurs mainly in individuals aged 30-70 years[2]. The etiology of PG is still unknown, alcohol consumption, chronic gastritis, mucosal injury, drugs, immunosuppression and malignancy have been suggested to cause PG[3]. In the current case, PG occurred after esophagectomy, which has been rarely reported in the published literature. It was noted that this patient had a history of splenectomy 18 mo before esophagectomy, although there is no known relationship between PG and splenectomy, decreased immunity due to splenectomy combined with the post-surgical stress response may have facilitated the entry of bacteria into the gastric submucosa and the induction of PG.

The typical clinical presentation of PG is the acute onset of a triad of symptoms consisting of: epigastric pain, vomiting and fever. Other symptoms such as nausea, chills, hematemesis have also been reported[1,4]. The pain is sometimes relieved by sitting in an upright position (Deininger's sign), and purulent emesis is said to be pathognomonic for PG regardless of its rarity[5]. High leukocytosis, accelerated erythrocyte sedimentation rate,

and dysproteinemia are usually seen in peripheral blood analysis.

Imaging examinations are helpful in PG diagnosis. On CT scanning, marked gastric wall thickening and intramural hypodense areas can be seen[6]; while endosonography (EUS) can reveal thickened gastric wall and anechoic areas[4]. On endoscopy, PG gastric mucosa is usually edematous and appears purple in color with dirty necrotic materials covering the surface[7], which indicate serosa penetration or peri-serosa effusion which is rarely seen and is mostly restricted to the gastric wall. In the present case, the patient had severe left lower lobe pneumonia with pleural and pericardial effusions, which suggested bacterial colonization of the gastric mucosa through sputum swallowing due to pulmonary infection.

The culture of gastric pus or a sample taken during the endoscopic procedure may help to identify the bacteria involved and provides a reference for antibiotic selection. Nevertheless, many cases of PG fail to document the presence of bacteria in the gastric wall, but the correct diagnosis can be made without histological documentation or bacterial culture from the gastric aspirate[6], suggesting that a positive culture of gastric secretions is not necessary for the diagnosis of PG. Our patient showed many of the manifestations mentioned above, although no organism was isolated from gastric contents. Laboratory findings of *Staphylococcus aureus* in sputum, CT imaging and endoscopic findings of pus justified the diagnosis of PG.

The standard of treatment for PG is still not well established. Treatment mainly involves conservative management with antibiotics and surgical gastrectomy. As in our case, which was diagnosed in the early phase, PG can be treated with the correct antibiotics and supportive treatment. Otherwise, gastrectomy should be considered. Surgery can be partial or total dissection depending on the extent of inflammation. However, a review by Kim *et al*[4] revealed that surgical interventions are superior to antibiotic therapies with mortality of 20% and 50%, respectively. Other treatments such as endoscopic mucosal resection and simple aspiration under endoscopic ultrasonography have also been reported[7].

In conclusion, PG is a rare and fatal condition that needs early diagnosis and appropriate management. Endoscopy, CT, EUS, and pathogen culture can be helpful in the diagnosis. In this article, we present an unusual case of PG after esophagectomy, through early diagnosis and effective antibiotic treatment, the patient recovered successfully. Surgeons should be aware of the possibility of PG when dealing with complex acute abdominal diseases.

REFERENCES

1 **Orel R**, Mlinaric V, Stepec S, Luzar B, Brencic E, Cerar A. Acute phlegmonous gastritis associated with Helicobacter heilmannii infection in a child. *Dig Dis Sci* 2006; **51**: 2322-2325 [PMID: 17103036 DOI: 10.1007/s10620-006-9193-2]

2 **Stephenson SE**, Yasrebi H, Rhatigan R, Woodward ER. Acute phlegmasia of the stomach. *Am Surg* 1970; **36**: 225-231 [PMID: 5436103]

3 **Park CW**, Kim A, Cha SW, Jung SH, Yang HW, Lee YJ, Lee HIe, Kim SH, Kim YH. A case of phlegmonous gastritis associated with marked gastric distension. *Gut Liver* 2010; **4**: 415-418 [PMID: 20981225 DOI: 10.5009/gnl.2010.4.3.415]

4 **Kim GY**, Ward J, Henessey B, Peji J, Godell C, Desta H, Arlin S, Tzagournis J, Thomas F. Phlegmonous gastritis: case report and review. *Gastrointest Endosc* 2005; **61**: 168-174 [PMID: 15672083]

5 **Nicholson BW**, Maull KI, Scher LA. Phlegmonous gastritis: clinical presentation and surgical management. *South Med J* 1980; **73**: 875-877 [PMID: 7384846]

6 **Sood BP**, Kalra N, Suri S. CT features of acute phlegmonous gastritis. *Clin Imaging* 2000; **24**: 287-288 [PMID: 11331158]

7 **Munroe CA**, Chen A. Suppurative (phlegmonous) gastritis presenting as a gastric mass. *Dig Dis Sci* 2010; **55**: 11-13 [PMID: 19757050 DOI: 10.1007/s10620-009-0961-7]

P- Reviewer Triantopoulou C **S- Editor** Gou SX
L- Editor Webster JR **E- Editor** Zhang DN

百世登
Baishideng®

Published by Baishideng Publishing Group Co., Limited
Room 1701, 17/F, Henan Building,
No. 90 Jaffe Road, Wanchai, Hong Kong, China
Fax: +852-31158812
Telephone: +852-58042046
E-mail: bpgoffice@wjgnet.com
http://www.wjgnet.com



ISSN 1007-9327

0 1>

© 2013 Baishideng. All rights reserved.